Signs of photoreceptor neurodegeneration in a wild type Canton-S strain of *Drosophila melanogaster* as revealed by electron microscopy characterization of ommatidia morphology.

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Abstract

Drosophila has a long and fruitful history in the use of photoreceptor morphology to study neuronal degeneration. Even subtle early changes in the cytoplasm can be easily noticed by transmission electron microscopy of photoreceptor sections. In the course of characterizing the mutant phenotype of our gene of interest, we have unexpectedly noted major ultrastructural abnormalities in the wild type Canton-S photoreceptors. These flies were obtained from the Bloomington stock center and were not contaminated by Wolbachia or any other micro-organism sensitive to tetracycline treatment. The observed "phenotype" disappeared after crossing the flies to different stocks indicating that the causative agent is not contagious and that it has a genetic basis. Our results demonstrate that caution should be practiced by scientists who are about to embark on the labor-intensive electron microscopic characterization of their mutant phenotypes to first ascertain the normal morphology of a control genotype, especially if it is related to the Canton-S genotype recently obtained from the Bloomington stock center.

Introduction

The optic lobe of the fruit-fly is ideally suited for histological analysis both by light and transmission electron microscopy as its symmetric and patterned structure yields itself to easy identification and sample preparation. EM analysis of numerous genes involved in cell polarity (Pellikka *et al.*, 2002), phototransduction (Harris and Stark, 1977), polyglutamine expansion induced neurodegeneration (Jackson *et al.*, 1998), etc. have revealed different aspects of abnormal photoreceptor subcellular morphology and provided significant insight into the pathology and molecular pathways affected in these mutants. Many aspects of neurodegeneration can be readily discerned in the eyes of mutant flies or in mutant eye clones, and in this way EM is well suited for the characterization of mutations in novel genes that are expressed in the optic lobes.

This was our rationale to study the morphology and subcellular appearance of a mutant that we have been working with during the past few years. However, in the course of our experiments we have noted that our control Canton-S fly eyes did not have a healthy appearance. Here we briefly report the electron microscopic appearance of the #1 Bloomington stock, Canton-S wild-type fly ommatidia.

Materials and Methods

Fly Stocks

Canton-S flies were from Bloomington (stock number 1), unless specified otherwise. All flies were maintained at 25^{o}C on a 12 hr dark : 12 light cycle.

Transmission Electron Microscopy

Appropriately aged eyes from adult flies were dissected using razor blades and fixed in 2% paraformaldehyde, 2% glutaraldehyde, 0.1 M Na-cacodylate (pH 7.2) overnight at 4°C and postfixed in 2% OsO_4 for 2 hr at ambient temperature. After dehydration and embedding, 50 nm thin sections were cut. Sections were stained with 4% uranyl acetate and 2.5% lead nitrate. TEM was performed on JEOL 1200 EX electron microscope; negatives were scanned and processed in Adobe Photoshop.

Wolbachia PCR

Primers specific for the 16S rDNA were designed according to (O'Neill *et al.*, 1992), namely Wolbachia_99F: 5'-TTGTAGCCTGCTATGGTATAACT-3' and Wolbachia_994R: 5'-GAATAGGTATGATTTTCATGT-3'. PCR was performed as described in (O'Neill *et al.*, 1992).

Tetracycline treatment

Tetracycline was added to regular fly food to yield 0.25 mg/ml final concentration. Flies were passed through two generations of tetracycline treatment.

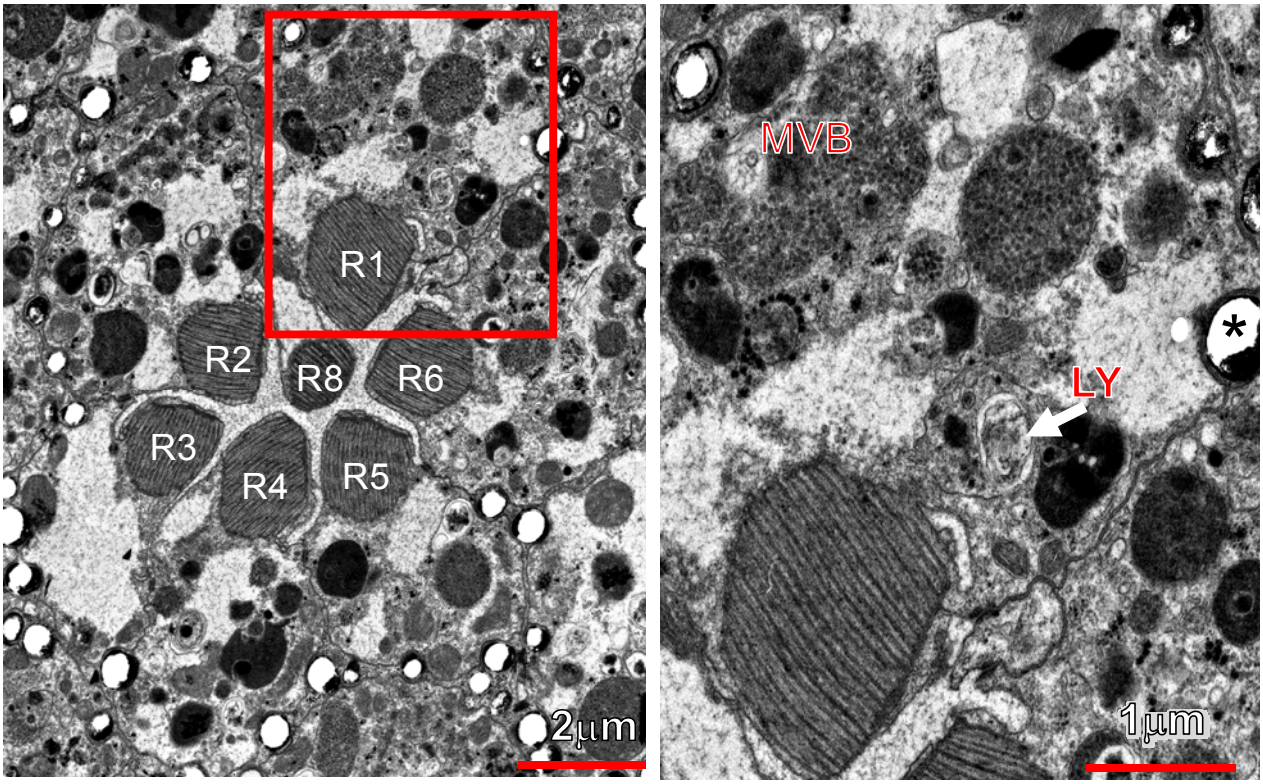

Figure 1. Bloomington #1 Canton-S fly ommatidium at 2000 × magnification. On the right, inset magnified to show the cytoplasm in more detail, characterized by the absence of the characteristic clean cytoplasm, and instead cluttered with lysosomes (white arrow), large multivesicular bodies (MVB) and dark aggregates. The rhabdomeres (dark stacks of cisternae in each individual photoreceptor cell) do not appear to be affected at this stage, although in some cases degenerative changes can also be noted in these structures. * indicates the pigment granules.

Results and Discussion

Since our gene of interest (CG4871) was shown to be expressed in the optic lobes and specifically in the photoreceptor cell by *in situ* hybridization, we were curious to examine the photoreceptor cells in the mutant background of CG4871. Wild type (Canton-S from Bloomington, stock number 1) and mutant flies in the same Canton-S background were aged for 20 days and then processed for transmission electron microscopy (see Methods). To our surprise, wild-type flies had indications of quite severe pathological changes in the cytoplasm of all the photoreceptors (R1-R8) examined (Figure 1).

We have subsequently re-ordered the same Canton-S stock from Bloomington (approximately 2 years after the ordering of the first batch of Canton-S flies), and to our surprise we found the very same morphological changes (data not shown). It is known that Wolbachia infection can result in the accumulation of bacteria inside the photoreceptor cells and lead to significantly abnormal ommatidium morphology somewhat similar to neurodegeneration phenotype detected in our experiments (Min and Benzer, 1997). Thus, we have tested for the presence of Wolbachia infection using a PCR protocol (O'Neill *et al.*, 1992), but found no evidence of Wolbachia contamination (Figure 2).

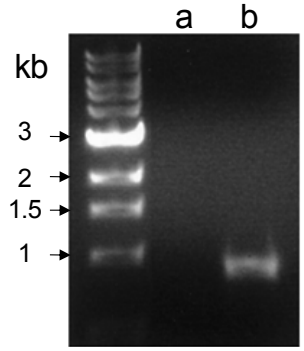

Figure 2. PCR screen of a) CS wild-type flies from Bloomington, and b) FTF1 control flies (from David Rand, Brown University) for Wolbachia infection. Absence of the PCR product from 16S rDNA in wild type Canton-S flies suggests the absence of Wolbachia infection.

Repeated tetracycline treatment did not improve the photoreceptor morphology of Canton-S flies (data not shown). Outcrossing to different genotypes did not lead to the "infection" of the progeny and the eye phenotype became indistinguishable from the one of the Canton-S flies received from an alternative source and used as "healthy" control (Figure 3). These observations suggest that a certain combination of genetic factors has accumulated in the Bloomington Canton-S fly stock that results in the abnormal appearance of the photoreceptors.

We have not examined in further detail the nature of detected defects and whether the putative genetic factors causing the phenotype are linked to particular genomic locations. Similar unexpected defects have not been unnoticed in the past, *e.g*,. a *Drosophila virilis* stock that did not have R7s (also from Bloomington) have previously been reported in DIS (William Stark, personal communication). We wanted to raise awareness among members of the *Drosophila* community about this issue, especially if their near-future research plan includes the ultrastructural characterization of photoreceptors. The morphological abnormalities described in this work (Figure 1) could be used for comparison in electron microscopy characterization of photoreceptor morphology of various genetic backgrounds. The sensitivity of ultrastructural eye morphology to a genetic background (even in the case of not isogenized "wild type" strain, as it was demonstrated in our experiments) underlines the importance of generating control strains with the genetic background as close as possible to the genetic background of the flies under investigation.

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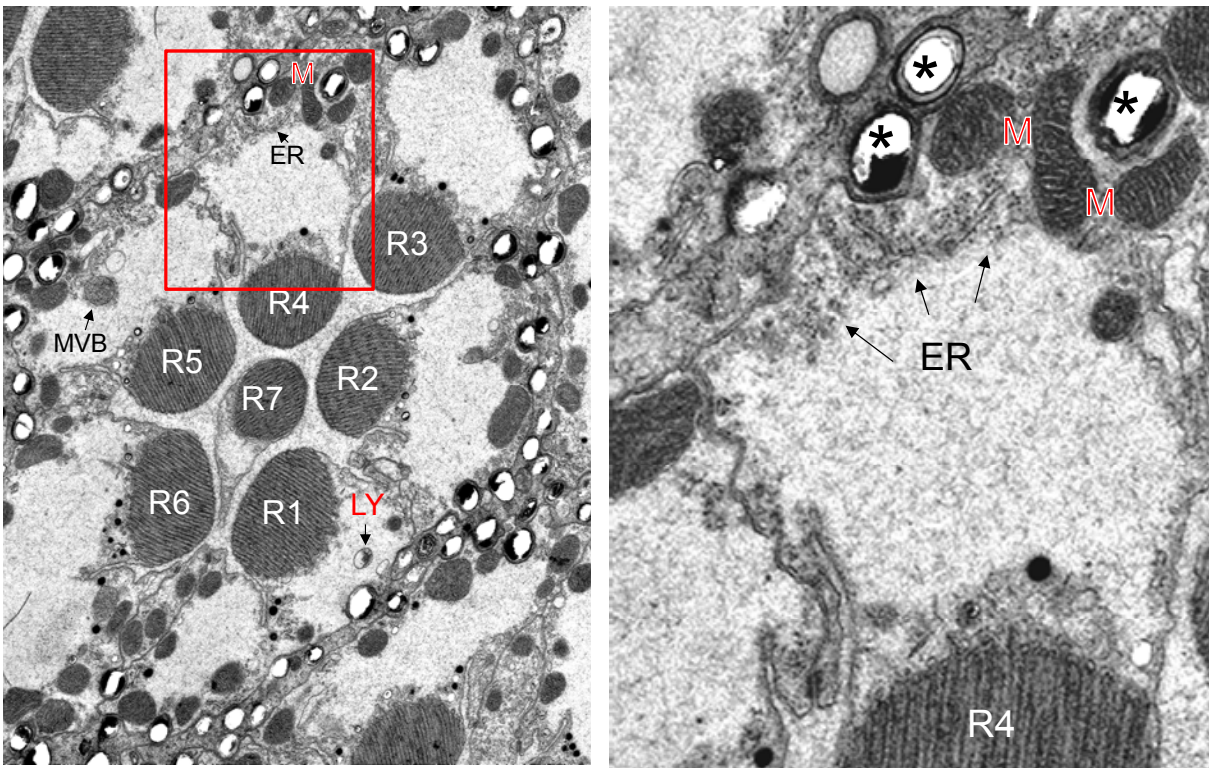

Figure 3. A healthy ommatidium with normal morphology from an alternative source of wild-type Canton-S flies (obtained from Howard Nash, NIMH, Bethesda, MD). On the right side panel the cytoplasm of R4 photoreceptor cell is shown at higher magnification. Note the relatively clean, uncluttered appearance of the cytoplasm, with the endoplasmic reticulum (ER, arrows) and a few mitochondria (M) near the cell border. * indicates the pigment granules; these often fall out during thin sectioning, hence the empty appearance of these structures.

References: Pellikka, M., G. Tanentzapf, M. Pinto, C. Smith, C.J. McGlade, D.F. Ready, and U. Tepass 2002, Nature 416: 143-149; Harris, W.A., and W.S. Stark 1977, J. Gen. Physiol. 69: 261-291; Jackson, G.R., I. Salecker, X. Dong, X. Yao, N. Arnheim, P.W. Faber, M.E. MacDonald, and S.L. Zipursky 1998, Neuron 21: 633-642; O'Neill, S.L., R. Giordano, A.M. Colbert, T.L. Karr, and H.M. Robertson 1992, Proc. Natl. Acad. Sci. U S A 89: 2699-2702; Min, K.T., and S. Benzer 1997, Proc. Natl. Acad. Sci. U S A 94: 10792-10796.

Growth temperature, duration of development, preadult viability, and body size in *Drosophila melanogaster*.

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