Freeze-fracture Study of the *Drosophila* Photoreceptor Membrane: Mutations Affecting Membrane Particle Density

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ABSTRACT The photoreceptor membrane of *Drosophila melanogaster* (wild type, vitamin A-deprived wild type, and the mutants $ninaA^{P228}$, $ninaB^{P315}$, and ora^{JK84}) was studied by freeze-fracture electron microscopy. The three mutations caused a decrease in the number of particles on the protoplasmic face of the rhabdomeric membrane. The $ninaA^{P228}$ mutation affected only the peripheral photoreceptors (R1-6), while the $ninaB^{P315}$ mutation affected both the peripheral (R1-6) and the central photoreceptors (R7). The ora^{JK84} mutation, which essentially eliminates R1-6 rhabdomeres, was found to drastically deplete the membrane particles in the vestigial R1-6 rhabdomeres but not in the normal rhabdomeres of R7 photoreceptors, suggesting that the failure of the ora^{JK84} mutant to form normal R1-6 rhabdomeres may be due to a defect in a major R1-6 photoreceptor-specific protein in the mutant. In all cases in which both the rhabdomeric particle density and rhodopsin content were studied, the mutations or vitamin A deprivation was found to reduce both these quantities, supporting the idea that at least the majority of the rhabdomeric membrane particles are closely associated with rhodopsin. Vitamin A deprivation and the mutations also reduced the number of particles in the plasma membrane as in the rhabdomeric membrane, suggesting that both classes of membrane contain rhodopsin.

Freeze-fracture studies of the photoreceptors have shown that there are numerous membrane particles on the fracture face of both the outer segment disk membrane of vertebrate photoreceptors (36, 37, and references cited therein) and the rhabdomeric microvillar membrane of invertebrate photoreceptors (3, 5, 8, 9, 14, 22, 26, 32). Several lines of evidence suggest that these membrane particles are correlated with the presence of rhodopsin. For example, vitamin A deprivation, which reduces the rhodopsin content, has been found to reduce the number of disk membrane particles in vertebrate photoreceptors (16) and rhabdomeric membrane particles in invertebrate photoreceptors (3, 14, 22).

In the case of *Drosophila*, it is also possible to reduce the rhodopsin content by means of single-gene mutations. Among the mutants of *Drosophila melanogaster* that we have isolated for the study of the photoreceptor process are those with drastically reduced rhodopsin content (22, 29, 30). The studies that have been carried out on some of these mutants suggest that the mechanism of rhodopsin depletion in these mutants can be very different from that of vitamin A deprivation (22). It thus appeared worthwhile to examine the photoreceptor membrane microstructure of several representative rhodopsin-deficient mutants not only to characterize the mutants in terms of membrane microstructure but also to reexamine some of the questions regarding the nature of the particles in the photoreceptor membrane.

The structure of the *Drosophila* compound eye is well-known from several studies on larger flies (2, 25, 40) as well as on *Drosophila* (6, 33). It consists of ~800 ommatidia, each containing a group of eight retinula cells (photoreceptors). Each retinula cell has a rhabdomere composed of hexagonally packed microvilli. On the basis of the position of the rhabdomeres in the ommatidium, the eight photoreceptor cells of each ommatidium are classified into the six peripheral (R1-6) and the two central (R7 and R8) cells. A cross section through a distal region of the ommatidium shows the rhabdomeres arranged in a characteristic trapezoidal pattern (Fig. 1). The rhabdomeres of R1-6 cells are located in the periphery of the trapezoid, and the rhabdomeres of R7 and R8 cells are located near the center of the trapezoid (Fig. 1), with R7 rhabdomere

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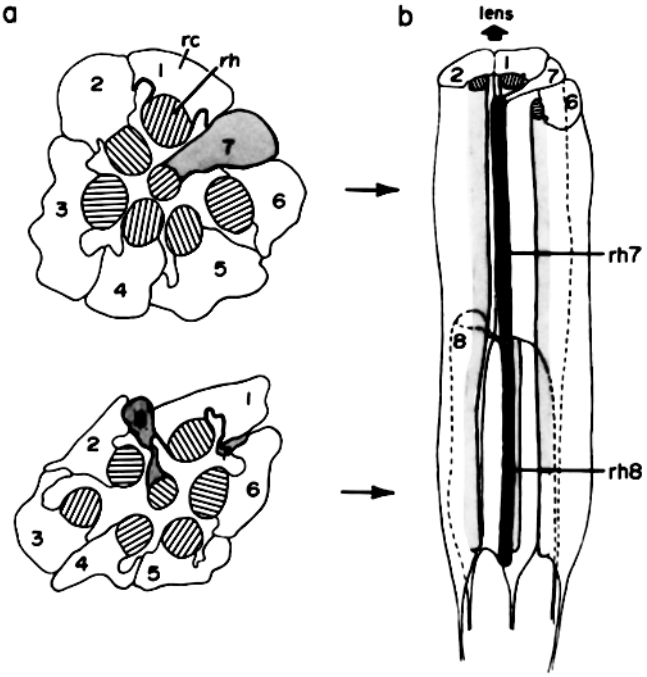

FIGURE 1 Schematic diagram showing the positions of eight retinula cells (numbered as shown) and their rhabdomeres within an ommatidium. *a* presents cross-sectional views at the levels indicated by the arrows. In *b*, retinula cells R3-5 are omitted for clarity; *rc*, retinula cell; *rh*, rhabdomere. That portion of the plasma membrane examined in this work is shown in thick lines for R1 retinula cell in *a* (see text). Reproduced with permission from Cosens and Perry (6) and Pergamon Press.

on top of R8 rhabdomere. The rhabdomere is known to contain the visual pigment (11, 18, 21). In the case of muscoid diptera, all R1–6 photoreceptors contain the same visual pigment, which absorbs maximally at ~480 nm and photointerconverts with a metarhodopsin absorbing maximally at ~580 nm (12, 15, 28, 38). The visual pigments contained in the other classes of photoreceptors, R7 and R8, are spectrally different from those in R1–6 photoreceptors (13, 15, 17).

In this paper we report on freeze-fracture analyses of the photoreceptor membranes of three membrane particle-deficient mutants, *ninaA*^{P228}, *ninaB*^{P315}, and *ora*^{JK84}. These mutants were chosen because they display substantially different phenotypes, suggesting that the mechanism of particle reduction in each may be different.

The following are some of the questions we investigated in this work:

(*a*) How do the mutations *ninaA*, *ninaB*, and *ora* affect the microstructure of the photoreceptor membrane?

(*b*) Do our data from these mutants support the hypothesis that the majority of the rhabdomeric intramembrane particles originate from rhodopsin?

(*c*) How does the microstructure of the nonrhabdomeric photoreceptor membrane located adjacent to the rhabdomeres differ from that of the rhabdomeric membrane?

MATERIALS AND METHODS

The following *Drosophila melanogaster* stocks were used in this work: wild-type flies of the Oregon-R strain, vitamin A–deprived wild-type flies of the same strain, the mutants *ninaA*^{P228}, *ninaB*^{P315}, and *ora*^{JK84}, and the double mutant *sev*^{LY3}; *ninaA*^{P228}. Most of the flies used had their screening pigments in the compound eye eliminated using the mutation white (*w*), because white-eyed flies are more convenient for determining the mutant phenotype. No statistically significant differences were observed between the freeze-fracture data obtained from normal, red-eyed flies and those from white-eyed flies of the same strain. All flies were raised in an incubator (25°C, 55% relative humidity, 12-h light/12-h dark cycle) on a cornmeal-yeast-agar medium. The age of the flies ranged between 2 and 18 d. Neither the age nor different illumination conditions had any significant effect on either the rhodopsin content or the microstructure of the rhabdomere.

The *nina* (neither inactivation nor afterpotential) mutants are a group of mutants, isolated in chemical mutagenesis, that as a class lack the prolonged depolarizing afterpotential (PDA)[1] in the R1–6 photoreceptors and have a substantially reduced rhodopsin content. The *nina* mutants studied to date fall into five complementation groups. The mutation *ninaA*^{P228} (superscript: allele designation) is recessive and maps on the second chromosome between aristaless (2-0.01) and dumpy (2-13.0) at ~1.4 (Scavarda and Wong[2]). It has been localized within the limits of the deficiency Df(2L)S3 (Kremer and Wong[3]), which has break points at 21D2-3 and 21F2-22A1 (23). The mutation *ninaB*^{P315} is also recessive and maps on the third chromosome between Acetylcholinesterase (3-52.2) and Stubble (3-58.2) at 53.5 ± 0.5 (Kremer and Wong[3]).

The mutation *ora*^{JK84} (outer rhabdomeres absent) was obtained from Dr. J. Merriam of the University of California at Los Angeles. It is a recessive, third chromosome mutation mapping at 65.3 ± 0.4 (19, 20). The mutation reduces the rhabdomeres of the R1–6 retinula cells to vestigial remains without affecting the rhabdomeres of the central retinula cells R7/8 (14, 19, 20). The mutation *sev*^{LY3} (sevenless) was obtained from the Benzer laboratory at California Institute of Technology. The mutation maps at 33.2 ± 0.2 on the X chromosome (15), and it specifically eliminates the R7 photoreceptors (4; see also reference 15). The double mutant *sev*^{LY3}; *ninaA*^{P228} was constructed by chromosome assortment.

Vitamin A–deprived flies were obtained by raising wild-type flies on vitamin A–deficient, Sang's medium C (35). To eliminate bacteria as a source of vitamin A, the medium was autoclaved and the antibiotics, penicillin G potassium and streptomycin sulfate, were added. To avoid the effects of individual variations among vitamin A–deprived flies, we carried out both freeze-fracture and spectrophotometric studies on the same eye of two 5- to 6-d-old flies, one that had been vitamin A deprived for a generation and the other for two generations.

The deep pseudopupil was used to measure in vivo the absorbance changes of rhabdomeres due to photoconversions of visual pigment between the rhodopsin ($\lambda_{max} \cong 480$ nm) and metarhodopsin ($\lambda_{max} \cong 580$ nm) states in R1–6 photoreceptors (35, 24). Because in *Drosophila* metarhodopsin has a higher extinction coefficient than rhodopsin (14, 27), measurements were made near the absorption peak of metarhodopsin. The transmitted light intensities at 578 nm were measured using a series of yellow test flashes (Ditric three-cavity interference filter with peak transmission at 578 nm; 0.3-s duration; $I \approx 3 \times 10^{14}$ photons $cm^{-2}\ s^{-1}$) after each alternately presented blue or yellow bleaching lights. The absorbance change at 578 nm (ΔA_{578}) was calculated by comparing transmission measurements obtained after the two different bleaching conditions.

For freeze-fracture work, an eye sliced from each anesthetized fly was fixed in a solution containing 2.5 ml of 70% gluteraldehyde, 3 ml of acrolein, 10 g of sucrose, and 100 ml of *Drosophila* saline (34), glycerinated in a graded series of glycerol-*Drosophila* saline, and frozen in liquid nitrogen-cooled Freon 22. The frozen eyes were fractured using a BA 360M freeze-etch device (Balzers high-vacuum technique; Balzers Corp., Nashua, NH) in a vacuum of $<2 \times 10^{-6}$ torr at a specimen stage temperature of −106° to −116°C. Immediately after the fracture, the specimen was covered with platinum to a depth of ~30 Å, coated with carbon, and cleaned in household bleach. A mirror-image replica device (Balzers high-vacuum technique) was also occasionally used.

Pictures of the replicas were taken with a Philips EM 300 electron microscope on 70-mm negatives. We monitored magnification using a "waffle" type carbon grating and took care to avoid lens hysteresis. Only those prints in which the particles appeared clearly as three-dimensional structures were used to determine the number and diameter of the membrane particles. Since the freeze-fracture photograph of a microvillus represents the projection of its cylindrical surface onto a plane, the measured area on the photograph was corrected for the distortion caused by the projection.

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[1] The PDA is a sustained potential that keeps the photoreceptor membrane depolarized after the termination of an intense blue stimulus that converts a substantial net amount of rhodopsin to metarhodopsin. It is terminated by an orange stimulus that photoconverts a substantial net amount of metarhodopsin back to rhodopsin.

[2] N. J. Scavarda and F. Wong, Department of Biological Sciences, Purdue University, West Lafayette, IN 47907, unpublished data.

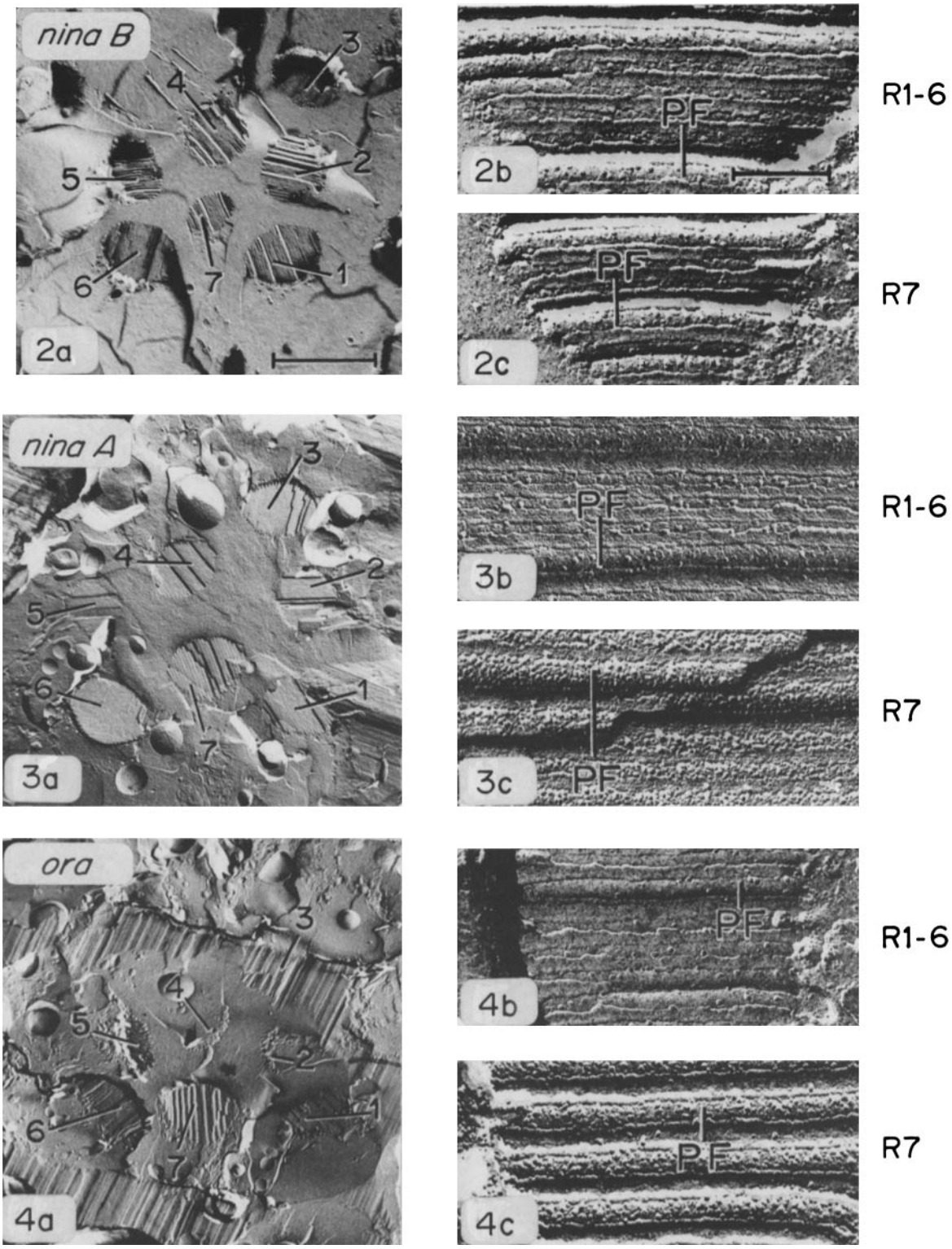
[3] N. E. Kremer and F. Wong, Department of Biological Sciences, Purdue University, unpublished data. Their present address is Marine Biomedical Institute, University of Texas Medical Branch, Galveston, TX 77350.

To obtain the particle diameter density distribution, particle diameters were grouped into size classes (bins) of 20-Å width, and the particle density (number/unit area) was determined for each diameter class for each cell studied. The means and standard errors for each diameter class were calculated from a population of cells. Statistical comparison of particle densities was carried out using the t distribution. The level of significance was $P = 0.01$.

RESULTS

Morphology of the Retinula Cells

The relative positions (Figs. 2 *a* and 3 *a*) and size (data from thin-sectioned eyes) of the rhabdomeres of the two *nina* mu-



FIGURES 2–4 Freeze-fracture replicas of ommatidia cross-fractured at a distal level displaying the seven rhabdomeres (labeled *1–7*), obtained from *ninaB*^{P315} (Fig. 2 *a*), *ninaA*^{P228} (Fig. 3 *a*), and *ora*^{JK84} (Fig. 4 *a*); and enlarged views of portions of rhabdomeres of the peripheral retinula cells R1–6 (Fig. 2 *b*, *ninaB*; Fig. 3 *b*, *ninaA*; Fig. 4 *b*, *ora*) and of the central retinula cell R7 (Fig. 2 *c*, *ninaB*; Fig. 3 *c*, *ninaA*; Fig. 4 *c*, *ora*). Bar: 2 μm, for Figs. 2 *a*, 3 *a*, 4 *a*; 0.2 μm for Figs. 2 *b* and *c*, 3 *b* and *c*, and 4 *b* and *c*. *PF*, protoplasmic face.

tants were not substantially different from those of wild type. In the mutant *ora*^{JK84}, however, the peripheral rhabdomeres were drastically reduced in size (Fig. 4*a*). Furthermore, in all the replicas of the *ora* compound eye examined, the rhabdomere of at least one peripheral retinula cell was missing in each ommatidium. Since the vestigial rhabdomeres are presumably located near the distal tip of the retinula cells (10, 15), the missing rhabdomeres could indicate either these retinula cells had no rhabdomeres at all or the fracture plane happened to fall proximal to their vestigial rhabdomeres. The cell bodies of the retinula cells R1–6 appeared normal.

The rhabdomeres of all three mutants, including the vestigial rhabdomeres of *ora*^{JK84}, consisted of numerous tightly packed microvilli, each of which had a diameter of ~500 Å, as in wild type.

Microstructure of the Rhabdomeric Membrane

The protoplasmic face (PF) of the freeze-fractured rhabdomeric membrane of the wild-type fly showed numerous membrane particles (see also references 14, 22), while the exoplasmic face (EF) showed only a very few particles and appeared smooth. The three mutants studied also displayed membrane particles on the PF of the microvillar membrane, and their EF appeared smooth and showed only a few particles. All three mutants, however, differed from wild type in having a markedly lower number of rhabdomeric membrane particles in the peripheral retinula cells R1–6 (Figs. 2*b*, 3*b*, 4*b*, and Table I). In the case of *ninaB*^{P315}, a similar decrease in rhabdomeric particles was also observed in the central retinula cell R7 (Fig. 2*c*). The other two mutants, on the other hand, appeared to have a normal number of particles on the rhabdomeres of R7 cells (Figs. 3*c* and 4*c*).

TABLE I

Comparison of Rhabdomeric Membrane Particle Density in Two Classes of Retinula Cells

	Retinula cells R1–6	Retinula cell R7*
	particles/μm^2	
Wild type	2,870 ± 750 (15)	3,160 ± 870 (5)
ninaB^{P315}	1,270 ± 610 (10)	770 ± 260 (5)
ninaA^{P228}	1,020 ± 270 (22)	3,030 ± 980 (5)
ora^{JK84}	450 ± 110 (20)	2,630 ± 780 (5)

The data are presented in the form, mean ± SD (*n*), where *SD* and *n* stand for the standard deviation, and number of cells, respectively. The data were obtained from both red- and white-eyed flies and were corrected for the curvature of microvilli (Materials and Methods).

* The number of fractured eyes were 3, 2, 4, and 2 for wild type, *ninaB*^{P315}, *ninaA*^{P228}, and *ora*^{JK84}, respectively. Only the data from unequivocally identified R7 cells are included in this column.

Table I displays the results of membrane particle density measurements. In wild type, the membrane particle densities in the two classes of rhabdomeres were nearly the same: ~3,000 particles/μm^2. This is somewhat lower than that reported by Harris et al. (14) probably because we corrected for the curvature of microvilli (Materials and Methods). In the case of the mutants *ninaA*^{P228} and *ora*^{JK84}, R1–6 rhabdomeres had substantially reduced particle counts while the particle density in R7 rhabdomeres was comparable to that in wild-type rhabdomeres. By contrast in the case of the mutant *ninaB*^{P315}, the particle density was significantly lower than that of wild type in both R1–6 and R7 rhabdomeres. Thus, the effect of the mutations *ninaA*^{P228} and *ora*^{JK84} on rhabdomeric membrane particles appears to be confined to R1–6 cells, while *ninaB*^{P315} affects the central retinula cell R7 as well.

To determine whether *ninaA*^{P228} spares R8 rhabdomeres as well as R7 rhabdomeres, we examined a double mutant carrying both the *ninaA*^{P228} and *sev*^{LY3} mutations (Materials and Methods), because in wild-type flies it is difficult to distinguish the rhabdomeres of the two central retinula cells (R7 and R8) from each other unambiguously. As far as we could determine, the phenotype of the double mutant was the sum of the effects of the two constituent mutations. Thus, the only central cell rhabdomeres remaining in the double mutant were those of R8 photoreceptors. Fig. 5 shows a replica of a cross-fractured ommatidium of the double mutant. Although the rhabdomeres do not form a clear trapezoidal pattern in this fracture plane, one can readily identify the rhabdomere designated by rh_c as the one belonging to the central retinula cell. The enlargements of the rhabdomeres shown in insets to the left of the figure show that this is the only rhabdomere with a normal complement of membrane particles. Thus, *ninaA*^{P228}, indeed, appears to have no effect on the R8 particle count.

Rhodopsin Content

Table II displays the absorbance changes at 578 nm (ΔA_{578}), obtained by deep pseudopupil spectrophotometry as a measure of R1–6 rhodopsin content (Materials and Methods), normalized to ΔA_{578} for wild-type flies (0.147 ± 0.022; n = 12 eyes). We have included in the table the results of Larrivee et al. (22), obtained from digitonin extracts of head homogenate. Relative

TABLE II

Comparison of Relative Rhodopsin Content with Relative Rhabdomeric Membrane Particle Density

	Relative rhodopsin content*		
	In vivo measurements	Digitonin extracts	Relative particle density‡
Wild type	1.00 ± 0.15 (12)	1.00 ± 0.09 (5)	1.00 ± 0.24 (19)
ninaB^{P315}	0.20 ± 0.08 (17)	—	0.37 ± 0.16 (28)
ninaA^{P228}	0.15 ± 0.04 (10)	0.11 ± 0.04 (5)	0.34 ± 0.09 (15)
Vitamin A-deprived wild type	≤0.09 (2)§	<0.03 (4)	0.24 ± 0.09 (11)§
ora^{JK84}	Not determined	—	0.15 ± 0.04 (20)

Data presented in the form mean ± SD (*n*) where *n* = number of eyes, number of extracts, and number of cells, for the first, second, and third column, respectively. All values were normalized to the corresponding wild-type value to obtain "relative values" shown. All data are from white-eyed flies only.

* The rhodopsin measurements obtained from spectrophotometry of digitonin extracts (1,000 heads/extract) by Larrivee et al. (22) are included in the second column of the table for comparison. No attempts were made to correct for small differences in the size of rhabdomeres that might be present in some mutants in calculating the "relative rhodopsin contents."

‡ The relative particle densities (third column) were calculated from the data displayed in the first column of Table III. For *ninaA*^{P228} and *ora*^{JK84}, only the data from the rhabdomeres of R1–6 cells are presented in the table. For other classes of flies, the data from other cell types are included.

§ For vitamin A-deprived flies, the same eyes were used for both in vivo spectrophotometry (first column) and particle density measurements (third column).

rhodopsin contents determined by the two methods agree reasonably well. As is apparent from the table, the two *nina* mutants and vitamin A-deprived (A^-) flies all display rhodopsin contents significantly lower than that of wild type. No attempts were made to determine absorbance changes in *ora* because of its small R1–6 rhabdomeres.

Also shown in Table II are rhabdomeric particle densities normalized to that of wild type. In the case of the mutants $ninaA^{P228}$ and ora^{JK84}, only the data from identified R1–6 cells are included in the table. In the case of the other classes of flies, the data from R1–6 and R7/8 rhabdomeres were combined, since in these flies no significant difference in particle density was observed between R1–6 and R7 rhabdomeres (Table I). In the descending order of particle density, the flies

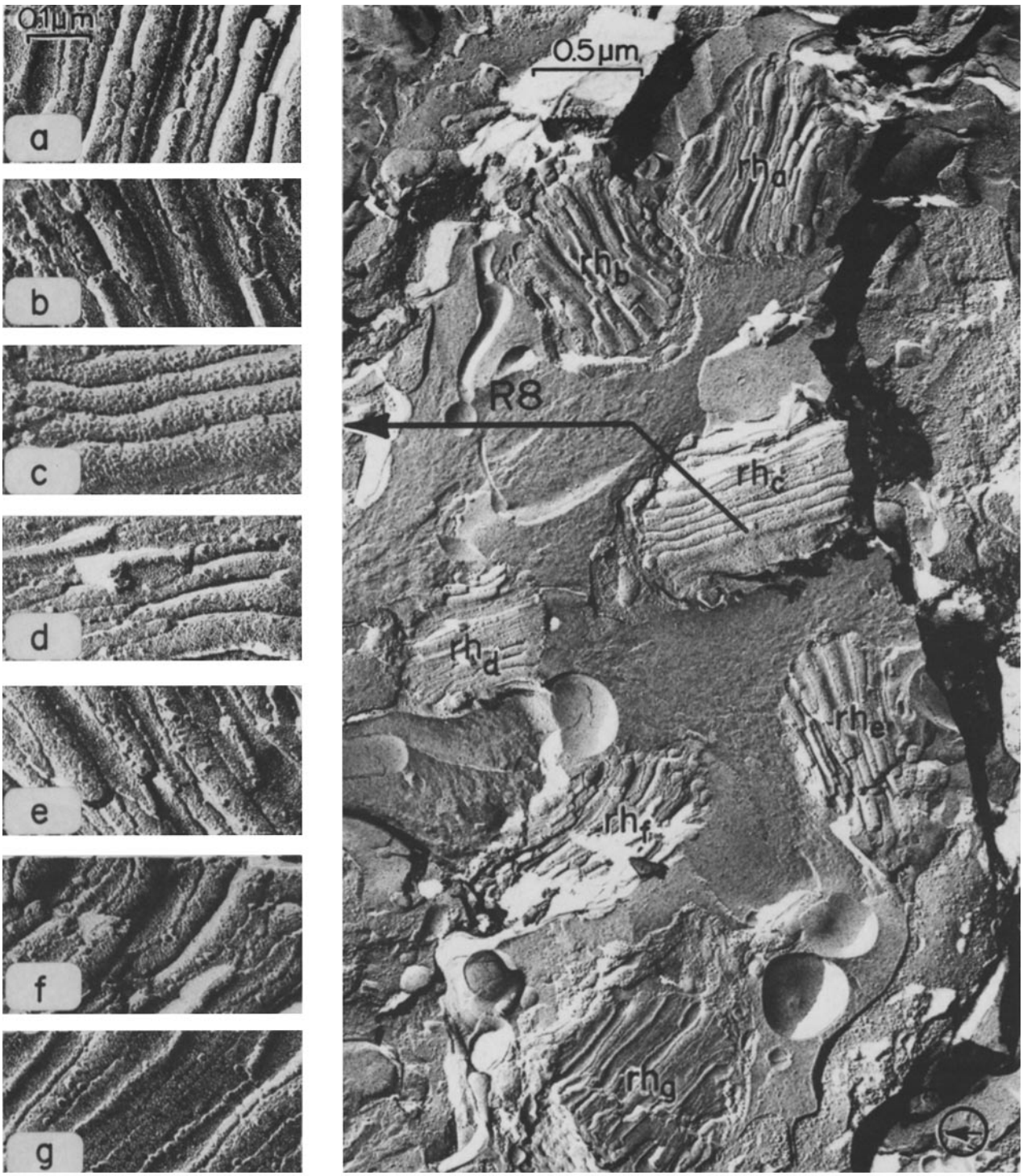


FIGURE 5 Fracture replica of an ommatidium of the double mutant sev^{LY3}; $ninaA^{P228}$ cross-fractured at a proximal level of the ommatidium. The seven rhabdomeres that are seen are labeled rh_a–rh_g. Enlarged views of these rhabdomeres are shown in *insets* labeled *a*–*g* to the left of the figure. The rhabdomere labeled rh_c belongs to the central retinula cell R8. Note that it is the only rhabdomere with a normal number of particles. The arrow in a circle in the lower right hand corner indicates the direction of platinum shadowing.

listed in Table II form the following sequence: wild type > *ninaB*^{P315} ≅ *ninaA*^{P228} > A$^-$ flies > *ora*^{JK84}. The same sequence also describes the order of rhodopsin content. In all of the particle-deficient flies with data on both the particle density and rhodopsin content, however, the decrease in R1–6 rhodopsin level was consistently greater than the decrease in R1–6 rhabdomeric particle density (see also references 14 and 22).

Particle Diameter Density Distribution

To compare the size of particles among the different classes of flies, we constructed for each class of flies a "particle diameter density distribution," which plots the density (number/μm^2) of particles of each diameter class against the diameter (Materials and Methods). Displayed in Fig. 6 are diameter density distributions of R1–6 rhabdomeric membrane particles for wild type (Fig. 6*a*, open histogram), *ninaB*^{P315} (Fig. 6*a*, shaded histogram), *ninaA*^{P228} (Fig. 6*b*, open histogram), A$^-$ flies (Fig. 6*b*, shaded histogram), and *ora*^{JK84} (Fig. 6*c*). The diameter classes for which the particle densities of the deficient flies differ from that of wild type by a statistically significant amount are indicated by a bracket below each set of histograms. It may be seen that the diameter distributions of the particle-deficient flies differ from that of wild type over a considerable range of sizes, although there is a tendency for these differences to occur in the smaller diameter range.

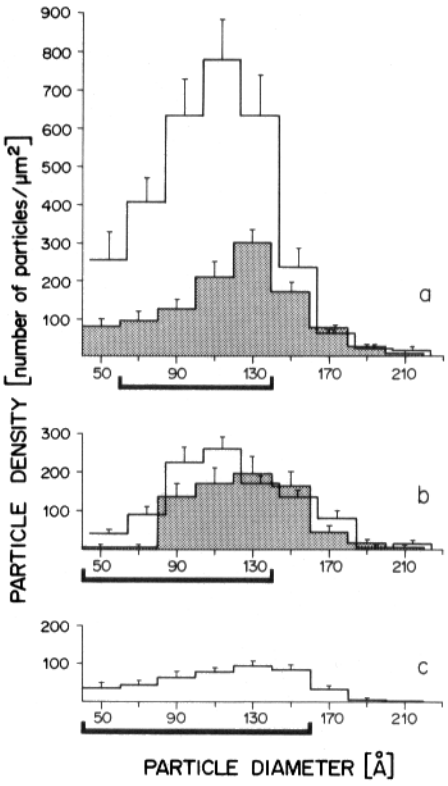


FIGURE 6 Density of rhabdomeric particles of various diameter classes: wild type (*a*, open histogram), *ninaB*^{P315} (*a*, shaded histogram), *ninaA*^{P228} (*b*, open histogram), vitamin A-deprived wild type (*b*, shaded histogram), *ora*^{JK84} (*c*). The diameters (abscissa) were grouped into size classes of 20 Å each. The sample sizes (number of cells) used to calculate the means and standard errors (shown in one direction only) of particle density (ordinate) were: 19 (wild type), 28 (*ninaB*), 22 (*ninaA*), 11 (vitamin A-deprived flies), and 20 (*ora*). In *a* and *b*, the open histogram was displaced by 4 Å along the abscissa to faciliate comparison of histograms. The brackets below the horizontal axes indicate the size classes that differ in density by a statistically significant amount from that of wild type raised on a normal medium. Except for the case of *ninaA*^{P228}, the same eyes from which the data in Table II were obtained were used to obtain these data. In the case of *ninaA*^{P228}, additional eyes were used.

Nonrhabdomeric Membrane

We also examined that portion of the nonrhabdomeric plasma membrane of the photoreceptor located between the rhabdomere and the region of contact between neighboring photoreceptor cells (indicated by dark lines in Fig. 1*a* for retinula cell R1), referred to simply as the "plasma membrane" in this paper. The membrane particles of the "plasma membrane" did not appear qualitatively different from those of the rhabdomeric membrane in either wild type (Fig. 7) or any of the particle-deficient flies (Figs. 8–11). Moreover, all four classes of particle-deficient flies showed a marked decrease in the number of particles in the PF of the plasma membrane when compared to that of wild type (Figs. 7–11).

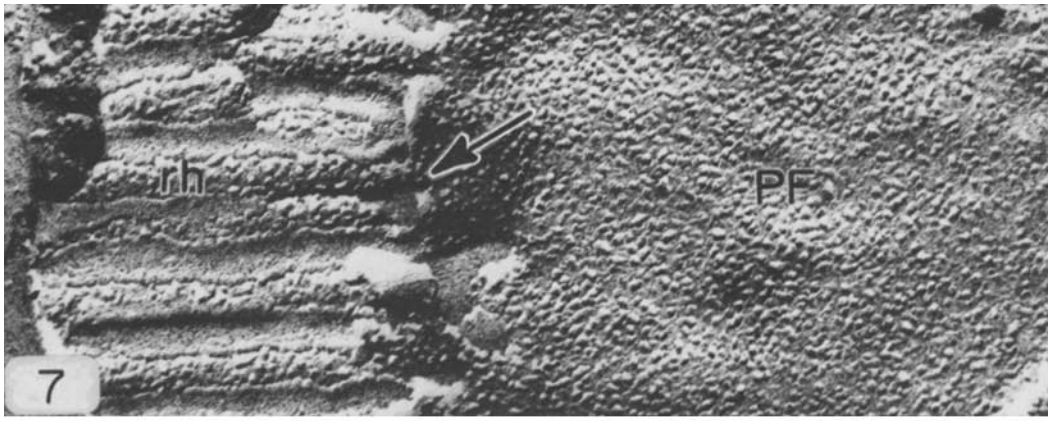
Table III compares the membrane particle densities of the plasma membrane with those of the rhabdomeric membrane for *ninaA*, *ninaB*, A$^-$, and wild-type flies. The data for rhabdomeres are the same ones from which the relative particle densities shown in Table II were calculated. As may be seen in the table, the particle density was reduced by a significant amount in both the plasma and rhabdomeric membranes in all three classes of particle-deficient flies examined.

In the case of wild-type flies, we also examined the diameters of membrane particles in the two types of membrane. The mean diameter obtained for the rhabdomeric membrane particles was 106 ± 12 Å (standard deviation, n = 24 cells), while that for the plasma membrane particles was 112 ± 9 Å (n = 12 cells). The diameter density distributions for the plasma and rhabdomeric membrane particles also showed no statistically significant differences in the density of particles at any diameter class (data not shown).

DISCUSSION

One of the objectives of this study was to obtain information on the rhabdomeric microstructure of the three mutants, *ninaA*^{P228}, ninaB^{P315}, and *ora*^{JK84}. Our results show unambiguously that the *ninaA*^{P228} mutation reduces the membrane particle density in R1–6 rhabdomeres but not in R7 rhabdo-

FIGURES 7–11 Freeze-fracture replicas of a portion of the plasma membrane obtained from wild type (Fig. 7), *ninaB*^{P315} (Fig. 8), vitamin A-deprived wild type (Fig. 9), *ninaA*^{P228} (Fig. 10), and *ora*^{JK84} (Fig. 11). In the case of *ninaA* (Fig. 10) and *ora* (Fig. 11), the pictures were obtained from identified R1-6 cells. It may be seen that the PF of the plasma membrane is continuous with that of the rhabdomeric membrane (*rh*), as indicated by arrows in Figs. 7, 8, 10, and 11. The hill-like structures above the arrow in Fig. 8 are tips of short rhabdomeric microvilli (also observed in wild type). All three mutants and vitamin A-deprived flies display a markedly lower plasma membrane particle density than wild type. Bar, 0.2 μm.


rh
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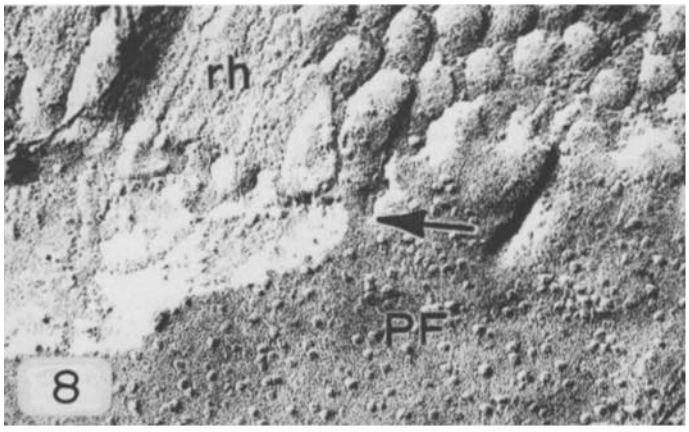
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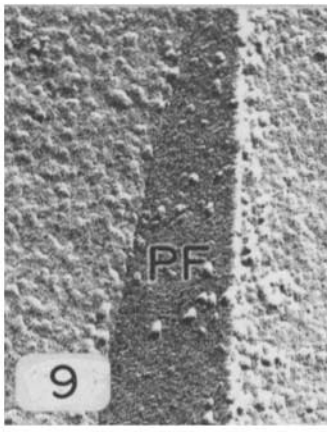
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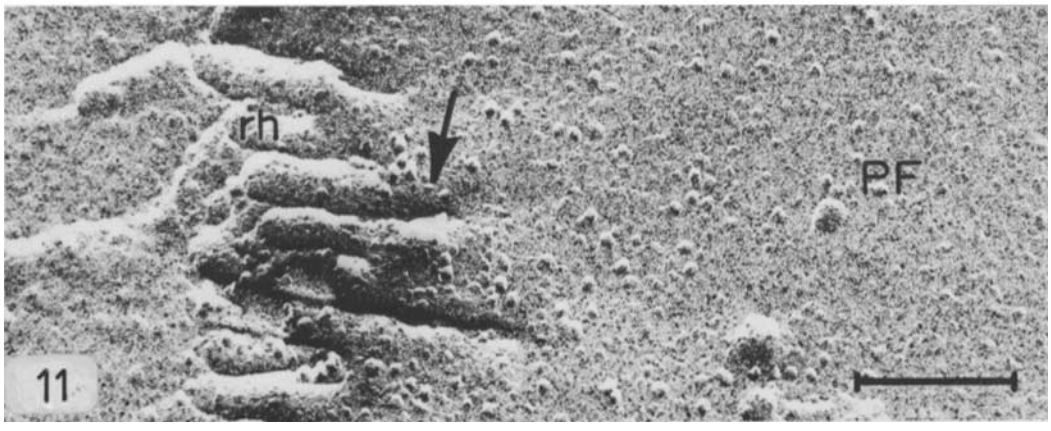
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TABLE III

Comparison of Particle Densities in Rhabdomeric and Plasma Membranes

	Rhabdomere*	Plasma membrane‡
	particles/μm²	
Wild type	2,960 ± 710 (19)	2,600 ± 510 (12)
ninaB^{P315}	1,100 ± 470 (28)	1,800 ± 780 (11)
ninaA^{P228}	1,020 ± 280 (15)	1,330 ± 790 (12)
Vitamin A-deprived wild type	710 ± 280 (11)	1,270 ± 780 (6)

Data presented in the form mean ± SD (*n*) where *n* = number of cells. All data are from white-eyed flies only.

* These data were used to calculate the relative particle densities shown in Table II. Corrected for curvature of microvilli. The number of fractured eyes was 5, 8, 3, and 5 for wild type, *ninaB*^{P315}, *ninaA*^{P228}, and *ora*^{JK84}, respectively.

‡ The numbers of freeze-fractured eyes were 5, 5, 3 and 2 for wild type, *ninaB*^{P315}, *ninaA*^{P228}, and vitamin A-deprived wild type, respectively.

meres (Fig. 3; Table I), consistent with the earlier results of Larrivee et al. (22). To see what effect the mutation *ninaA*^{P228} might have on the other class of central rhabdomeres, R8, we examined the double mutant *sev*^{LY3}; *ninaA*^{P228}. The results showed that the R8 rhabdomere of the double mutant has a normal number of membrane particles, (Fig. 5), indicating that the effect of *ninaA*^{P228} is, indeed, specific for R1–6 photoreceptors. The specificity of the *ninaA*^{P228} mutation for R1–6 photoreceptors, containing only R1–6 rhodopsin, suggests that *ninaA*^{P228} affects the apoprotein, opsin, of R1–6 rhodopsin.

The *ninaB*^{P315} mutation does not show similar specificity (Fig. 2*b* and *c*; Table I), as is also the case with vitamin A deprivation. Indeed, the *ninaB* phenotypes that have been uncovered to date are virtually indistinguishable from those of A^- flies, providing that the amount of rhodopsin remaining in the A^- flies is made comparable to that in *ninaB*^{P315}, suggesting that the mechanism of action of the mutation *ninaB* may be to restrict the amount of chromophore available for rhodopsin formation. In fact, Stephenson and Pak (39) have shown recently that the *ninaB* defect can be "cured" by raising the mutant on a medium that contains an excess amount of retinal. The same treatment, however, had no effect on the *ninaA* mutant. Thus, while the mutations *ninaA* and *ninaB* both apparently exert their effects on rhodopsin, one (*ninaA*) appears to express its effect on the opsin portion of one particular class of rhodopsin (see also 22), whereas the other (*ninaB*) seems to affect the availability of chromophore.

One of the more surprising findings of this study is that the membrane particle density in the vestigial R1–6 rhabdomeres of *ora*^{JK84} is extremely low (Fig. 4; Tables I and II). In fact, the particle density in these vestigial rhabdomeres is the lowest we have obtained in any rhabdomeres of any particle-deficient flies studied (Tables I and II). It has been known for some time that *ora*^{JK84} interferes with the formation of the R1–6 rhabdomeres (15, 19, 20). One plausible mechanism for the failure of R1–6 rhabdomeres to form in *ora*^{JK84} is that the mutation blocks the differentiation of R1–6 rhabdomeres during development. Such a mechanism, however, need not necessarily affect the density of membrane particles in R1–6 photoreceptors. The fact that the membrane particle density is extremely low in R1–6 photoreceptors suggests another possibility: that the *ora*^{JK84} mutation might block the synthesis of (a) major polypeptide(s) specific for R1–6 cells and that the loss of polypeptide(s) in turn leads to loss of rhabdomeres.

Another objective of the present study was to assess to what extent the results obtained from the particle-deficient *Drosophila* mutants support the view that the majority of the rhabdomeric membrane particles are structural correlates of rhodopsin. As may be seen in Table II, whenever a decrease in rhabdomeric membrane particle density was observed in a class of flies, the rhodopsin content was also found to be reduced in the same class of flies. In fact, the particle-deficient flies arranged in descending order of particle density (Table II) were found to be in descending order of rhodopsin content as well. Moreover, in *ninaA*^{P228} there is evidence suggesting that the amount of visual pigment is reduced in R1–6 photoreceptors but not in R7 photoreceptors (22). All these observations are in strong support of qualitative correlation between the rhabdomeric particles and rhodopsin molecules.

Quantitative relationships are difficult to establish, however. One of the difficulties is that the fractional decrease in rhodopsin content is not equal to the fractional decrease in the membrane particle density in any given class of particle-deficient flies considered (Table II).[4] In every case so far examined, the amount of decrease in rhodopsin level was consistently greater than the amount of decrease in rhabdomeric particle density (Table II; see also references 14 and 22). To consider the significance of this difference between rhodopsin content and rhabdomeric particle density, it is necessary to have independent measurements of opsin content, because opsin molecules, with no chromophore, could contribute to rhabdomeric particle measurements but not to spectrophotometric rhodopsin measurements. Available evidence suggests, however, that opsin does not account for the observed difference between rhodopsin content and particle density (22).

Another difficulty in quantitatively relating the rhabdomeric particles to rhodopsin molecules is that the diameter of the rhabdomeric membrane particles are on the average relatively large (106 Å for wild type) and vary over a wide range (40–220 Å; Fig. 6). The molecular weight of *Drosophila* rhodopsin is reported to be ~37,000 daltons (27), corresponding to a diameter of about 50 Å if globular in shape. Thus, the rhabdomeric particles seem too large and vary too widely in size to correspond to individual rhodopsin molecules. A part of the discrepancy between the calculated rhodopsin diameter and observed particle diameters probably is due to freeze-fracture artifacts. These artifacts, however, do not seem likely to be solely responsible for the discrepancy because techniques that should have minimized artifacts did not materially reduce the average particle size (~90 Å) or eliminate the size variation.[5]

Nevertheless, the present work is in strong support of the conclusion that at least the majority of the membrane particles on the rhabdomeres of *Drosophila* photoreceptors are formed

[4] Rhodopsin contents shown in Table II represent the total rhodopsin level in the rhabdomeres viewed by the deep pseudopupil technique. Therefore, if the rhabdomere sizes of the particle-deficient flies differ significantly from that of wild type, the relative rhodopsin contents shown will not correspond to "relative rhodopsin concentrations." We found no obvious differences in rhabdomere size among the particle-deficient flies examined for rhodopsin content and made no attempt to correct our rhodopsin measurements.

[5] Unpublished data by G. Bellin, Department of Cell Biology, Swiss Federal Institute of Technology, communicated to R. H. Schinz; and unpublished data by T. Suda and R. H. Schinz, Strahlenbiologisches Institut der Universität Zürich. The techniques used were rapid freezing with pressurized liquid nitrogen and fracturing at low specimen temperature in high vacuum (x − 170°C and 1.5 × 10^{-8} torr). These techniques should have eliminated artifacts due to chemical treatment of specimens and minimized plastic deformations of the membrane components.

by or in association with rhodopsin molecules, even if a precise correlation between a single rhodopsin molecule and a single membrane particle cannot be made.

The third objective of the present work was to see if there are any microstructural differences between the rhabdomeric membrane and the adjacent nonrhabdomeric plasma membrane. Three criteria have been employed to compare the two types of membrane: (*a*) the density of membrane particles (Table III), (*b*) particle diameter distribution (data not shown), and (*c*) the effects of the mutations and vitamin A deprivation on particle density (Table III; Figs. 8–11). None of the three criteria succeeded in revealing any striking differences between the two membranes.

The similarity in particle density (Table III) and particle density distribution between the two classes of membrane suggest that the same population(s) of membrane particles are present in the two membranes. Moreover, the parallel decrease in the number of rhabdomeric and plasma membrane particles in particle-deficient flies support the view that rhodopsin is present in both classes of membrane, at least for that part of the nonrhabdomeric membrane examined in this work. Brown and Schwemer (P. K. Brown, personal communication) have reached similar conclusions from their studies of normal and vitamin A–deprived blowflies. Fernandez and Nickel (9), on the other hand, have reported that in the crayfish the particle density in the nonrhabdomeric membrane is considerably lower than that in the rhabdomeric membrane, as did Chi and Carlson (5) for the housefly. The source of disagreement is not clear.

Thus, as in vertebrates (1, 7, 16, 31, 41), rhodopsin does not appear to be confined to the differentiated membrane of the light-receptive organelle in certain invertebrates (at least in certain species of flies). Nevertheless, because the rhabdomeres contain most of the rhodopsin-bearing membrane, these differentiated membrane structures are expected to be responsible for most of the photon capture by the photoreceptor.

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REFERENCES

1. Basinger, S., D. Bok, and M. Hall. 1976. Rhodopsin in the rod outer segment plasma membrane. *J. Cell Biol.* 69:29–42.
2. Boschek, C. B. 1971. On the fine structure of the peripheral retina and lamina ganglionaris of the fly, *Musca domestica*. *Z. Zellforsch.* 118:369–409.
3. Boschek, C. B. and K. Hamdorf. 1976. Rhodopsin particles in the photoreceptor membrane of an insect. *Z. Naturforsch Sect. C. Biosci.* 31c:763.
4. Campos-Ortega, J. A., G. Jürgens, and A. Hofbauer. 1979. Cell clones and pattern formation: studies on *sevenless*, a mutant of *Drosophila melanogaster*. *Roux's Arch. Dev. Biol.* 186:27–50.
5. Chi, C. and S. D. Carlson. 1979. Ordered membrane particles in rhabdomeric microvilli of the housefly (*Musca domestica* L.). *J. Morphol.* 161:309–322.
6. Cosens, D. J. and M. M. Perry. 1972. The fine structure of the eye of a visual mutant, A-type, of *Drosophila melanogaster*. *J. Insect Physiol.* 18:1773–1786.
7. Dewey, M. M., P. K. Davis, J. K. Blasie, and L. Barr. 1969. Localization of rhodopsin antibody in the retina of the frog. *J. Mol. Biol.* 39:395–405.
8. Eguchi, E. and T. H. Waterman. 1976. Freeze-etch and histochemical evidence for cycling in crayfish photoreceptor membranes. *Cell Tissue Res.* 169:419–434.
9. Fernandez, H. R. and E. Nickel. 1976. Ultrastructural and molecular characteristics of crayfish photoreceptor membranes. *J. Cell Biol.* 69:721–732.
10. Fischbach, K. F. 1976. Funktionelle Differenzierung und Wechselwirkungen der Rezeptorsysteme im Komplexauge von *Drosophila melanogaster*. Ph.D. Thesis, Albert-Ludwigs-Universität, Freiburg, i. Br.
11. Goldsmith, T. H. and M. S. Bruno. 1973. Behavior of rhodopsin and metarhodopsin in isolated rhabdoms of crabs and lobsters. *In* Biochemistry and Physiology of Visual Pigments. H. Langer, editor. Springer-Verlag, Berlin. 147–153.
12. Hamdorf, K., and G. Rosner. 1973. Adaptation und Photoregeneration im Fliegenauge. *J. Comp. Physiol.* 86:281–292.
13. Hardie, R. C., N. Franceschini, and P. D. McIntyre. 1979. Electrophysiologial analysis of fly retina. II. Spectral and polarization sensitivity in R7 and R8. *J. Comp. Physiol.* 133:23–29.
14. Harris, W. A., D. F. Ready, E. D. Lipson, A. J. Hudspeth, and W. S. Stark. 1977. Vitamin A deprivation and *Drosophila* photopigments. *Nature (Lond.)* 266:648–650.
15. Harris, W. A., W. S. Stark, and J. A. Walker. 1976. Genetic dissection of photoreceptor system in the compound eye of *Drosophila melanogaster*. *J. Physiol.* 256:415–439.
16. Jan, L. Y. and J.-P. Revel. 1974. Ultrastructural localization of rhodopsin in the vertebrate retina. *J. Cell Biol.* 62:257–273.
17. Kirschfeld, K. 1979. The function of photostable pigments in fly photoreceptors. *Biophys. Struct. Mechanisms* 5:117–128.
18. Kirschfeld, K., N. Franceschini, and B. Minke. 1977. Evidence for a sensitising pigment in fly photoreceptors. *Nature (Lond.)* 269:386–390.
19. Koenig, J. H. 1975. The isolation and preliminary characterization of autosomal electroretinogram-defective mutants in *Drosophila melanogaster*. Ph.D. thesis, University of California, Los Angeles.
20. Koenig, J. H. and J. R. Merriam. 1977. Autosomal ERG mutants. Drosophila Information Service. 52:50–51.
21. Langer, H. 1966. Spektrophotometrische Untersuchung der Absorptionseigenschaften einzelner Rhabdomere im Facettenauge. *Zool. Anz. Supplementband* 29:329–338.
22. Larrivee, D. C., S. K. Conrad, R. S. Stephenson, and W. L. Pak. 1982. Mutation that selectively affects rhodopsin concentration in the peripheral photoreceptors of *Drosophila melanogaster*. *J. Gen. Physiol.* 78:521–545.
23. Lindsley, D. L. and E. H. Grell. 1968. Genetic variations of *Drosophila melanogaster*. Carnegie Institution of Washington, Wash. D.C.
24. Lo, M.-V. C. 1977. Darkening of deep pseudopupil in *Drosophila:* an optical indication of inactivation of the peripheral photoreceptors. PhD thesis, Purdue University, West Lafeyette.
25. Meinertzhagen, I. A. 1973. Development of the compound eye and optic lobe of insects, *In* Developmental Neurobiology of Arthropods, D. Young, editor. Cambridge Univ. Press, Cambridge. 51–104.
26. Nickel, E. and R. Menzel. 1976. Insect UV- and green-photoreceptor membranes studied by the freeze-fracture technique. *Cell Tissue Res.* 175:357–368.
27. Ostroy, S. E. 1978. The characteristics of *Drosophila* rhodopsin in wild-type and *norpA* vision transduction mutants. *J. Gen. Physiol.* 72:717–732.
28. Ostroy, S. E., M. Wilson, and W. L. Pak. 1974. *Drosophila* rhodopsin: photochemistry, extraction and differences in the *norpA*^{P12} phototransduction mutant. *Biochem. Biophys. Res. Commun.* 59:960–966.
29. Pak, W. L. 1979. Study of photoreceptor function using Drosophila mutants. *In* Neurogenetics: Genetic Approaches to the Nervous System. X. Breakefield, editor. Elsevier North Holland, New York. 67–99.
30. Pak, W. L., S. K. Conrad, N. E. Kremer, D. C. Larrivee, R. H. Schinz, and F. Wong. 1980. Photoreceptor function. *In* Development and Neurobiology of Drosophila. O. Siddiqi, P. Babu, L. M. Hall, and J. C. Hall, Plenum Publ. Corp., New York. 331–346.
31. Papermaster, D. S., B. G. Schneider, M. A. Zorn, and J. P. Kraehenbuhl. 1978. Immunocytochemical localization of opsin in outer segments and Golgi zones of frog photoreceptor cells. An electron microscope analysis of cross-linked albumin-embedded retinas. *J. Cell Biol.* 77:196–210.
32. Perrelet, A., H. Bauer, and U. Fryder. 1972. Fracture faces of an insect rhabdom. *J. Microscopie.* 13:97–106.
33. Ready, D. F., T. E. Hanson, and S. Benzer. 1976. Development of the *Drosophila* retina, a neurocrystalline lattice. *Dev. Biol.* 53:217–240.
34. Robb, J. A. 1969. Maintenance of imaginal discs of *Drosophila melanogaster* in chemically defined media. *J. Cell Biol.* 41:876–885.
35. Sang, J. H. 1956. The quantitative nutritional requirements of *Drosophila melanogaster*. *J. Exp. Biol.* 33:45–72.
36. Sjöstrand, F. S. and M. Kreman. 1978. Molecular structure of outer segment disks in photoreceptor cells. *J. Ultrastruct. Res.* 65:195–226.
37. Sjöstrand, F. S. and M. Kreman. 1979. Freeze-fracture analysis of structure of plasma membrane of photoreceptor cell outer segments. *J. Ultrastruct. Res.* 66:254–275.
38. Stavenga, D. G., A. Zantema, and J. W. Kuiper. 1973. Rhodopsin process and the function of pupil mechanisms in flies. *In* Biochemistry and Physiology of Visual Pigments. H. Langer, editor. Springer-Verlag, Berlin. 175–180.
39. Stephenson, R. S. and W. L. Pak. 1981. *Drosophila* visual mutants defective in vitamin A utilization. *Invest. Ophthalmol. Vis. Sci. (Suppl.)* 20:112 (abstr.).
40. Trujillo-Cenóz, O. and J. Melamed. 1966. Electron microscope observations on the peripheral and intermediate retinas of Dipterans. *In* The Functional Organization of the Compound Eye. C. G. Bernhard, editor. Pergamon Press, London. 339–361.
41. Yoshikami, S. and G. Nöll. 1979. The outer segment plasma membrane reveals the pigment epithelium to the source of the 11-cis retinaldehyde in rods. *Biophys. J.* 25:314a (abstr.).