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Rhodopsin Transport and Rab Expression in the Carotenoid-Deprived *Drosophila melanogaster*

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ABSTRACT—*Drosophila* rhodopsin consists of an apoprotein, opsin, and a chromophore, 11-*cis*-3-hydroxyretinal. When flies are raised in carotenoid-free medium, rhodopsin transport is blocked between the rough endoplasmic reticulum (rER) and the Golgi body, and immature opsin accumulates in the rER. To elucidate how carotenoid controls the protein transport between these organelles, we investigated the morphological and molecular-biological defects caused by carotenoid deprivation. The results demonstrated that this deprivation causes rER elongation and a shortening of the rhabdomeric microvilli, whereas the density and morphological features of the Golgi body are not significantly affected. Moreover, the content of rhabdomeric proteins other than rhodopsin is not decreased by the deprivation. These results indicate that carotenoid deprivation does not affect the overall traffic of vesicle transport, but selectively suppresses rhodopsin transport between the rER and the Golgi body. We further investigated the gene and protein expression of Rab proteins that control the vesicle transport between cell organelles. The results demonstrated that neither gene nor protein expression of RAB1 and RAB2 is influenced by carotenoid deprivation, which also supports the above conclusion.

INTRODUCTION

Drosophila rhodopsin covalently binds a chromophore, 11-*cis*-3-hydroxyretinal, which is usually synthesized from dietary carotenoid (Goldsmith *et al*., 1986; Seki *et al*., 1986; Tanimura *et al*., 1986; Isono *et al*., 1988; Stark *et al*., 1990; Lee *et al*., 1996). When flies are raised on a carotenoid-deprived medium, very little of this chromophore is synthesized, and thus holoproteins of rhodopsin that are able to absorb visible light are not formed in the retinas. Furthermore, carotenoid deprivation blocks opsin (an apoprotein of rhodopsin) synthesis (Boschek and Hamdorf, 1976; Harris *et al*., 1977; Schinz *et al*., 1982; deCouet and Tanimura, 1987). We have demonstrated in a previous study that a small amount of immature opsin accumulates in the deprived fly (Ozaki *et al*., 1993). Because this immature opsin bears a high-mannose oligosaccharide, it was suggested that carotenoid deprivation inhibits rhodopsin maturation before its arrival at the Golgi body. In addition, electron microscopy revealed that carotenoid deprivation reduces the size of rhabdomere, the terminal of rhodopsin transport (Sapp *et al*., 1991). Although these data suggest that carotenoid participates in rhodopsin transport, especially between the rough endoplasmic reticulum (rER) and the Golgi body, it still remains unclear whether carotenoid specifically facilitates the rhodopsin transport or controls the overall traffic of proteins.

In order to answer this question, we here carried out the quantitative electron microscopy to examine the effects of carotenoid deprivation, especially on the rER, the Golgi body, and rhabdomere. Moreover, we investigated the protein compositions of rhabdomeric membranes in carotenoid-replete and carotenoid-deprived flies. The results suggested that carotenoid deprivation specifically suppresses rhodopsin transport. However, several lines of evidence have shown that retinoic acid, a derivative of carotenoid, controls the gene expression of Rab proteins (Motoike *et al*., 1991), which are active participants in the vesicle transport of various proteins (Novick and Brennwald, 1993; Simons and Zerial, 1993). More than 30 members of the Rab family have been reported in a wide variety of organisms (Simons and Zerial, 1993). The differential distribution of Rab proteins to distinct organelles suggests that different members of the family are involved in controlling traffic in different pathways of vesicle transport. In a previous study, we cloned and sequenced several kinds of cDNAs encoding *Drosophila* Rab proteins (DRABs) (Satoh *et al*., 1997a). RAB1 is the only protein clearly shown to be involved in vesicle transport between the rER and the Golgi body in *Drosophila* (Satoh *et al*., 1997b), as well as in yeast and mammals (Plutner

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et al., 1991; Rexach and Schekman, 1991; Tisdale *et al*., 1992). DRAB2 has also been shown to participate in vesicle transport between the rER and the Golgi body in mammalian cells (Tisdale *et al*., 1992; Tisdale and Balch, 1996), although no evidence of the involvement of DRab2 has been demonstrated in *Drosophila* cells. In the present study, we therefore examined the gene and protein expression of these DRABs in the carotenoid-deprived fly.

MTERIALS AND METHODS

Fly stocks

All experiments were carried out on white-eyed (*w*) *Drosophila melanogaster* (*A35*). Flies were ordinarily raised on a carotenoid-rich medium containing 6% yellow cornmeal, 5% dry yeast, 3.2% sucrose, 0.32% methyl-p-hydroxybenzoate, and 2% agar (Car+). Carotenoid deprivation (Car–) was achieved by raising flies from egg to adult on a medium composed of 10% dry yeast, 10% sucrose, 0.02% cholesterol, 0.32% methyl-p-hydroxybenzoate, and 2% agar ("yeast-food"). Both Car+ and Car– flies were raised in a room at 25°C with a 12-hr light : 12-hr dark cycle of fluorescent lighting at an intensity of 50 lux.

Electron microscopy

Tissues including compound eyes and laminas were dissected out of flies (7 days after eclosion) in a fixative solution (2% paraformaldehyde, 2% glutaraldehyde, 0.1 M cacodylate buffer (pH 7.4)), and kept in the ice-cold fixative solution for 2 hr. Samples were then post-fixed (2% OsO_4, 0.1 M cacodylate buffer (pH 7.4)) on ice for 2 hr, dehydrated through a graded series of ethanol, and embedded in epoxy resin (Quetol-812, NISSIN EM). Ultrathin sections were stained with uranyl acetate and lead hydroxide, and were observed and photographed with an electron microscope (JEM1010, JEOL). In order to quantitate subcellular structures, a single section was prepared from each individual fly, and approximately 20 cells were subjected to measurement in each section. Four sections (flies) were used for measurement for each of the carotenoid-replete and deprived groups. The sizes of the rER and rhabdomeric microvilli and the number of Golgi bodies were manually measured on electron micrographs.

Analysis of rhabdomeric proteins

A hundred flies (6 – 8 days) were used for each preparation. Rhabdomeric membranes were prepared according to a method previously used in the isolation of rhabdomeres from blowflies (Paulsen and Schwemer, 1983). Compound eyes of the carotenoid-deprived or carotenoid-replete flies were dissected out and immediately immersed in ice-cold/deionized water. After 10 min on ice, retinas were homogenized in water, and the homogenate was centrifuged at 2,600 × g for 10 min (4°C). The precipitate was suspended in 200 µl of 2.25 mM Na-phosphate buffer (pH 6.5) containing 10% Percoll (final density 1.13 g/ml). After removing the corneal cuticles, the suspension was centrifuged at 14,000 × g for 12 min (4°C). The upper layer containing rhabdoms (30 µl) was recovered and mixed with 200 µl of 5 mM Tris-Cl buffer (pH 6.8) containing 10% Percoll and 2 mM EGTA. The mixture was incubated on ice for 10 min followed by centrifugation at 14,000 × g for 12 min (4°C). The lower layer (30 µl) was then recovered as a rhabdomeric membrane fraction and mixed with 10 µl of 4 × SDS sample buffer. Proteins in the fraction were separated by SDS-PAGE (Laemmli, 1970), and the gel was stained with silver (Morrissey, 1981).

Preparation of poly(A)$^+$ RNA

Poly(A)$^+$ RNA was extracted from the dehydrated tissue of *Drosophila* (Satoh *et al*., 1997a). Flies (4-8 days after eclosion) were rapidly frozen in liquid nitrogen and separated into heads and bodies. They were immersed in cold acetone (–30°C), and kept at –30°C for 10 days to substitute acetone for water. After evaporating acetone at room temperature, the dried heads were dissected to separate the retina from the brain. Optic lobes were included in the brain fraction. A body fraction was also prepared without further dissection. Poly(A)$^+$ RNA was directly extracted from each part of the fly (1,000 eyes, 300 brains, or 30 bodies) by the guanidine thiocyanate method (Chirgwin *et al*., 1979), combined with the purification with oligo(dT)-cellulose using a QuickPrep Micro mRNA Purification Kit (Pharmacia).

Northern analysis

Poly(A)$^+$ RNA extracted from each part of the Car+ or Car– flies was roughly quantified spectrophotometrically. Approximately 100 ng of poly(A)$^+$ RNA from each fraction was then subjected to northern analysis to determine the relative amount of histone H3.3Q mRNA in each fraction. Based on the results, the amount of poly(A)$^+$ RNA charged in each lane was finely readjusted to give an equal density of histone signal between Car+ and Car– flies. Poly(A)$^+$ RNA was separated on a 1.4% agarose gel containing 6% formaldehyde, vacuum transferred onto a nylon membrane (Hybond-N, Amersham), and fixed on the membrane by UV irradiation. cDNA probes for DRabs were labeled with [α-^{32}P]dCTP, and used for hybridization. Hybridization was carried out at 53°C for 12 hr, and the membrane was washed with 2 × SSC at 25°C, followed by washing with 0.2 × SSC containing 0.1% SDS at 60°C. Hybridization signals were detected with X-ray films (X-Omat AR, Kodak). After removing the DRab mRNA probes, the membrane was reused for hybridization using histone and opsin cDNA probes.

Western analysis

To raise antisera against DRAB1 and DRAB2, 6 × His - DRAB fusion proteins were synthesized in *E. coli* cells, purified on a polyhistidine affinity resin (Qiagen), and injected into mice. Samples for SDS-PAGE were prepared as follows. Eyes were dissected out of flies and immediately immersed in water. After removing the corneal cuticles, retinas were homogenized in water and dissolved in SDS-containing buffer. The brain fraction was prepared from heads without eyes in the same manner as described above. Proteins were separated by electrophoresis in 12.5% SDS-polyacrylamide gels. For immunoblotting, separated proteins were transferred onto PVDF membrane, which was then incubated with anti-NINAE monoclonal antibodies, anti-DRAB1 antiserum, or anti-DRAB2 antiserum. Immunoreactive proteins were detected using an avidin-biotin amplification system (VECTOR).

RESULTS

Morphological changes in photoreceptor cells in response to carotenoid deprivation

To investigate the rhodopsin transport in the carotenoid-deprived fly, we first examined the morphological changes in photoreceptor cells caused by carotenoid deprivation. Electron micrographs of the retinal sections that include the nuclei of R1-6 photoreceptor cells were used to quantitate the sizes of the rhabdomere and the rER, and the density of the Golgi bodies. As shown in Fig. 1A-D, the size of the rhabdomere is drastically reduced by carotenoid deprivation. In addition, rhabdomeric microvilli of the deprived fly have disordered alignment and largely invaginate into the cytoplasm. Figure 1D also displays the elongated rER residing in the carotenoid-deprived photoreceptor cells. These features are quantitated and summarized in Fig. 2. The rER in the deprived fly is approximately three times longer than that in the normal fly (Fig. 2, left panel). In contrast, the maximum length of rhabdomeric microvilli in

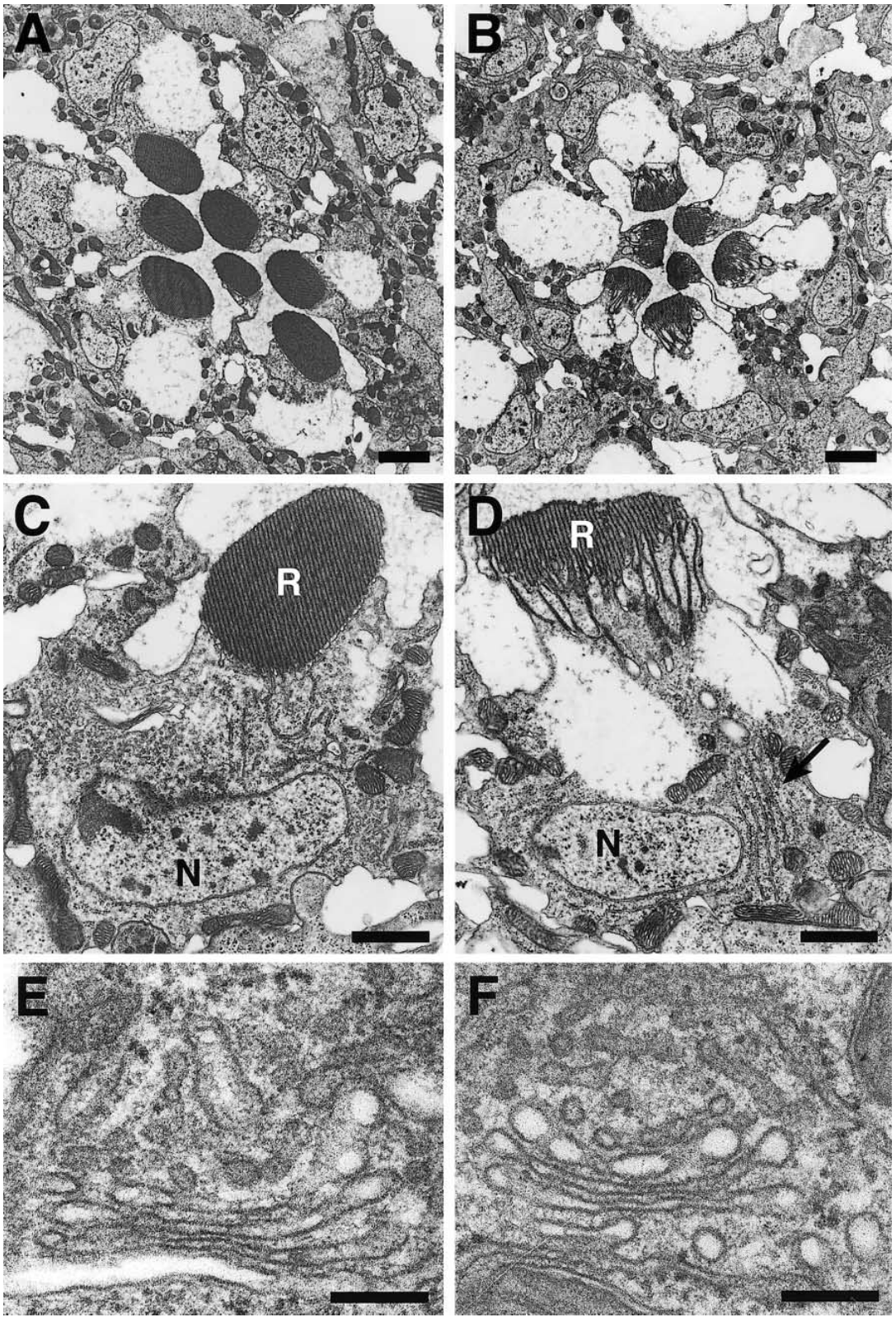

Fig. 1. Electron micrographs of the *Drosophila* retina. (**A**, **B**) Cross-section of an ommatidium at the layer including the nuclei of R1-6 photoreceptor cells from the carotenoid-replete (**A**) and deprived (**B**) fly. All rhabdomeres of an ommatidium are degenerated in the deprived fly. Bar = 2 μm. (**C**, **D**) Cross-section of a single photoreceptor cell from the carotenoid-replete (**C**) and deprived (**D**) fly. Rhabdomeric microvilli of the deprived fly are disordered and deeply invaginated into the cytoplasm. rERs (arrow) are elongated in the deprived fly. N, nucleus; R, rhabdomere. Bar = 1 μm. (**E**, **F**) Golgi body in the photoreceptor cell of the carotenoid-replete (**E**) and deprived (**F**) fly. The deprived fly has normal Golgi bodies showing profiles that are morphologically the same as those in the replete fly. Bar = 0.2 μm.

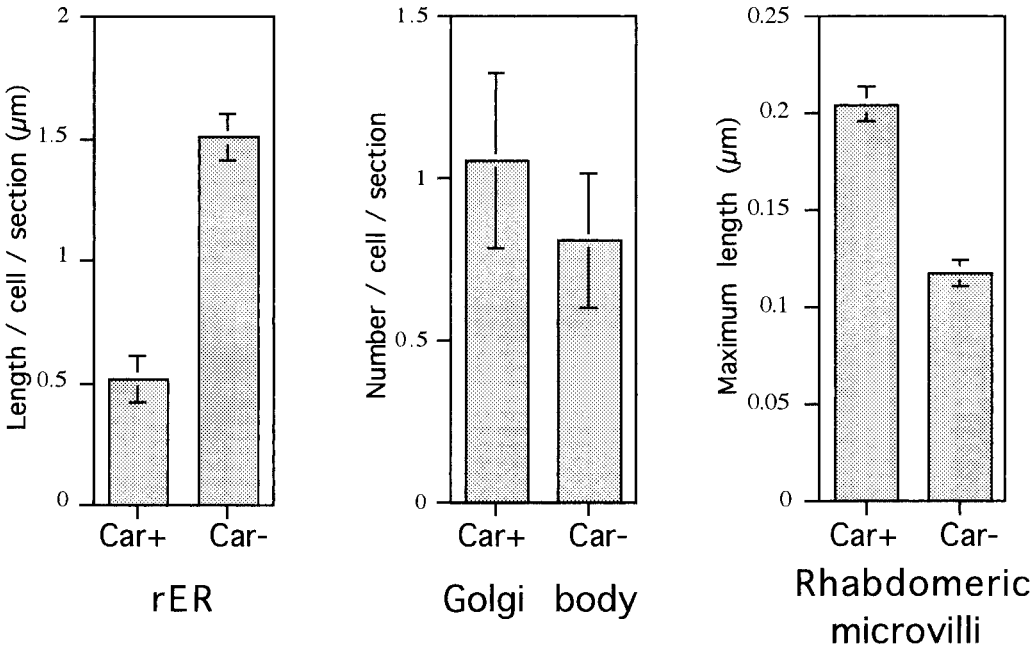

Fig. 2. Quantitation of the sizes of the rER and rhabdomeric microvilli, and the density of the Golgi body in the photoreceptor cells of carotenoid-replete and deprived *Drosophila*. Details on quantitation are described in the Materials and Methods section. The length of the rER in the deprived fly (Car–) is significantly ($P < 0.005$) larger than that in the replete fly (Car+), whereas the size of the microvilli is significantly ($P < 0.005$) reduced in the deprived fly. The mean value of the density of the Golgi body is reduced in the deprived fly, but the difference is not statistically significant. Data are expressed as mean ± SE (n = 4).

the deprived fly is reduced to about 60% of that in the replete fly (Fig. 2, right panel). We next looked at the effect of carotenoid deprivation on the Golgi body. Golgi bodies in the carotenoid-deprived fly show similar morphological profiles to those in the carotenoid-replete fly (Fig. 1E, F). Specifically, each Golgi body contains a collection of cisternae having narrow inner lumens, many electron-dense vesicles on the *trans* side, and several ER-Golgi vesicles on the *cis* side. *Trans* Golgi networks are poorly developed in both cases. Furthermore, no statistically significant difference in the average number of Golgi body was found between the carotenoid-deprived and replete flies, although the number appears to be slightly reduced by the deprivation (Fig. 2, middle panel). In addition to these cell organelles presumably involved in rhodopsin synthesis and transport, we further carried out a close observation of other parts of photoreceptor cells. However, no definite aberration besides the abnormal microvilli and elongated rER could be found in the carotenoid-deprived photoreceptor cells. For example, the morphological features in the axonal region and synaptic vesicles, as indicated in Fig. 3, display no essential difference between carotenoid-replete and deprived flies.

Protein composition of rhabdomeric membranes

SDS-PAGE analyses of rhabdomeric proteins in the carotenoid-deprived and carotenoid-replete flies are shown in Fig. 4 (lanes 2 and 3). The protein composition of the total retinal membranes of carotenoid-replete flies is also displayed in the figure (lane 1). Through the purification procedure, the concentration of rhodopsin (35 k) is greatly increased, accompanied by significant changes in the composition and content of other proteins (lanes 1 and 2). This result indicates that this procedure effectively concentrated the rhabdomeric membranes that contain dense amounts of rhodopsin. Based on this result, we compared the protein composition of rhabdomeric membranes between carotenoid-deprived and carotenoid-replete flies (lanes 2 and 3). The results demonstrated that the content of rhabdomeric proteins other than rhodopsin was not affected by the deprivation, whereas the amount of rhodopsin in the deprived fly is dramatically decreased and is undetectable in the silver-stained gel. This result indicates that carotenoid deprivation specifically suppresses the incorporation of rhodopsin into rhabdomeric membranes.

Gene and protein expression of DRab1 and DRab2

In order to examine whether carotenoid deprivation affects the expression of Rab proteins potentially functioning in the vesicle transport between the rER and the Golgi body, we next measured the gene and protein expression of DRab1 and DRab2. Gene expression of these DRabs was visualized by northern analysis for the eyes (corneas and retinas), brains (including optic lobes) and bodies (all parts except eye and brain). To separately prepare poly(A)$^+$ RNA from each tissue, flies were quickly frozen in liquid nitrogen, dehydrated with acetone at –30°C, and micro-dissected into each part. mRNA was directly extracted from these dried tissues. Using this technique, we could isolate the target tissue from neighboring tissues, and could extract the intact poly(A)$^+$ RNA without any

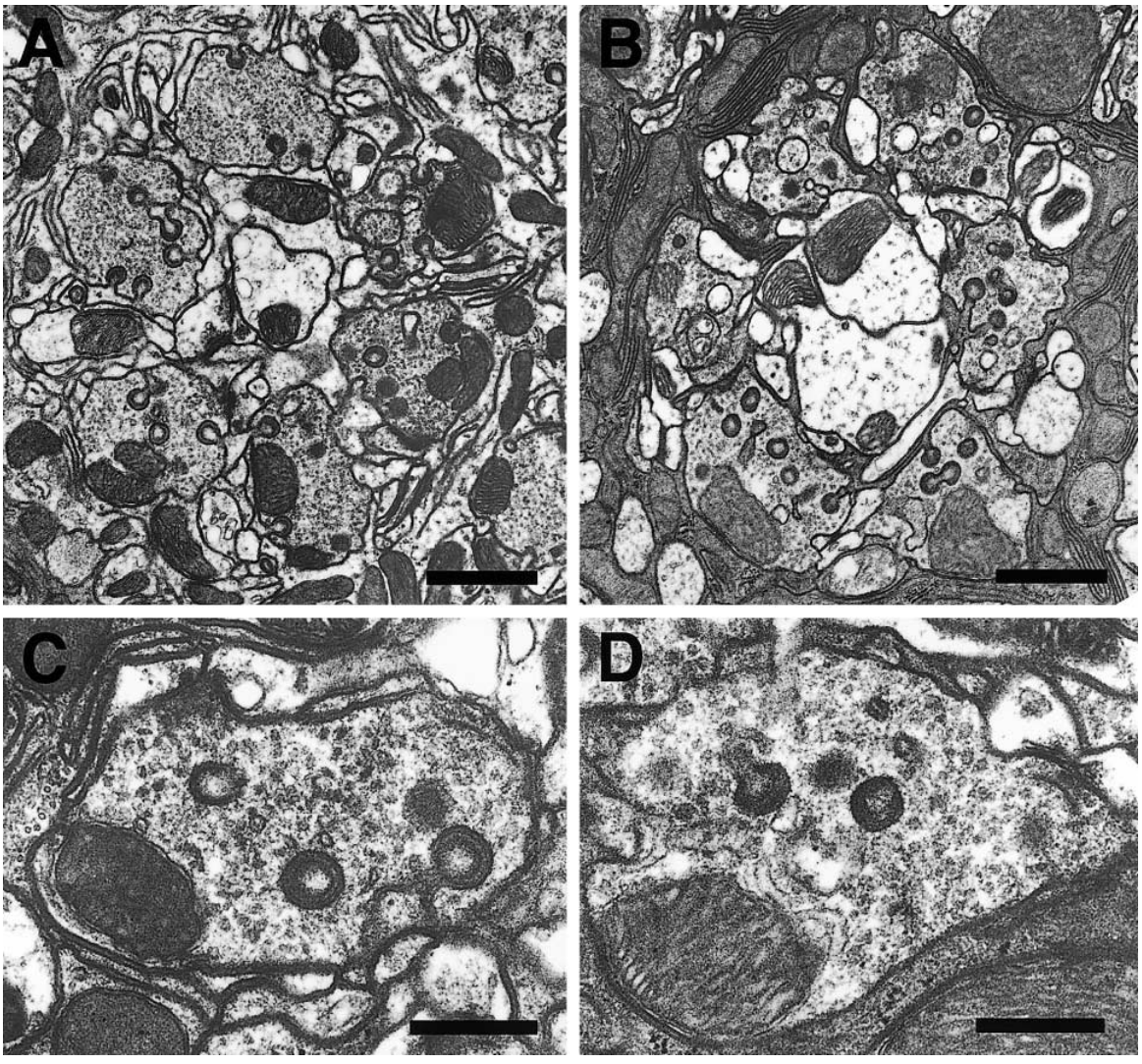

Fig. 3. Electron micrographs of *Drosophila* lamina. (**A**, **B**) Cross-sections of an optic cartridge in the carotenoid-replete (**A**) and deprived (**B**) fly. Six photoreceptor axons surround two lamina monopolar cell axons. Bar = 1 μm. (**C**, **D**) Photoreceptor axons in the carotenoid-replete (**C**) and deprived (**D**) fly. No significant differences in the profiles of the optic cartridge and the distribution of synaptic vesicles and capitate projections are found between these flies. Bar = 0.5 μm.

degradation and contamination from other tissues.

As shown in Fig. 5A, mRNAs of DRab1 and DRab2 were found in all parts of the fly. Two hybridization bands at ~900 and ~1600 bases, and three bands at ~1100, ~1400, and ~1700 bases were detected by DRab1 and DRab2 probes, respectively. However, as discussed later, transcripts of different sizes for each DRab are most likely to be produced from a single gene of corresponding DRab.

Figure 5A shows the carotenoid-dependency of the expression of DRab1 and DRab2 mRNAs in the eye, brain, and body fractions of the fly. In most cases, transcription of DRab1 and DRab2 genes was insensitive to the carotenoid deprivation. Exceptionally, slight changes of mRNA expression were observed in the brain DRab1 and eye DRab2, which, however, do not influence the protein expression of these DRabs (see below). A northern analysis of the major *Drosophila* rhodopsin (*ninaE*) gene is also shown in Fig. 5A, indicating that opsin mRNA, as well as DRab1 and DRab2 mRNA, is unaffected by carotenoid deprivation.

We next examined the carotenoid dependency of the protein expression of DRab1 and DRab2 using the immunoblotting method (Fig. 6). Neither mRNA transcription nor protein expression of DRab1 (lanes 3 - 6) and DRab2 (lanes 7 - 10) was affected by carotenoid deprivation either in the eye or the brain. In contrast, expression of mature rhodopsin is remarkably suppressed in the carotenoid-deprived fly (lanes 1, 2). These results thus exclude the possibility that carotenoid controls the expression of DRab1 and DRab2 proteins, which potentially regulate the vesicle traffic between the rER and the Golgi body.

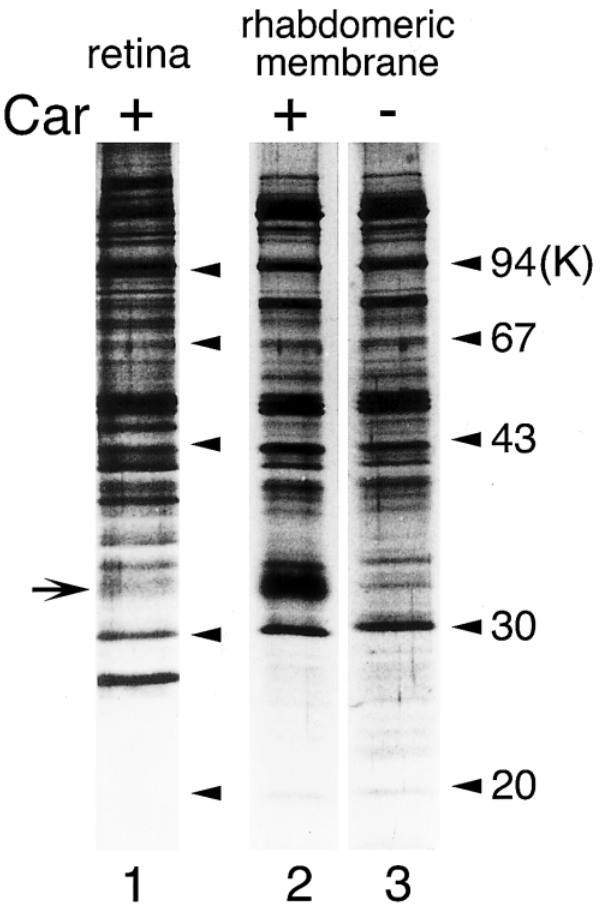

Fig. 4. SDS-PAGE analysis of the protein compositions of rhabdomeric membranes in carotenoid-replete and carotenoid-deprived *Drosophila*. No significant difference in the protein composition is found between carotenoid-replete (lane 2) and carotenoid-deprived (lane 3) flies, except that the rhodopsin (arrow) content is greatly reduced in the deprived fly. The protein composition of total retina (lane 1) shows a much lower concentration of rhodopsin than that of rhabdomeric membranes. Arrowheads and numbers on the right indicate the positions and sizes of protein molecular weight markers.

DISCUSSION

Transcripts of DRab1 and DRab2 genes

The use of DRab1 probe enable the detection of two kinds of transcripts at ~900 and ~1600 bases. Since the nucleotide sequence of DRab1 cDNA (D84312) includes 4 polyadenylation hexanucleotide signals (AATAAA) at 900-905, 930-935, 1624-1629, and 1736-1741 in the 3'-noncoding region (Fig. 5B, arrows), the larger and smaller mRNAs are probably polyadenylated by the hexanucleotide signals at 1624-1629 (and/or 1736-1741), and 900-905 (and/or 930-935), respectively. DRab2 cDNA (D84313) has two AATAAA signals at 1020-1025 and 1671-1676 (Fig. 5B, arrows). DRab2 mRNAs of ~1100 and ~1700 bases indicate that both AATAAA signals are implicated in polyadenylation. In addition, another DRab2 transcript of ~1400 bases was detected by northern hybridization. No AATAAA is found around the corresponding position of DRab2 cDNA, but the analogous sequence AATATA at 1340-1345 (Fig. 5B, arrowhead) might be implicated as a signal for polyadenylation (Nevins, 1983). In both DRab1 and DRab2, it should be noted that the larger transcript occurs more abundantly than the smaller one in the eye, while the smaller is predominantly expressed in the body. Although the functional significance of the plural transcripts from a single DRab gene has not been elucidated, mRNAs with 3'-noncoding regions of different length may have different localization or lifespan in the cells, as has been reported in several kinds of cells (Jackson, 1993).

Rhabdomeric microvilli in the carotenoid-deprived fly

In the present study, we have indicated that carotenoid deprivation induces the shortening of rhabdomeric microvilli. This result coincides with the previous results reported by Sapp *et al*. (1991). Because it has also been demonstrated that the P-face particles of rhodopsin molecules are greatly reduced in the deprived fly (Boschek and Hamdorf, 1976; Harris *et al*., 1977; Schinz *et al*., 1982), the shortening of the microvilli might be interpreted simply as a loss of areas occupied by rhodopsin molecules. In contrast to the results of Sapp *et al*. (1991), however, our present study demonstrates that the denaturation of rhabdomeric structure also occurs in deprived flies. Similar denaturation is reported in various *ninaE* (a structural gene of *Drosophila* major rhodopsin) mutants whose rhodopsin is largely reduced or completely lost even under carotenoid-replete conditions (O'Tousa *et al*., 1989; Leonard *et al*., 1992; Colley *et al*., 1995; Kurada and O'Tousa, 1995; Bentrop *et al*., 1997). Together with our present results, these data thus suggest that rhodopsin molecules may participate in the construction and/or maintenance of microvillar structures.

Rhodopsin maturation and transport

In addition to the shortening of rhabdomeric microvilli, carotenoid deprivation induced an elongation of the rER. These results indicate that rhodopsin export from the rER and its import into rhabdomeric microvilli is reduced by carotenoid deprivation. In contrast, no significant effect of carotenoid deprivation was found in the Golgi bodies and other subcellular structures of photoreceptor cells. Previous studies have demonstrated that disassembly of a Golgi body occurs when total transport from the rER to the Golgi body is severely inhibited (Wilson *et al*., 1994; Satoh *et al*., 1997b). Furthermore, the protein composition of the rhabdomeric membrane in the carotenoid-deprived fly is not significantly different from that in the carotenoid-replete fly, except that the rhodopsin content is greatly reduced. These observations indicate that carotenoid deprivation does not completely block ER-Golgi transport. In contrast, we have demonstrated that rhodopsin maturation occurring after export from the rER is completely inhibited by carotenoid deprivation (Ozaki *et al*., 1993). These results therefore suggest that carotenoid deprivation specifically blocks rhodopsin transport between the rER and the Golgi body. Alternatively, one could consider that rhodopsin transport might bypass the Golgi body. However, the following find-

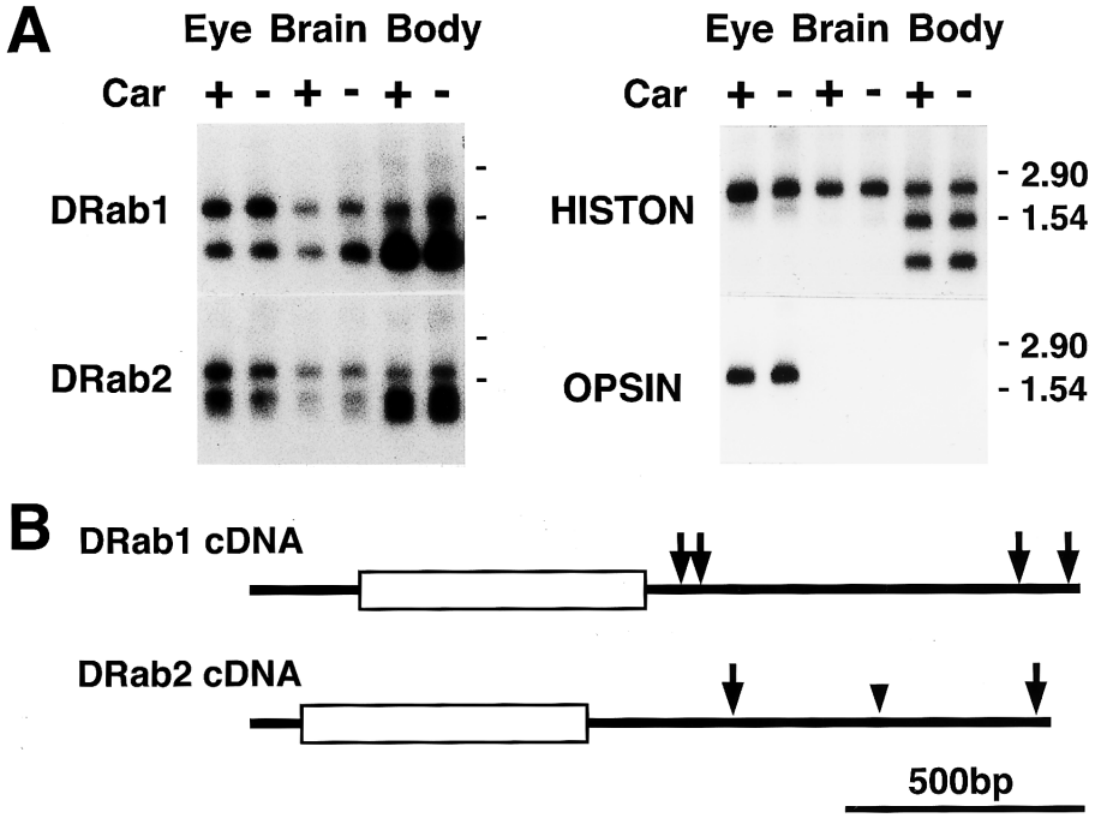

Fig. 5. Northern analysis of DRab1, DRab2, and opsin mRNAs. (**A**) DRab1 and DRab2 have plural kinds of transcripts whose distribution is different between each tissue. Opsin has a single kind of transcript and is exclusively expressed in the eyes both under carotenoid-replete (Car+) and deprived (Car–) conditions. The mRNAs of DRab1 and DRab2 are also expressed in carotenoid-independent manner in every tissue. The amount of poly(A)$^+$ RNA in each lane is adjusted with a histone probe so as to show an equal signal-density between carotenoid-replete and deprived conditions. Numbers (2.90 and 1.54) on the right indicate the positions and sizes (kilobases) of the RNA size markers. (**B**) Schematic presentation of DRab1 and DRab2 cDNA. Open boxes show the open reading frames. Arrows indicate the positions of polyadenylation hexanucleotide signals (AATAAA). Arrowhead in DRab2 cDNA represents the position of an analogous sequence for polyadenylation (AATATA). For detailed information regarding these sequences, refer to Satoh *et al.* (1997a).

ings eliminate this possibility: (1) The processing of an oligosaccharide chain binding to rhodopsin is similar to that which occurs in Golgi bodies of various organisms (Ozaki *et al.*, 1993). (2) The inhibition of rER-Golgi transport with dominant negative DRab1 protein causes an accumulation of immature rhodopsin (Satoh *et al.*, 1997b). Therefore, we would conclude that carotenoid deprivation selectively inhibits rhodopsin transport from the rER to the Golgi body.

As mentioned in the Introduction, several lines of evidence have shown that retinoid controls the expression of Rab proteins participating in vesicle transport. We therefore examined the gene and protein expression of DRab1 and DRab2 proteins under carotenoid-deprived and replete conditions. The results, however, indicated that neither DRab1 nor DRab2 shows a carotenoid-dependent gene or protein expression. This result thus supports the above conclusion that it is not the total traffic but the specific transport of rhodopsin that is controlled by carotenoid supplementation.

In the present study, the expression of opsin mRNA was not influenced by the carotenoid deprivation using “yeast-food”. Recently, Stark’s group has demonstrated that the complete deficiency of carotenoid with Sang’s medium suppresses the expression of opsin mRNA, and has suggested that the “yeast-food” possibly contains a trace amount of retinoids which could activate an opsin promoter (Sun *et al.*, 1993; Picking *et al.*, 1996). Therefore, it might be likely that DRab-expression could be suppressed under more severe conditions of carotenoid deprivation. However, it should be emphasized here that rhodopsin maturation and transport can be completely blocked by carotenoid deprivation using “yeast-food”, as shown in Fig. 6 (lanes 1, 2) and as has been reported by several groups including ours and Stark’s (deCouet and Tanimura, 1987; Ozaki *et al.*, 1993; Picking *et al.*, 1996). It is therefore suggested that the carotenoid-dependent transport of rhodopsin, rather than the carotenoid-dependent transcription of these genes, preferentially controls rhodopsin content in the *Drosophila* photoreceptor cells.

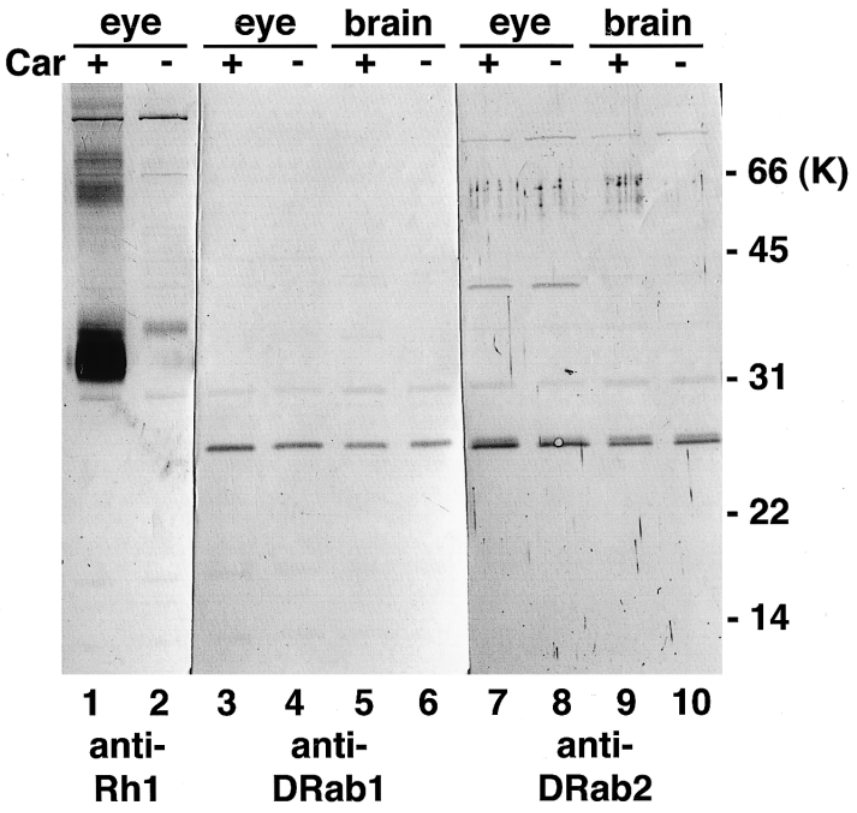

Fig. 6. Immunoblot analysis of opsin, DRAB1, and DRAB2. The expression of opsin (lanes 1, 2), DRAB1 (lanes 3, 4, 5, 6), and DRAB2 (lanes 7, 8, 9, 10) was compared in the flies raised under carotenoid-replete (Car+; lanes 1, 3, 5, 7, 9) and carotenoid-deprived (Car–; lanes 2, 4, 6, 8, 10) conditions. In the eye (lanes 3, 4, 7, 8) and brain (lanes 5, 6, 9, 10), no significant difference in DRAB1 and DRAB2 expression is detectable between carotenoid-replete and deprived flies. In contrast, only a small amount of 40 k immature opsin is detected in the carotenoid-deprived fly, while large expression of mature (35 k) opsin is found in the carotenoid-replete fly. Numbers on the right indicate the positions and sizes of the protein molecular weight markers.

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