Supplemental Data

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Calnexin is Essential for Rhodopsin Maturation, Ca^{2+} Regulation and Photoreceptor Cell Survival

Erica E. Rosenbaum[1], Roger C. Hardie[2] and Nansi J. Colley[1]

Figure S1. Northern Blot Analysis of *cnx* mutants.
Rh1 transcript was normal in the *cnx* mutants. Northern blot analysis showing that the *ninaE* gene encodes a 1.5 kb transcript for Rh1. (1) WT (Canton S), (2) *cnx*^{1}, (3) *cnx*^{2}, (4) *ninaE*^{I17}, and (5) flies lacking eyes (*eya*^{1}). mRNA was isolated from heads of 0-7 day old *cnx* mutants, prior to retinal degeneration. Ten micrograms of polyA^{+} selected RNA were loaded into each lane (same as 2B). An internal control for loading was a DIG labeled actin RNA probe (Roche, Indianapolis, IN) (data not shown).

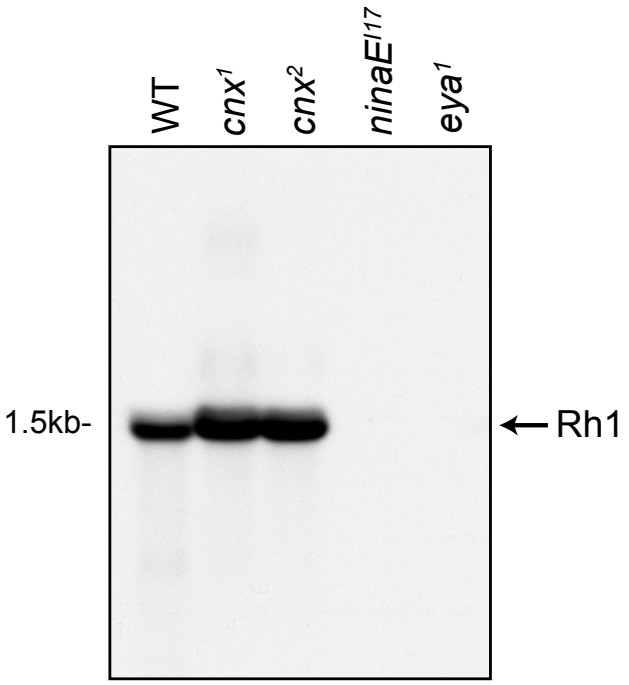
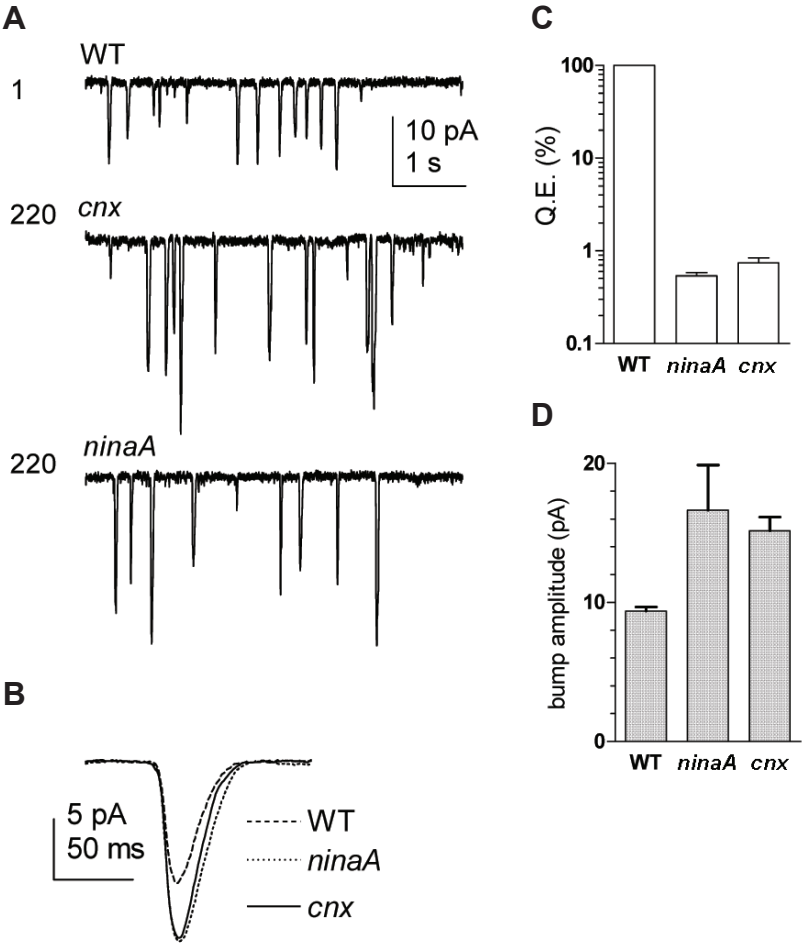


Figure S2. Whole Cell Recordings in WT, *cnx* and *ninaA* mutants.

Quantum bumps recorded in WT, *cnx* and *ninaA* mutants taken from dissociated ommatidia from recently eclosed adult flies and transferred to a recording chamber on an inverted Nikon Diaphot microscope. Whole-cell voltage clamp recordings were made using electrodes with a resistance of ~10-15 MOhm. Data were collected and analyzed using an Axopatch 1-D amplifier and pCLAMP 8 or 9 software (Axon Instruments, Foster City, CA). Cells were stimulated via a green LED. **(A)** Trains of quantum bumps induced by dim light (approximately 2 effective photons s^{-1}) in WT, *ninaA*^{P269} and *cnx* photoreceptors. Intensity relative to WT shown alongside each trace was 220x brighter in *cnx* and *ninaA*^{P269}. **(B)** Average quantum bumps (after aligning > 40 bumps by their rising phases). **(C)** Relative quantum efficiency (Q.E. normalized to WT) in both *ninaA*^{P269} (0.55%) and *cnx* (0.75%) was reduced approximately 200-fold. Mean ± S.E.M., n = 7 (*ninaA*^{P269}) and n= 10 for *cnx* (both alleles pooled). **(D)** Quantum bump amplitudes, n = 4 (*ninaA*^{P269}) and n = 7 (*cnx)*.



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University of Wisconsin-Madison

NEWS

Scientists link a gene to degenerative blindness

January 18, 2006

by Paroma Basu (mailto:basu1@wisc.edu)

Researchers have labored for decades to understand blindness-inducing neurodegenerative diseases such as age-related macular degeneration (AMD) and retinitis pigmentosa (RP).

It has been a painstaking scientific journey as AMD and RP each belong to a complex family of disorders, in which every disorder has many forms and each form is encoded with a distinct genetic recipe. Even AMD, a major cause of vision loss in people over 60, is actually a collection of more than 50 diseases.

Now, a team of researchers at UW-Madison has taken a small but crucial step forward in the ongoing fight against retinal degeneration. Working with fruit flies, the scientists have discovered that a mutation in a common gene called calnexin can derail the light-processing activity of cells and set in motion the gradual breakdown of vision. They report their findings today in the journal Neuron.

Calnexin-found in both fruit flies and humans-functions as a cellular chaperone, ensuring that proteins "fold" or orient properly and get to the parts of the cell they need to go. It also modulates calcium levels, which is critical for proper vision.

When calnexin goes awry, however, calcium levels build up and the proteins that depend on it malfunction, says senior author Nansi Jo Colley (http://www.genetics.wisc.edu/faculty/profile.php?id=100) , a medical geneticist at the UW-Madison departments of ophthalmology (http://wieyemd.ophth.wisc.edu/) and genetics (http://www.genetics.wisc.edu/faculty/profile.php?id=100) , and an affiliate of the Eye Research Institute.

At a time when more than 103 genes are known to be involved with AMD and RP, the UW-Madison work could one day help doctors deliver tailor-made treatments to patients who specifically carry calnexin mutations. Because the calnexin protein and other chaperones are also present in the brain, the work can help to answer broader questions about neurodegenerative disease, Colley adds.

"Understanding the basic mechanisms of how proteins are folded holds the key to finding treatments for not only retinal degenerative diseases but also other neurodegenerative diseases such as Alzheimer's, Huntington's and Parkinson's."

To detect the calnexin mutation, the UW-Madison team used genetic mapping to zero in on the exact region harboring the mutant. Subsequent DNA sequencing of that target area pinpointed calnexin as the culprit gene, explains lead author Erica Rosenbaum, a researcher in Colley's

laboratory.

Colley plans to continue searching for other genetic mutations that might help trigger retinal degeneration. "The more mutations we identify the easier it will be to step back and look at the big picture of the general principles of neurodegeneration," she says.

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Clues to blindness causes uncovered
Wisconsin State Journal Article
January 22, 2006

Clues to blindness causes uncovered

Diseases that cause progressive blindness, much like cancer, are actually a collection of disorders stemming from a variety of genetic defects, scientists are learning.

UW-Madison researchers, by recently discovering a mutation in a gene in fruit flies, may have moved a step closer to understanding what causes some forms of macular degeneration and retinitis pigmentosa. Both diseases gradually destroy vision.

Nansi Jo Colley, a medical geneticist, and her colleagues found a mutation in a gene that produces a protein called calnexin; the mutation can interfere with the ability of cells to process light. Though the researchers studied fruit flies, humans also have the gene.

The findings were reported in last week's edition of the journal Neuron.

When the gene mutation is present, calcium can build up in cells and important proteins can become misshapen, the researchers said.

The calnexin protein is also present in the brain, so the mutation could also be related to neurodegenerative diseases such as Alzheimer's, Huntington's and Parkinson's.

DISCOVERIES

Medical and science news from around Wisconsin

Mineral mystery cracked

The dogged, retired director of UW-Madison's Geology Museum has discovered the answer to a mystery involving minerals: Where did a box of minerals given to the museum more than 20 years ago come from originally?

Klaus Westphal was director of the museum when the old wooden cigar box was delivered. It contained a small collection of minerals, some wood, a stone knife and some labels.

The box came from Clarence Olmstead, then a retired professor of geography at the university. He had picked it up during World War II, while working in Germany for the Office of Strategic Services, the forerunner of the CIA.

Olmstead, who died in 2000, couldn't remember specifically how he had obtained the box. After he gave it to the university, the box sat on a shelf, mostly forgotten, in a museum storeroom.

But Westphal, a native of Berlin, recently took more of an interest.

Using a magnifying glass and a black light to analyze a faded red splotch of ink, he finally made out the name of a city: Wurzburg, Germany.

Westphal sent some of the labels to the University of Wurzburg, and a professor there confirmed the minerals had come from that city. Last month, Westphal shipped the specimens to the German university, where they are to be displayed in a museum.

"I sat in front of that box for I don't know how long," Westphal said. "It had a story to tell."

— David Wahlberg