INHERITED AND ENVIRONMENTALLY INDUCED RETINAL DEGENERATIONS IN *Drosophila*

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I. INTRODUCTION

The present era of research involving *Drosophila* mutants to analyse the visual system was initiated with early publications from Pak's laboratory[e.g.1] "Nonphototactic mutants in a study of vision in *Drosophila*" and from Benzer's laboratory[e.g.2] "Genetic dissection of the *Drosophila* nervous system..." This latter publication introduced two genes, originally named the receptor degeneration I and II cistrons, now ***rdgA*** and ***rdgB*** respectively.[3] These genes have since been the subject of scores of studies [references[4, 5]].

The purpose of this chapter is to critically evaluate issues in the use of ***Drosophila*** as an animal model of retinal degeneration. Exactly what is meant by retinal degeneration? The importance of this issue is exemplified by the suggestion[3] that one mutant, namely *ora* [= *o*uter *r*habdomeres *a*bsent] had specific nonformation of the visual organelles [rhabdomeres, the fly equivalent of the vertebrate outer segments] while it was later shown that the rhabdomeres form then diminish [degenerate?] without cell death.[6, 7] What are the forms of retinal degeneration and how do these compare with vertebrate models? It is now recognized that, in addition to "degeneration" mutants, there are other degeneration syndromes caused by [1] pharmacological manipulations relevant to cell signalling;[8,9] [2] "transduction" mutants;[5,10-13] and [3] light stimuli in white-eyed [otherwise non-mutant] flies [the *Drosophila* equivalent of the vertebrate albino] such as [i] intense ultraviolet [UV] and blue light,[14,15] [ii] prolonged exposure to cyclic room light,[16] and [iii] exposure to constant room light.[12] Finally we discuss what the recent progress tells us about the mechanisms of normal photoreceptor function and the pathogenesis when that function is disrupted.

II. RESULTS AND DISCUSSION

A. LIGHT INDUCED DEGENERATION IN *rdgB* AND OTHER MUTANTS

When Harris and Stark[17] extensively analysed retinal degeneration in *Drosophila*

In Retinal Degenerations, edited by Robert E. Anderson, Joe G. Hollyfield and Matthew M. LaVail, CRC Press, Boca Raton - Ann Arbor - Boston - London, 1991.

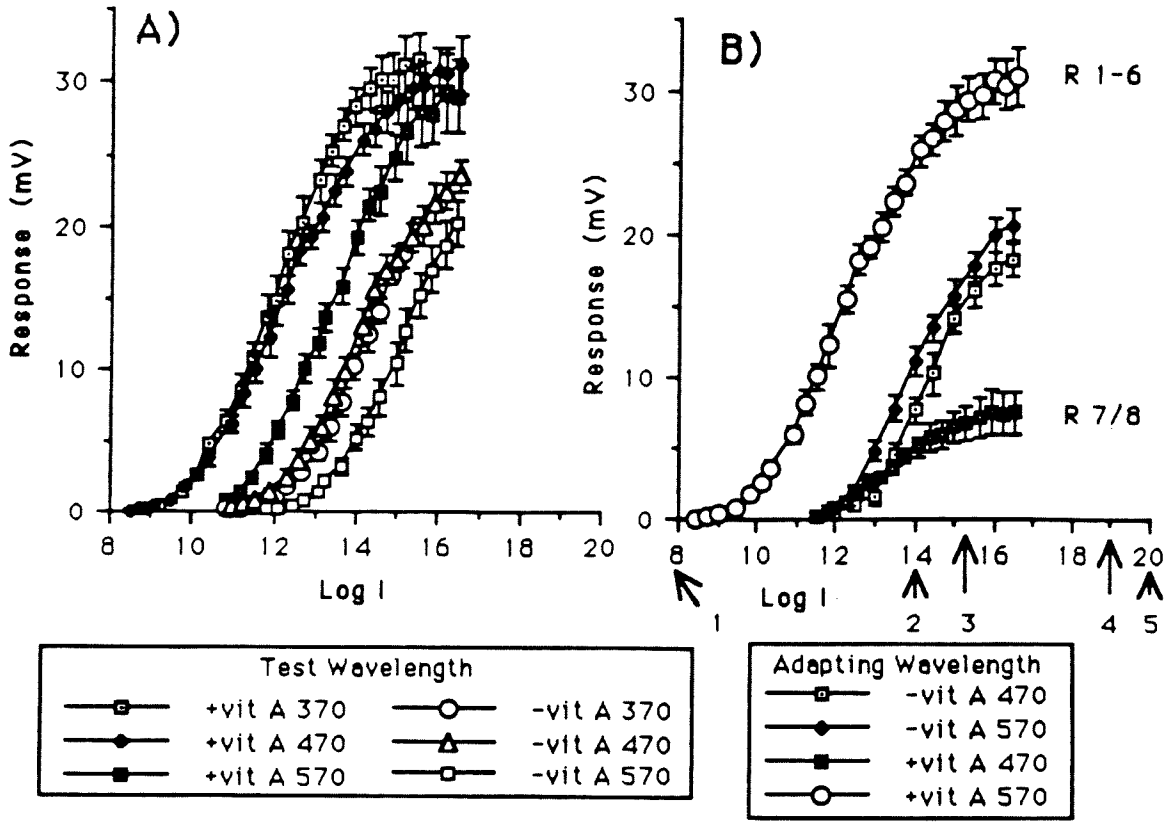

FIGURE 1. Intensity - response data for the ERG receptor component of white-eyed *Drosophila* [averaged from 4 - 8 eyes per curve with standard errors shown] helps to understand many of the findings discussed.

Graph A shows functions from dark adapted eyes for test wavelengths of 370 nm [near UV], 470 nm [blue] and 570 nm [yellow] for carotenoid replete [+vit A] and deprived [-vit A] flies. Note that the responsivity is surprisingly high, with maxima around 30 mV; this helps to explain the usefulness of ERG analyses applied to *Drosophila*. Sensitivity is highest in the blue and UV; deprivation decreases sensitivity throughout the spectrum but preferentially in the UV.[18]

Graph B shows the responses to a 470 nm test wavelength after adaptation with 570 nm [which nearly maximizes rhodopsin] vs. 470 [which favors metarhodopsin] in carotenoid replete [+vit A] vs. deprived [-vit A] flies. Note that, in replete flies, 570 nm adaptation maintains an ERG dominated by R1-6 as labeled while 470 nm adaptation inactivates R1-6 via a PDA [see text] leaving a diminished ERG generated by R7/8 as labeled. There is no PDA in carotenoid deprived flies.

In Graph B, the arrows on the abscissa help to put these intensities into perspective. Arrow # 1 marks a threshold at which approximately 1 quantum is being absorbed per rhabdomere per second.[18] Arrow # 2 very approximately [with the proviso that comparisons of white vs. monochromatic intensities are very difficult] indicates a photopic intensity of white room light from a desk lamp [perhaps 100 lux]. Arrow #3 marks an intensity which, if it were blue and delivered for 1 sec, would convert about half of the rhodopsin to metarhodopsin and induce a maximal PDA.[19] Arrow #4 is the threshold for UV induced intense light damage if delivered for 1 sec[14] and the intensity for nearly complete conversion of the rhodopsin - metarhodopsin visual pigment to M' [see text].[15] Finally, Arrow #5 marks the corresponding blue damage threshold[14] and blue induced visual pigment alteration.[15]

mutants, *rdgB* was considered to be more interesting than *rdgA* in that *rdgB*'s degeneration was light induced. Electrophysiological recording during the response to the light which initiated the demise [enough blue to maximally convert the 480 nm absorbing rhodopsin to its 570 nm absorbing stable metarhodopsin] showed that one specific subset of receptors in the compound eye, namely R1-6, was unable to maintain its poststimulus depolarization [PDA = prolonged depolarizing afterpotential, see Fig. 1]. Many factors which should inhibit the effectiveness of stimulation inhibited degeneration. These factors included dark rearing, lower temperature, eye color pigments, carotenoid deprivation [since deprivation decreases visual pigment], and the *ora* mutant [which eliminates visual pigment, see below]. The *norpA* [*no* receptor potential, see below] mutants also blocked degeneration. The most exciting suggestion was the finding that an allele specific suppressor of degeneration in *rdgB* was an allele of *norpA* which did not block the receptor potential. This suggested, on the basis of genetic evidence [in an era when molecular biology was in its infancy], that the gene products of these two genes interact directly in the process of phototransduction.

The *rdgB* gene has been frustratingly difficult to characterize biochemically or to clone. Thus, at the present time, the most thorough investigation of the degeneration mechanism is the pharmacological work of Minke and coworkers.[e.g.8] They suggest that a phosphoprotein phosphatase is deficient in the mutant.[9] This is consistent with the finding that structural degeneration is first witnessed in the receptor cell terminals.[5] However, the molecular biology is progressing rapidly, and a more direct answer to the cause of *rdgB*'s degeneration will probably be available in the near future.

Meanwhile, there has been substantial progress on other *rdg* genes in *Drosophila.* An example is *rdgA*, whose degeneration is independent of light.[17] Different alleles of *rdgA* differ in their severity and receptor specificity;[20-22] an extreme allele, namely *BS12* is very degenerate upon pupal emergence but does not suffer the ravages of time as much as another allele, *PC47*, suggesting that *BS12* has even lost the competence for further degeneration.[21] *rdgA* was shown to be deficient in the activity of diacylglycerol kinase,[23] responsible for the conversion of the putative cellular signalling molecule and product of phospholipase C, namely diacylglycerol, into phosphatidic acid. Also another gene, namely *rdgC*, was identified, and mutants were shown to have light induced retinal degeneration.[24] This mutant's degeneration depends on substantial rhodopsin activation in R1-6 but is curiously independent of phospholipase C in that *norpA* does not block the degeneration. This, as well as other mutants will continue to be of significant value in determining the mechanisms of visual excitation and of maintenance of the photoreceptor cells.

B. THE OPSIN GENE IN *Drosophila* AND HUMAN

The *ninaE* gene was cloned and shown to code for the opsin [Rh1] specific to the R1-6 subset of receptors in the compound eye.[25,26] *NinaE* is the *E* gene whose mutants suffer from *n*either *i*nactivation *n*or *a*fterpotential. The afterpotential is a "locking-in" of R1-6's depolarization [PDA, see above] caused by maximal conversion of its rhodopsin to metarhodopsin;[27,28] such maximal conversion inactivates R1-6.[28,29] It

turns out[6,30] that one of the first[3,31] and best characterized[7,32] visual mutants, namely *ora* [see I. INTRODUCTION], is a strong allele of *ninaE*. The designation *o*uter *r*habdomeres *a*bsent is actually an overstatement in that *ora* actually begins its adult life with small rhabdomeres;[6,7] the microvilli of *ora* are devoid of the P-face particles that are normally interpreted as opsin molecules in freeze fracture electron microscopy.[32,33] As mentioned in the introduction, the suggestion[3] that *ora* had nonformation of the rhabdomeres is now more accurately stated: rhabdomeres form then diminish, making the boundary of distinction concerning the diagnosis of "degeneration" subtle indeed. An extreme example of this situation is provided by the example of *ey*-2.[34] This is a mutant with an adult *ey*eless phenotype; an examination of the larval eye imaginal disk, the pre-metamorphosis eye primordium, shows extensive cell death, once again suggesting degeneration rather than nonformation.

Recent research has left us with a fascinating parallel since several genetic lines of human autosomal dominant retinitis pigmentosa[35-37] turn out to be rod rhodopsin missense mutations. It seems odd, at first glance, that a defect in a protein as fundamental as rhodopsin, which comprises 90% of the outer segment protein, would cause a disorder taking years to fully express itself. However, in the autosomal dominant case, a heterozygous normal rhodopsin allele can compensate for the defective allele. In the human disorder, the mechanism of degeneration is not clear yet. It will be important to eventually determine whether the mutant rhodopsin is actually functional and / or forms a "toxic" product.

In the example of the *ora* mutant of the fly, opsin is neither synthesized nor deployed and the rhabdomeres, small at first, "degenerate" [diminish, however, cell death does not occur[32]]. However, the rhabdomere decline is not associated with the appearance of normal autophagic bodies [multivesicular bodies = MVB's].[7] In flies, autophagy, rather than the vertebrate's phagolysosomal system involving the retinal pigment epithelium, recycles membrane, and the MVB is the fly equivalent of the vertebrate phagosome.[16] Unfortunately, the mechanism by which the absence of opsin leads to the demise of the organelle is not known; further, carotenoid deprivation, which also produces a similar opsin decrease, does not lead to such complete demise of the rhabdomeres [see next section].

C. CAROTENOID DEPRIVATION & REPLACEMENT IN *Drosophila* & RAT

In the 1970s, a most remarkable finding was made concerning carotenoid deprivation in the fly: P-face particles are greatly reduced[17,38] while the microvilli of the rhabdomere remain intact. Sensitivity as measured by the ERG[28,29,39] and visual pigment content, as measured using *in vitro* spectrophotometry[17] and *in vivo* microspectrophotometry[40,41] are also drastically reduced in *Drosophila*. Pertinent to retinal dystrophy, what was not known until recently was that rhabdomeres are substantially smaller when *Drosophila* are reared from egg to adult on carotenoid deprivation.[42]

Using carotenoid deficiency to decrease rhabdomeric opsin, unlike using *ora* [discussed above], does not lead to the total loss of the rhabdomere. In order to

investigate this phenomenon further, we followed the electroretinographic [ERG] sensitivity of white-eyed deprived flies as a function of time. Sensitivity increases rapidly when deprived flies are given carotenoid "replacement therapy" by placing the deprived flies in a vial with nothing but carrot juice to drink. Surprisingly, in deprived flies maintained on the deprivational medium, sensitivity increases as a function of adult age after pupal emergence by 1.64 log units as of 3 days, then decreases again 2.47 log units by 11 days. Although we suspected that this decline might be associated with degeneration, we found that sensitivity rebounds by 3.98 log units in 1 day when 11-day deprived flies are given carrot juice. This means that, even after deprived flies' sensitivity has waxed and waned, visual receptors are still healthy.

We further investigated trophic effects of vitamin A on rhabdomere and opsin maintenance in visual receptors using microspectrophotometry as well as electron micrographic morphometry and immunocytochemistry. Rhabdomeres enlarge[41] and visual pigment increases[16,43] when adults are carotenoid-replaced using carrot juice. We used a monoclonal antibody to the rhodopsin [called Rh1] in R1-6 receptors in the compound eye to further quantify opsin recovery in such carotenoid replacement therapy. Density of immunogold, specific to R1-6 [vs. R7], increases between days 1 and 3 of replacement as visual pigment and rhabdomeres recover. In summary, sensitivity, visual pigment, opsin and the opsin-containing organelle recover in unison during carotenoid replacement therapy in carotenoid deprived *Drosophila*. At one day of replacement therapy, when opsin synthesis and deployment are high, opsin immunogold labeling is especially high in the rough endoplasmic reticulum of the retinula cells.[42] While this is consistent with the possibility that vitamin A may even regulate fly opsin gene expression at the mRNA level,[44] the situation in the invertebrate is far from resolved since R. White [personal communication] did not observe a decrease in opsin mRNA, assayed with a *ninaE* probe in the moth [*Manduca*].

In the fly, carotenoid deprivation and replacement is relatively straightforward; in the vertebrate this is not the case, which may explain the relative paucity of such data in higher animals. The long-standing dogma for the rat is that deprivation leads to degeneration.[45,46] The effects of vitamin A deprivation were recently reexplored in the rat with the surprising finding that vitamin A's control of opsin is strikingly different in the fly vs. rat.[47,48] Deprivation for 26 weeks decreases rhodopsin by over 85% as measured spectrophotometrically but it does not decrease opsin density as determined by P-face particle counts or quantification of EM immunogold labeling of an opsin antibody. However, the volume of the outer segments is reduced to 42%, and the amount of vitamin A remaining even after 26 weeks of deprivation is still sufficient to support the synthesis of the outer segment's opsin. Even though bleached opsin is regenerated to rhodopsin by chromophore supplied from the adjacent retinal pigment epithelial cells, there is evidence that the chromophore is added in the inner segment during synthesis.[49] It would be interesting to determine whether further deprivation does, in fact, lead to degeneration and if replacement would allow for the reestablishment of normal outer segments as it does for rhabdomeres in *Drosophila*.

D. OTHER REGULATORS OF SYNTHESIS AND DEPLOYMENT

In the vertebrate, rhodopsin is glycosylated, and tunicamycin, which blocks N-linked glycosylation at asparagine residues decreases opsin incorporation into the outer segment and eventually causes degeneration.[50,51] Tunicamycin experiments had also been done in the moth.[52] During pupal development tunicamycin caused a withdrawal of microvilli within a few hours after injection suggesting that it affects certain proteins which are necessary for rhabdomere maintenance; however it did not affect well developed adult microvilli. It was claimed in the fly that the rhodopsin is not glycosylated[53] which seems counterintuitive because of the prevalence of glycosylation among membrane proteins. However, this suggestion was verified and extended by the finding that the mature rhabdomeric opsin is not glycosylated while, during synthesis or deployment it is, and only at one of the two possible sites.[54,55]

We pursued this finding in *Drosophila* with tunicamycin injections much as White and Bennett did in the moth.[52] Carotenoid deprived white-eyed flies within 36 hrs after emergence were fixed to a microscope slide with nail polish. Each fly was then pressure injected [through a glass micropipette[12]] with a solution of 20 mg tunicamycin [B family from Sigma which is fairly specific] per ml of DMSO or a control saline solution. [We had attempted applying tunicamycin onto the eye with DMSO, a good solvent and potential carrier, without an effect; further, we tried to feed the drug but could not find a sublethal dose which had an effect.] The visual pigment was immediately measured using microspectrophotometry, and the flies were then carefully removed from the microscope slide. After removal, flies were carotenoid

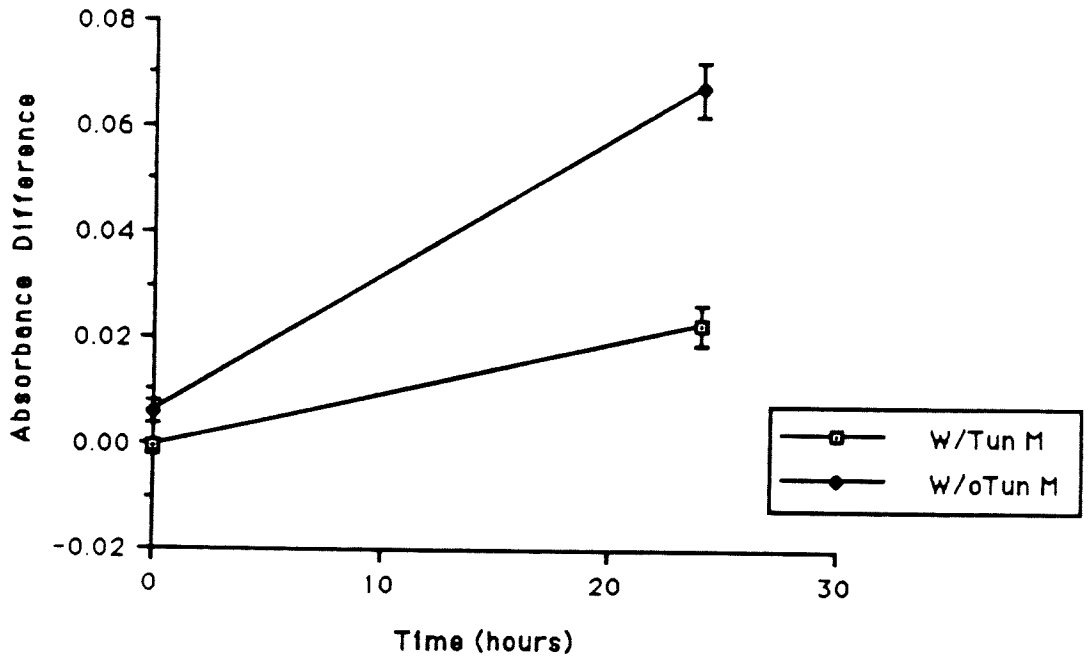

FIGURE 2. Carotenoid replacement therapy experiments on two groups of animals: those injected with tunicamycin have their recovery greatly diminished relative to those given a sham injection. N = 10 for each group with the standard error shown.

replaced by introduction to a vial with 2 ml of carrot juice applied to a kimwipe. At 24 hrs ± 30 min, flies were fixed on the microscope slide and their visual pigment was again measured. Within the 24 hr period, approximately 20% of the flies died due to the injection procedure, and these flies were not counted. The results for male white-eyed *Drosophila* are shown in Fig. 2 As expected, the visual pigment at the beginning of the experiment was virtually zero. Tunicamycin significantly decreased the recovery afforded by carotenoid replacement therapy relative to the "sham" injected controls. This finding is consistent with the data that opsin is glycosylated during cellular synthesis but not in the mature rhabdomeric form.[54,55]

Because *norpA* lacks phospholipase C [see below], the phospholipid and fatty acid composition of *Drosophila* heads is of interest.[56] Organic extracts of heads broken off after freezing in liquid nitrogen were separated by 2 or 3 solvent system HPTLC. Phospholipids were labeled by feeding flies with ^{32}P phosphate. Spots on TLC plates were counted. The profile of labeling after 24 hr of feeding was approximately as follows: phosphatidylethanolamine=PE - 47%, PE plasmalogen=PE_{pl} - 1.5%, phosphatidylcholine=PC - 24%, PC_T [an unknown, likely PC, but with different fatty acids] - 4%, phosphatidylinositol=PI - 12%, lysophospholipids LPE - 2.5%, LPC - 1.6%, LPI - 1.4%, poly-PI's PIP_2 - 0.2%, PIP - 0.2%, phosphatidic acid=PA - 0.4%, phosphatidylserine=PS - 1.6% and the cardiolipins CL_1 - 2.5% and CL_2 - 1.1%. A fraction of a percent of cerebroside is likely present. Relative to rat brain[57] the most notable differences were that, in *Drosophila* heads, PE is higher, PC is lower, PC_T is present and there is probably no sphingomyelin. Fatty acids of PS, PI, PE and PC were analysed by GC. Fatty acid composition differed for the above phospholipids: 14:0 - 2%, 16:0 - 9-34%, 16:1 - 2-10%, 18:0 - 8-25%, 18:1 - 22-33%, 18:2 - 18-28% 18:3 - 1-15%. Triglycerides comprise around 70% of head lipids and have high 14:0 and 14:1. Although docosahexaenoic acid [22:6] is high in vertebrate photoreceptors and nervous system,[58] *Drosophila* heads had none. We confirm and extend the earlier finding that PI lacks arachidonic acid [20:4].[59]

There are no fatty acids longer than 18 carbons in fly heads, but neither are there any in the food. Using several different media which differed in fatty acid composition, it was shown that the fatty acid composition of the heads could be readily manipulated. Thus, we decided to supplement the fly food with menhaden oil, a rich source of long chain fatty acids. We found that the ultrastructure of the photoreceptors of the fish oil supplemented flies was normal and that there was a small but significant increase in the electrophysiological sensitivity, especially in the UV. While these ERG findings should be considered as preliminary, they open the possibility of important trophic influences of dietary fatty acids.

E. THE *norpA* [*no* receptor *p*otential] MUTANT

The mechanism of signal transduction has long been of interest. For photoreceptors, it has long been suspected that transduction defects can lead to retinal dystrophies. In vertebrate vision, a unique G protein mechanism in which cGMP phosphodiesterase [PDE] is inhibited is well established, and the recent evidence that

the *rd* [*r*etinal *d*egeneration] gene[60] in mice codes for cGMP's PDE β subunit[61] adds credence to the transduction - integrity link.

In *Drosophila* vision, one of the oldest[1] and best studied[5,12,13,22] mutants, *norpA* [*no r*eceptor *p*otential] has a transduction defect involving a much more commonly used means of signal transduction, namely G protein mediated activation of phospholipase C [PLC]: mutants lack activity of a PLC[62] which acts on PIP_2 as well as PI,[63] and the gene, now cloned, proves to be a PLC gene.[64] [In the strictest sense, PLC is a PDE,[65] so these mechanisms may not be so far removed from each other after all.] Visual pigment is normal in newly emerged *norpA* flies.[11-13,66,67] In *norpA*, there is a demise of the rhabdomeres and an accumulation of membranous material called "zippers," and these phenomena are the subject of recent reports.[5,12,13] Interestingly, there are vertebrate models in which receptors apparently have rhodopsin, but the transduction is deficient; the parallel between *norpA* and congenital stationary night blindness in the human has already been discussed.[13] The newly hatched *rd* chicken has rhodopsin but no receptor potential; however an abnormal cone visual pigment protein is the current explanation of the chicken's "*norpA*"-like phenotype.[68]

Curiously, in a white-eyed strain of the *norpA* mutant, microspectrophotometry [MSP], electron microscopy [EM] and electroretinography [ERG] revealed that light mediates receptor demise in *norpA* even though *norpA* lacks phototransduction.[11, 12] Visual pigment and the rhabdomere which houses it decrease with increasing age in *norpA* but not in *w* with rearing on a 12 hr light / 12 hr dark cycle. At warm room temperature [about 28°C] in *norpA;cn bw* and *w* reared in constant light, visual pigment decreases, rhabdomeres diminish and cells die. This finding, incidentally, introduces an entirely new form of retinal degeneration in *Drosophila* in that white-eyed otherwise nonmutant flies show a retinal demise when placed in a constant light [L / L] regimen for about 10 days. Importantly, dark rearing blocked visual pigment loss in *norpA;cn bw*;[12] the M-potential, an ERG reflection of visual pigment level,[18,20,67,69,70] corroborated this finding.[12]

It seemed amazing to us that the rhabdomere demise should be light-dependent in a mutant which did not have a response to light. We explained the result by suggesting that light initiated the need for "housekeeping" or receptor maintenance, but that depolarization [or at least the transduction steps leading to depolarization] was necessary to put proper photoreceptor maintenance into effect. The ease with which additional manipulations can be applied in *Drosophila* is exemplified by our further experiments [Fig. 3]. We found that decreasing the rhodopsin activation by carotenoid deprivation blocked the rhabdomere demise in the constant light condition in white-eyed otherwise wild-type flies and in the light / dark cycle as well as in the constant light condition in white-eyed *norpA* flies. Of course the rhabdomeres were smaller to begin with [i.e. in constant darkness] in flies which had been reared without carotenoids in the diet [see above, C. CAROTENOID DEPRIVATION ...]. Further, we found that decreasing the photic stimulation by making the flies red-eyed afforded the same protections [with the caveat that different but equally strong alleles of *norpA* were used: $norpA^{P24}$ in the white-eyed strain and $norpA^{EE5}$ in the red eyed strain].

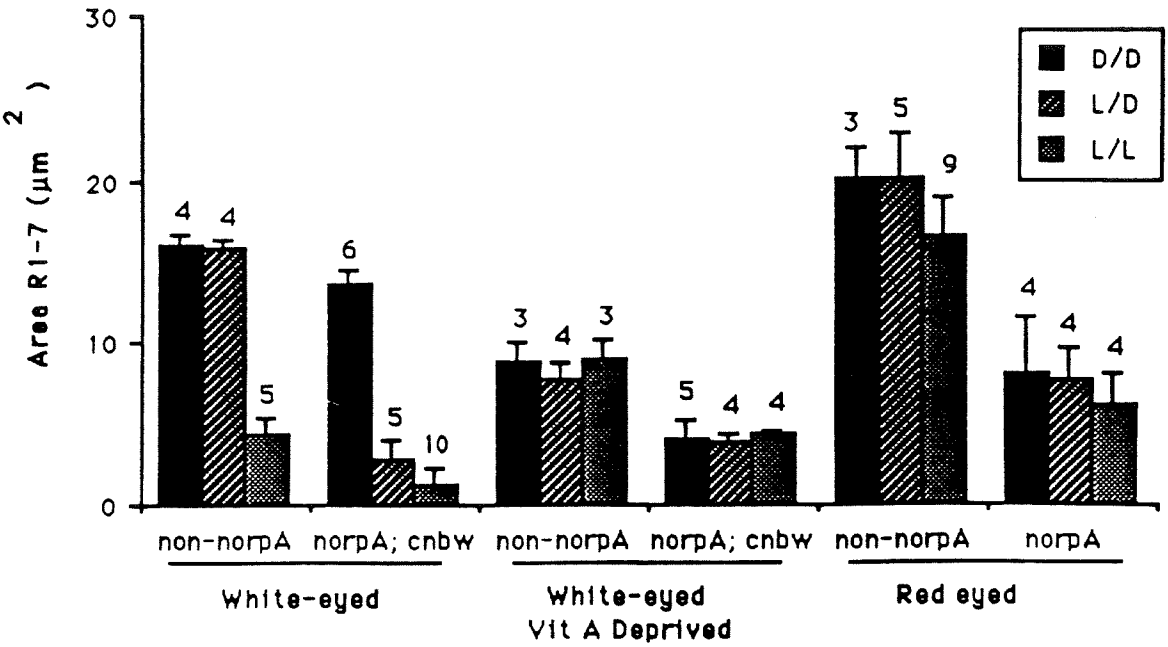

FIGURE 3. The cross sectional area of 10-day post-eclosion white-eyed non-*norpA* vs. *norpA* rhabdomeres with rearing on normal medium vs. on carotenoid [vit A] deprivation; also compared are flies which are red-eyed. N = the number of ommatidia with the standard deviation between ommatidia shown; one animal for each bar except *norpA;cnbw* L/L, vit A deprived *norpA;cnbw* D/D and wild-type [non-*norpA*, red-eyed] L / L where two animals each were pooled. These experiments go beyond those reported earlier[12] by establishing the protective effects of red eye color pigments and carotenoid deprivation.

Both *ora* and *norpA* have a rhabdomere demise. However, *norpA* has MVB's while *ora* does not. We decided to further analyse the resource of electron micrographs available from the Fig. 3 experiment for MVB counts to answer the question as to the interaction of lighting conditions, caroteniod rearing conditions, and genotype with respect to *norpA*. We were initially hesitant about this for the following reason: we have been regularly plagued with what seemed like bad fixation [we called it "ghosty cytoplasm"] in the deprived flies. We now think there is a reason, namely that there is less structure there. The most important finding in this analysis is so qualitative in this regard that we do not hesitate to present it: carotenoid deprivation brings the MVB count to zero, virtually; we say "virtually" because, if we look hard enough in the EM, we can find a few MVB's, though these micrographs turned up none.

F. ENVIRONMENTALLY INDUCED RETINAL DEGENERATION

Environmental manipulations have been used to accelerate or prevent retinal degeneration in mutants. For instance, the results on *norpA* show that light and visual pigment level play a role in the rhabdomere's demise. Further, in *rdgB*, decreases in temperature, visual pigment [via carotenoid deprivation as well as combination with *ora*] and light [by genetically replacing white eyes with wild-type red eyes] all inhibit

degeneration.[17] What is less well appreciated is that degeneration can occur in "normal" flies as a result of environmental, as opposed to genetic, manipulations. By "normal," we mean *w* [flies made white-eyed with *w*hite or other eye color mutations]. White-eyed flies have been used for decades to eliminate the spectrally selective effects of the red eye color pigments on the basis of a substantial body of information suggesting that it is only the screening function of these pigments which is deficient.

One of the most dramatic examples of light induced degeneration in white-eyed otherwise wild-type *Drosophila* is the effect of intense UV and blue stimulation. These effects were first noted as a consequence of using the intense light sources of the incident illuminator of a fluorescence microscope to study photopigments microspectrofluorometrically.[15,18,71,72] The threshold for UV-induced damage is about 10^{19} quanta/cm^2 [quantum flux multiplied by time], while for blue it is about 10^{20} [Fig. 1].[14] These thresholds are precisely the thresholds needed for conversion of the visual pigment to an intense light photoproduct termed M' to differentiate it from M [the normal metarhodopsin photoproduct of rhodopsin, induced by about 3 orders of magnitude lower illumination, Fig. 1]. This line of work, together with the finding that carotenoid deprivation protects against degeneration, suggests that it is overstimulation of the photopigment system itself which induces retinal degeneration. There is an amazing parallel with the results on aphakic rhesus monkeys[73] in the absolute thresholds of UV and blue damage as well as in the greater efficiency of UV light for inducing damage.

The fact that intense UV and blue stimulation caused retinal degeneration came as no surprise. More recently, it was shown that constant light rearing for 10 days, at warm room temperature, caused a dramatic receptor demise in white-eyed otherwise wild-type *Drosophila*.[12] It was our impression that the *norpA* mutant actually protected somewhat against this degeneration. Also, a scattering of visual receptor cells are lost when flies are reared for 3 weeks on a light / dark cycle of ordinary white room light.[16]

III. CONCLUSIONS AND FUTURE DIRECTIONS

Knowledge is an expanding sphere of light in a universe of darkness: as knowledge increases, so does its contact with the unknown. As pessimistic as this view sounds, it is the *sine qua non* of scientific progress in that each round of answers gives rise to the next, more probing, questions. So it has been through two decades in the application of interdisciplinary cell biological approaches to study *Drosophila* as an animal model of retinal degeneration. Some aspects of *Drosophila*'s visual system are uniquely different from that of the vertebrate, such as the nonbleaching metarhodopsin and the PDA. From a basic science standpoint, it is through studying the differences as well as the similarities of organisms that we can gain our fullest level of understanding. But as molecular biology is increasingly applied to the vertebrate as it has been to *Drosophila*, a new unity in the family tree of life paves the way for a new optimism; new

homologies such as the rhodopsin - G protein receptor family tree are constantly being elaborated. And, as *Drosophila* remains at the forefront of molecular technology[e.g.74], it remains likely that *Drosophila* will continue to answer questions about the mechanisms underlying one of our most precious gifts, our eyesight.

ACKNOWLEDGEMENTS

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