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Regulatory arrestin cycle secures the fidelity and maintenance of the fly photoreceptor cell

(termination of the light response/rhodopsin kinase/rhodopsin phosphatase/retinal degeneration/*Drosophila* visual mutants)

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ABSTRACT **Excitation of fly photoreceptor cells is initiated by photoisomerization of rhodopsin to the active form of metarhodopsin. Fly metarhodopsin is thermostable, does not bleach, and does not regenerate spontaneously to rhodopsin. For this reason, the activity of metarhodopsin must be stopped by an effective termination reaction. On the other hand, there is also a need to restore the inactivated photopigment to an excitable state in order to keep a sufficient number of photopigment molecules available for excitation. The following findings reveal how these demands are met. The photopigment undergoes rapid phosphorylation upon photoconversion of rhodopsin to metarhodopsin and an efficient Ca^{2+} dependent dephosphorylation upon regeneration of metarhodopsin to rhodopsin. Phosphorylation decreases the ability of metarhodopsin to activate the guanine nucleotide-binding protein. Binding of 49-kDa arrestin further quenches the activity of metarhodopsin and protects it from dephosphorylation. Light-dependent binding and release of 49-kDa arrestin from metarhodopsin- and rhodopsin-containing membranes, respectively, directs the dephosphorylation reaction toward rhodopsin. This ensures the return of phosphorylated metarhodopsin to the rhodopsin pool without initiating transduction in the dark. Assays of rhodopsin dephosphorylation in the *Drosophila* retinal degeneration C (rdgC) mutant, a mutant in a gene previously cloned and predicted to encode a serine/threonine protein phosphatase, reveal that phosphorylated rhodopsin is a major substrate for the rdgC phosphatase. We propose that mutations resulting in either a decrease or an improper regulation of rhodopsin phosphatase activity bring about degeneration of the fly photoreceptor cells.**

Photoexcitation of fly photoreceptor cells is caused by light-induced isomerization of 11-*cis*-retinal, the chromophore of rhodopsin, to the all-*trans*-retinal of metarhodopsin. Since metarhodopsin does not convert spontaneously into an inactive state, an efficient turn-off mechanism must exist to terminate its activity. In vertebrate rod membrane preparations, phosphorylation of metarhodopsin by rhodopsin kinase results in partial inactivation of metarhodopsin, followed by binding of a 48-kDa protein, arrestin, which brings about complete inactivation of metarhodopsin (1, 2). More recent studies, however, both *in vitro* (3) and on functionally intact rod outer segments (4), have suggested that photolyzed rhodopsin is fully inactivated by multiple phosphorylation, whereas arrestin quenches the activity of partially phosphorylated rhodopsin. Studies of the β-adrenergic receptor revealed a receptor-specific kinase and a second-messenger-stimulated kinase that phosphorylate the receptor (5), as well as an arrestin homologue with specificity toward the β-adrenergic receptor (6). It therefore seems that phosphorylation of the receptor and binding of arrestin is a general theme for inactivation of receptors that are coupled to guanine nucleotide-binding proteins (G proteins) (for review, see ref. 7).

While transduction in fly photoreceptors bears many similarities to transduction in vertebrate rods, it also manifests several important differences. In particular, the target for the rhodopsin-activated G protein is a phospholipase C (8–10); a second messenger appears to be inositol trisphosphate (11, 12) and there are at least two types of light-dependent ion channels, one of which is highly permeable to Ca^{2+} (13). Invertebrate photoreceptor channels open (14), whereas vertebrate rod channels close, in response to illumination. Thus, light increases the free Ca^{2+} in invertebrate photoreceptors and decreases Ca^{2+} concentration in vertebrate rods. Experiments on intact *Drosophila* eyes identified light-dependent phosphorylation of three eye-specific proteins of 39, 49, and 80 kDa (15).

In the blowfly *Calliphora*, illumination of retinal homogenate induced phosphorylation of opsin and three proteins of 48, 68, and 200 kDa, as well as a light-dependent reversible binding of the 48-kDa protein to the rhabdomal membranes (16). In none of these studies, however, have the functional consequences of these reactions been determined. Two of the *Drosophila* phosphoproteins, those of 39 and 49 kDa, have been identified as arrestin homologues by molecular cloning (17–20), suggesting that they may play a role in termination of the response to light. In contrast to vertebrate rods and the β-adrenergic system, in the fly, Ca^{2+} enhances dephosphorylation of rhodopsin and phosphorylation of the arrestins, suggesting that Ca^{2+} plays a feedback regulatory role. Our studies suggest a regulatory cycle whereby the binding of 49-kDa arrestin quenches the activity of metarhodopsin and keeps it in an inactive state by protecting it from phosphatase activity. Subsequent photoregeneration of phosphorylated metarhodopsin (p-M) to phosphorylated rhodopsin (p-R) releases the 49-kDa arrestin from the membrane and exposes the p-R to Ca^{2+}-dependent phosphatase without reinitiation of phototransduction in the dark. Concerted operation of this cycle is important for both the fidelity and the maintenance of the photoreceptor cell.

MATERIALS AND METHODS

[γ-^{32}P]ATP (3000 Ci/mmol; 1 Ci = 37 GBq) and [γ-^{32}P]GTP (>10 Ci/mmol) were purchased from Amersham. Na_2ATP (A2383) and GTP lithium salt type IX were obtained from Sigma. Leupeptin and pepstatin A were from the Peptide Institute (Osaka). All other materials were of analytical grade. The calmodulin-binding peptide M5 (21) was supplied by Y. Salomon (Weizmann Institute, Rehovot, Israel), and antiserum raised against the peptide sequence 280–300 of *Drosophila* 49-kDa arrestin was provided by H. Matsumoto

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Abbreviations: p-R, phosphorylated rhodopsin; p-M, phosphorylated metarhodopsin.

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(University of Oklahoma, Oklahoma City). This epitope does not contain serines or threonines and the antibodies recognize equally well both the phosphorylated and nonphosphorylated forms of *Drosophila* and *Musca* 49-kDa arrestin. Monoclonal antibodies against R1–6 *Drosophila* opsin were provided by D. Blest (Australian National University, Canberra, Australia).

Flies and Eye Membrane Preparations. All experiments were performed on white-eyed *Drosophila* melanogaster (Canton-S strain) and white-eyed *Musca domestica* flies. *Drosophila* flies were grown on formula 4-24 (Carolina Biological Supply) and *Musca* flies were grown on fly larvae medium 50/60 (Purina). Media were supplemented with water-miscible vitamin A (Type 100, Hoffman–La Roche) at 1% concentration of the liquid that wet the dry formulas. Flies were maintained at 25°C under 12 hr/12 hr dark/light cycle, except for $rdgC^{306}$ flies, which were maintained in the dark. Eye membranes were prepared as previously described (9), except that *Drosophila* eyes dissected under a binocular microscope rather than *Drosophila* heads were used for membrane preparations. When membranes were to be derived from eyes illuminated with orange or blue lights, illumination was for 2 min prior to homogenization. An orange edge filter (Schott RG-610) or blue light filter (Schott BG-28) and two KG3 heat-reducing filters from Schott were used to obtain orange and blue lights. The energies of the unattenuated orange or blue lights at the level of the sample were 12.7 and 2.6 mW/cm^2, respectively.

Phosphorylation of Eye Proteins. Membranes derived from 30 *Drosophila* or 5 *Musca* eyes (15–20 μg of protein) in 100 μl of homogenization buffer (10 mM Mops, pH 7.0/120 mM KCl/3 mM $MgCl_2$/1 mM dithiothreitol with leupeptin at 10 μg/ml and pepstatin A at 1 μg/ml) were illuminated for 15 sec with either orange or blue light as indicated. The reaction was initiated by the addition of 100 μl of reaction mixture to give the following final concentrations: 0.1 mM [γ-^{32}P]ATP (1–3 μCi); 10 mM Mops, pH 7.0; 3 mM $MgCl_2$; 120 mM KCl; 1 mM dithiothreitol; and 1 mM EGTA. When indicated, 0.1 mM $CaCl_2$ was substituted for EGTA. After incubation for 10 min at 25°C, the reaction was terminated by the addition of 100 μl of 10% (wt/vol) trichloroacetic acid. Proteins were precipitated by centrifugation at 14,000 × *g* for 10 min. The supernatant was removed and the pellet was dissolved in 40 μl of SDS/PAGE loading buffer.

Dephosphorylation of Eye Proteins. After phosphorylation, tubes were transferred to ice, and labeling of proteins was quenched by addition of unlabeled ATP to 0.5 mM concentration, followed by centrifugation and resuspension of the pellet in 100 μl of cold homogenization buffer. To initiate dephosphorylation, membranes were illuminated for 15 sec with either orange or blue light and transferred to 25°C, and 100 μl of dephosphorylation mixture was added to give the following final concentrations: 10 mM Mops, pH 7.0; 120 mM KCl; 3 mM $MgCl_2$; 1 mM dithiothreitol; and either 1 mM EGTA or 0.1 mM $CaCl_2$ as indicated. After 5 min at 25°C, the dephosphorylation reaction was terminated by the addition of 200 μl of 10% trichloroacetic acid, and proteins were precipitated by centrifugation, neutralized with 10 μl of 1 M Tris (pH 8.0), and dissolved in 40 μl of SDS/PAGE loading buffer.

Identification and Quantitation of 49-kDa Arrestin. Proteins were dissolved in loading buffer, separated by SDS/PAGE (22) in 7.5–15% polyacrylamide gradient gels, and electroblotted overnight at 4°C onto nitrocellulose paper with a constant current of 50mA. Phosphoproteins blotted to the nitrocellulose paper were visualized by radiography at −70°C using Agfa Curix film. The same blot was subsequently used to quantitate the 49-kDa arrestin. Antibodies generated against the peptide sequence 280–300 of 49-kDa *Drosophila* arrestin were employed with the enhanced chemiluminescence Western blot detection system (Amersham) to visualize the total amount of 49-kDa arrestin on x-ray film according to the manufacturer's instructions.

RESULTS

Phosphorylation and Dephosphorylation of Rhodopsin. A characteristic of fly photoreceptors is that their photopigment is thermostable and photoreversible. Blue light (<490 nm) converts rhodopsin to metarhodopsin (80%), and orange light (>580 nm) regenerates it to rhodopsin (100%) (23). *Drosophila* eye membranes, preilluminated with blue light, revealed substantial phosphorylation of metarhodopsin. This phosphorylation was strictly dependent on conversion of rhodopsin to metarhodopsin, as no phosphorylation of the photopigment took place in membranes that had been preilluminated with orange light (Fig. 1, lanes 1 and 2). The highest extent of metarhodopsin phosphorylation was obtained in the presence of the Ca^{2+} chelator EGTA, whereas Ca^{2+} considerably reduced the extent of metarhodopsin phosphorylation (Fig. 1, lanes 2 and 3). This effect was not dependent on calmodulin, as an efficient peptide inhibitor of calmodulin, M5 (21), did not change the extent of metarhodopsin phosphorylation (Fig. 1, lanes 3 and 4). Ca^{2+} was required for the dephosphorylation reaction, but the rate of dephosphorylation was much faster in membranes in which phosphorylated metarhodopsin had been first converted to phosphorylated rhodopsin by illumination with orange light and then subjected to dephosphorylation in the presence of Ca^{2+} (Fig. 2, lanes 2 and 4). Parallel assays were conducted on *Drosophila* eye preparations of the retinal degeneration C (rdgC) mutant. Recent cloning of the *rdgC* gene showed sequence similarity to mammalian serine/threonine protein phosphatases with an appended domain containing direct Ca^{2+} binding sites (24). Optimal dephosphorylation conditions resulted in removal of 95% of the phosphate from wild-type rhodopsin, whereas little if any dephosphorylation of the rdgC rhodopsin was observed (Fig. 2, lanes 1 and 1′), indicating that phosphorylated rhodopsin is a major substrate for the rdgC protein phosphatase. The much higher extent of 49-kDa arrestin phosphorylation in the rdgC preparation was due to higher content of Ca^{2+} in this preparation, as in the presence of 0.1 mM Ca^{2+} an equal extent of 49-kDa arrestin phosphorylation was obtained in both the wild-type and the rdgC preparations (data not shown). Because arrestin dephosphorylation in wild-type preparations was not Ca^{2+}-dependent (Fig. 2), it was not further studied.

Phosphorylation of Arrestin and Its Interaction with the Membrane. Phosphorylation of *Musca* eye cytosol derived from dark-adapted flies revealed Ca^{2+}-dependent phosphorylation of 39- and 49-kDa proteins that was blocked by the presence of excess EGTA. Addition of the calmodulin inhibitor M5 at 1 μM greatly diminished, and at 10 μM almost completely abolished, the phosphorylation of the 39- and 49-kDa proteins in the presence of Ca^{2+} (Fig. 3, lanes 3 and 4). Furthermore, addition of bovine calmodulin in excess

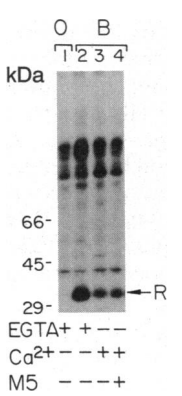

FIG. 1. Phosphorylation of *Drosophila* membrane proteins derived from eyes; illuminated with orange (O) or blue (B) light. Lanes in radiogram: 1, membranes of orange-illuminated eyes phosphorylated in the presence of 1 mM EGTA; 2–4, membranes of blue-illuminated eyes phosphorylated in the presence of 1 mM EGTA, or 0.1 mM Ca^{2+}, or 0.1 mM Ca^{2+} plus 10 μM M5, respectively. Arrow points to phosphorylated opsin. Opsin was identified on Western blot with monoclonal antibodies to *Drosophila* R1–6 opsin (data not shown).

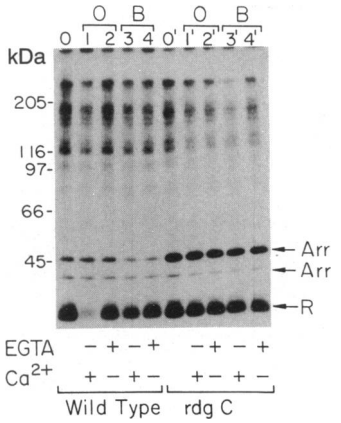

FIG. 2. Dephosphorylation of proteins in wild-type and rdgC eye membranes. Membranes were derived from blue-illuminated eyes and phosphorylated in the presence of 10 μM EGTA, followed by addition of unlabeled ATP to 0.5 mM and centrifugation. The membrane pellets were suspended in homogenization buffer and illuminated with orange (O) or blue (B) lights. Dephosphorylation was carried out for 5 min at 25°C, with the indicated additions. Lanes in radiogram: 0 and 0', phosphoproteins before dephosphorylation; 1, 1', 3, and 3', dephosphorylation in the presence of 0.1 mM Ca^{2+}; 2, 2', 4, and 4', dephosphorylation in the presence of 1 mM EGTA. Arr, arrestin; R, rhodopsin.

over M5 completely restored and even increased the phosphorylation of the 39- and 49-kDa proteins (Fig. 3, lane 5), indicating that the arrestins in the eye cytosol are phosphorylated *in vitro* by a Ca^{2+}/calmodulin dependent kinase. Phosphorylation of proteins in membranes derived from blue-illuminated *Musca* eyes revealed a phosphoprotein of 31 kDa corresponding to *Drosophila* R1–6 rhodopsin (Fig. 4*A*, lane 1), as well as phosphoproteins of 39 and 49 kDa. These proteins remained associated with the membrane upon subsequent illumination with blue light (Fig. 4*A*, lane 3). On the other hand, illumination with orange light, which regenerates all the photopigment into rhodopsin, resulted in an essentially quantitative release of the 49-kDa protein to the medium (Fig. 4*A*, lanes 2 and 4). The 49-kDa protein was the major phosphoprotein that was released into the medium upon illumination with orange light (Fig. 4*A*, lanes 4 and 5). Quantitation of the 49-kDa arrestin with antibodies raised against a peptide sequence of *Drosophila* 49-kDa arrestin showed that the 49-kDa arrestin was associated with membranes containing metarhodopsin and that it was quantitatively released into the medium upon regeneration of metarhodopsin to rhodopsin (Fig. 4*B*). Understanding the role of arrestin phosphorylation will require testing the eight possible combinations between phosphorylated and unphosphorylated rhodopsin, metarhodopsin, and arrestin.

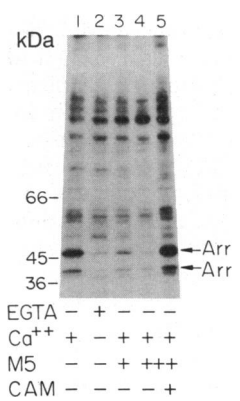

FIG. 3. Phosphorylation of *Drosophila* eye cytosol proteins, and inhibition by EGTA and the calmodulin-binding protein M5: Cytosol was derived from eyes illuminated with orange light (equivalent of 25 eyes per lane). Phosphorylation reaction mixtures contained the following additions: 0.1 mM Ca^{2+} (lane 1), 1 mM EGTA (lane 2), 0.1 mM Ca^{2+} plus 1 μM M5 (lane 3), 0.1 mM Ca^{2+} plus 10 μM M5 (lane 4), and 0.1 mM Ca^{2+} plus 1 μM M5 plus 2 μM bovine calmodulin (CAM) (lane 5).

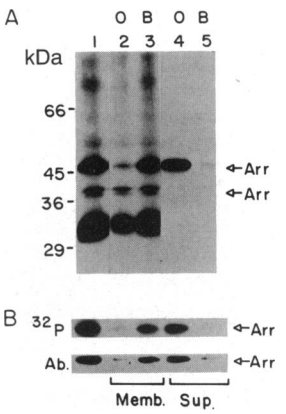

FIG. 4. Effect of illumination on the release of phosphorylated proteins from *Musca* eye membranes: (*A*) Membranes derived from blue-illuminated eyes were phosphorylated in the presence of 0.1 mM Ca^{2+}. At the end of the phosphorylation reaction, unlabeled ATP was added to 0.5 mM, and membranes were precipitated by centrifugation (14,000 × *g*, 10 min), suspended in 100 μl of homogenization buffer, and illuminated for 15 sec with orange (O) or blue (B) light. Membranes were separated from the supernatant by centrifugation (14,000 × *g*, 10 min) and each fraction was analyzed by SDS/PAGE and radiography. Lanes: 1, phosphorylated membranes before centrifugation; 2 and 3, membrane fractions obtained by centrifugation following illumination with orange or blue light, respectively; 4 and 5, supernatant fractions obtained by centrifugation following illumination with orange or blue light, respectively. Positions of arrestin (Arr) are indicated. (*B*) Phosphorylation, illumination, and separation of the membranes from the supernatant were carried out as described in *A*. Proteins separated by SDS/PAGE were electroblotted onto nitrocellulose paper and detected by radiography (^{32}P) or by antibodies to 49-kDa arrestin coupled with a chemiluminiscence detection system (Ab). Exposure for radiography was 16 hr, and for immunodetection 30 sec.

Arrestin Binding Inhibits Dephosphorylation of Metarhodopsin. As shown in Fig. 2, both an increase in free Ca^{2+} and regeneration of p-M to p-R are required to obtain substantial dephosphorylation of the photopigment. These findings raised the question whether the resistance of p-M to phosphatase activity is due to the metarhodopsin conformation or whether a protein component binds to p-M, thus making it inaccessible to the phosphatase. Indeed, it has been demonstrated that bovine arrestin inhibits dephosphorylation of freshly bleached bovine rhodopsin by protein phosphatase 2A. As the photolyzed rhodopsin becomes inactive, arrestin is released from the membrane, thus allowing the phosphatase to remove phosphate from the now inactive opsin (25). Incubation of p-M-containing, arrestin-depleted membranes in the presence of Ca^{2+} resulted in partial dephosphorylation of p-M with no significant difference from dephosphorylation of p-R (Fig. 5, lanes 6–9), indicating that once arrestin is removed, p-M becomes accessible to phosphatase activity. In contrast, in arrestin-containing membranes p-M was resistant to dephosphorylation (Fig. 5, lane 4).

Phosphorylation of Metarhodopsin and Binding of Arrestin to the Membrane Terminate Metarhodopsin Activity. Membranes depleted of or containing 49-kDa arrestin were prepared from eyes that had been illuminated with orange or blue light, respectively. To test the ability of metarhodopsin to sustain GTPase activity after illumination, each set of membranes was illuminated again at zero time, with blue light for 10 sec, followed by incubation in the dark in the presence of

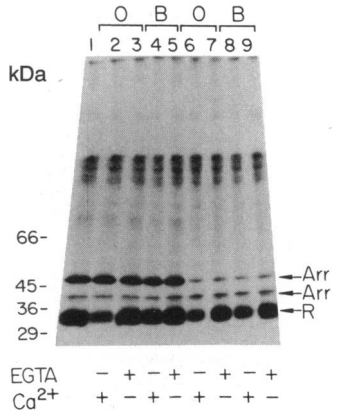

FIG. 5. Metarhodopsin in membranes depleted of arrestin is a substrate for dephosphorylation. *Musca* membranes were derived from blue-illuminated eyes, phosphorylated in the presence of 1 mM EGTA, and then subjected to illumination with orange (O) or blue (B) light followed by centrifugation to generate membranes depleted of (lanes 6–9) or containing (lanes 1–5) 49-kDa arrestin. Dephosphorylation was carried out in the presence of 0.1 mM $CaCl_2$ or 1 mM EGTA as indicated. Lane 1, phosphorylated membranes before dephosphorylation. Arr, arrestin; R, rhodopsin.

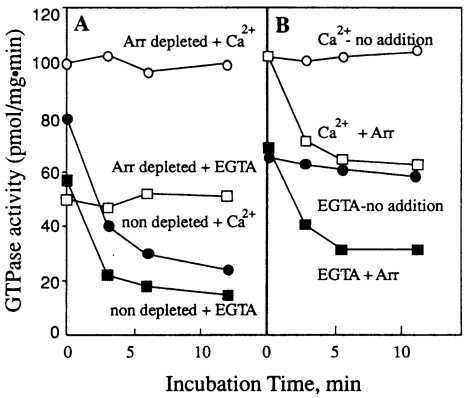

FIG. 6. Effect of 49-kDa arrestin on metarhodopsin-stimulated GTPase. At zero time, *Musca* eye membranes in homogenization buffer were illuminated for 10 sec with blue light, ATP was added to 0.5 mM, and the systems were incubated at 25°C in the dark. Where indicated, 0.1 mM Ca^{2+} or 1 mM EGTA was added together with ATP. At the times indicated, aliquots were taken for 1-min assay of GTPase activity at 0–4°C in the dark (26). (*A*) Open and filled symbols depict GTPase activities of membranes derived from eyes illuminated with orange (arrestin-depleted) and blue (nondepleted) light, respectively. Data are from a single experiment with similar results obtained in four other experiments. (*B*) Membranes derived from orange-illuminated *Musca* eyes were incubated with ATP together with 0.1 mM Ca^{2+} or 1 mM EGTA as in *A*. A partially purified arrestin was prepared by two rounds of release and binding to orange- and blue-illuminated membranes, respectively. The arrestin was estimated to be 90% pure by SDS/PAGE. Each GTPase assay mixture contained 10 μg of eye membrane proteins (equivalent to two eyes) and, when indicated, the amount of arrestin (Arr) added at zero time was derived from an equivalent number of eyes. Open symbols are GTPase activities of membranes incubated in the presence of 0.1 mM Ca^{2+} and filled symbols are GTPase activities of membranes incubated with 1 mM EGTA. Data are from a single experiment. Similar results were obtained in two other experiments with different preparations of arrestin.

MgATP. Assay of GTPase activity of these membranes in the dark gave a measure of the functional metarhodopsin in the system. Membranes depleted of 49-kDa arrestin showed an undiminished ability of metarhodopsin to catalyze GTPase activity. The GTPase activity was considerably higher in the presence of Ca^{2+} than in the presence of EGTA. In contrast, membranes that contained 49-kDa arrestin showed a progressive decline in the ability of metarhodopsin to sustain GTPase activity after a flash of blue light, with little difference between the systems incubated either in the presence or in the absence of Ca^{2+} (Fig. 6*A*). Addition of partially purified 49-kDa arrestin to eye membranes that were depleted of 49-kDa arrestin caused a progressive decrease in the ability of metarhodopsin to activate the G protein both in the presence and in the absence of Ca^{2+} (Fig. 6*B*).

DISCUSSION

Arrestin Secures the Fidelity of Phototransduction. The fly visual system accommodates both highly active rhodopsin kinase and phosphatase, yet inactivation of the photopigment goes to completion, and reversal of the inactivation reaction does not reinitiate transduction in the dark. The present results indicate that 49-kDa arrestin is the crucial component that prevents reinitiation of phototransduction in the dark, by inhibiting dephosphorylation of metarhodopsin. Inactivation of p-M by 49-kDa arrestin, however, is not a dead-end reaction. Once the p-M/49-kDa arrestin complex is hit by another photon, p-M regenerates back to p-R with concomitant release of 49-kDa arrestin. p-R then becomes an efficient substrate for rhodopsin phosphatase, which safely reintroduces it to the rhodopsin pool, ready for the next round of photoexcitation. A similar mechanism whereby binding of arrestin to the p-M2 intermediate protects it from dephosphorylation by phosphatase 2A has been described for vertebrate rods (25). However, in this system it is not known how the latent activity of phosphatase 2A is regulated nor is it clear whether dephosphorylation of the photopigment is important for the maintenance of the photoreceptor cell.

Disruption of the Termination Process. A most productive approach to genetic analysis of the *Drosophila* visual system stemmed from the observation that conversion of a large net amount of rhodopsin to metarhodopsin disrupts the termination of the transduction process. The functional consequence of this photoconversion is a prolonged depolarizing afterpotential (PDA) which far outlasts the light stimulus (33). With remarkable success this information was turned by Pak and colleagues (27) into a screening test for isolation of PDA mutants. Analysis of the PDA mutants revealed that reduction of the photopigment level in the photoreceptor cells, either by mutations in a number of different genes (27) or by vitamin A deprivation (28), completely eliminated the PDA response. The PDA response thus depends on the net amount of conversion, rather than on the fractional conversion, of rhodopsin to metarhodopsin. Taken together, these experiments are consistent with a mechanism in which a component that is present at a concentration lower than that of the photopigment inactivates an equivalent amount of metarhodopsin, leaving the excess metarhodopsin in a persistently active state. The present work corroborates this mechanism by demonstrating that membranes depleted of 49-kDa arrestin show undiminished GTPase activity after a flash of blue light, whereas membranes containing 49-kDa arrestin demonstrate a progressive decline in GTPase activity.

Relevance to Retinal Degeneration. Genetic analysis of the *Drosophila* mutant rdgC revealed that retinal degeneration is dependent on high levels of activated rhodopsin and placed the site of action of the *rdgC* gene product, before phospholipase C (29). More recently, molecular cloning of the *rdgC* gene showed sequence similarity with mammalian serine/threonine phosphatase and identified an appended domain containing putative direct Ca^{2+} binding sites (24). Our findings that phosphorylated rhodopsin is a major substrate for the rdgC phosphatase and that dephosphorylation of rhodopsin in wild-type flies requires Ca^{2+} but not calmodulin

(Figs. 1 and 2) are in perfect agreement with the above studies by O'Tousa and colleagues (24, 29). While the full range of substrates for the rdgC phosphatase has still to be established, these results suggest a similar mechanism of retinal degeneration in yet another *Drosophila* visual mutant. Studies of the no receptor potential A (norpA) mutant revealed that while *norpA* alleles at 2 days of age have close to normal rhodopsin levels, they later develop a substantial age-dependent decrease in rhodopsin concentration and retinal degeneration. Alleles with complete elimination of the receptor potential exhibit the largest decrease in rhodopsin levels and, astonishingly, this decrease was enhanced by exposure to light (30, 31). In view of the knowledge that norpA mutants are defective in an eye-specific phospholipase C (10) and the present findings of Ca^{2+} dependent dephosphorylation of rhodopsin, an analogy can be drawn between the norpA and the rdgC mutants. We suggest that in both mutants phosphorylation of the photopigment generated by photoconversion of rhodopsin to metarhodopsin is not adequately followed by dephosphorylation. In the rdgC mutant this is due to deficiency in rhodopsin phosphatase, and in the norpA mutant, due to a failure to generate the Ca^{2+} signal required for activation of rhodopsin phosphatase. Since arrestin phosphorylation does not take place in norpA mutants *in vivo* (19), excessive phosphorylation of arrestin as the cause of retinal degeneration of norpA can perhaps be excluded. Whether a protein phosphatase also plays a role in vertebrate retinal degeneration is unknown, nor is it known what are the physiological regulators of the latent protein phosphatase 2A that dephosphorylates rhodopsin. In view of the large number of mutations in the human opsin gene causing retinitis pigmentosa and the remarkable differences in the phenotypic expression among individuals afflicted by the same mutation (reviewed in ref. 32), the possibility that rhodopsin dephosphorylation may play a direct or indirect role in vertebrate retinal degeneration should be considered.

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