Protein Gq Modulates Termination of Phototransduction and Prevents Retinal Degeneration*§

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Background: Appropriate termination of photoresponse is critical for photoreceptors to achieve high temporal resolution and to prevent excessive Ca2+-induced cell toxicity.

Results: We isolated a novel Gαq mutant allele and revealed that metarhodopsin/Gq interaction affects Arr2-Rh1 binding.

Conclusion: Gq modulates the termination of phototransduction and prevents retinal degeneration.

Significance: Our study revealed the novel role of Gq in phototransduction deactivation and in retinal degeneration.

Appropriate termination of the phototransduction cascade is critical for photoreceptors to achieve high temporal resolution and to prevent excessive Ca2+-induced cell toxicity. Using a genetic screen to identify defective photoresponse mutants in Drosophila, we isolated and identified a novel Gαq mutant allele, which has defects in both activation and deactivation. We revealed that Gq modulates the termination of the light response and that metarhodopsin/Gq interaction affects subsequent arrestin-rhodopsin (Arr2-Rh1) binding, which mediates the deactivation of metarhodopsin. We further showed that the Gαq mutant undergoes light-dependent retinal degeneration, which is due to the slow accumulation of stable Arr2-Rh1 complexes. Our study revealed the roles of Gq in mediating photoresponse termination and in preventing retinal degeneration. This pathway may represent a general rapid feedback regulation of G protein-coupled receptor signaling.

Heterotrimeric G proteins play pivotal roles in mediating extracellular signals from hormones, neurotransmitters, peptides, as well as sensory stimuli to intracellular signaling pathways (1, 2). In Drosophila photoreceptors, G proteins are essential for the activation of the phototransduction cascade (3, 4). Photon absorption leads to the photoisomerization of chromophores, resulting in the formation of activated metarhodopsin. In turn, metarhodopsin activates heterotrimeric G proteins and PLC. The activation of PLC leads to transient receptor potential and transient receptor potential-like channels opening and extracellular Ca2+ influx (5–8).

It is also critical for each step of the phototransduction cascade to be terminated appropriately, which is essential for the high temporal resolution of fly vision (9, 10). The most important step in phototransduction termination is the deactivation of metarhodopsin. During this step, arrestin (Arr2) plays an important role by displacing the Gα subunit and allowing it to bind with rhodopsin (Rh1) (11, 12). Unlike other G protein-coupled receptors (GPCRs), the phosphorylation of fly rhodopsin is not required for its deactivation (13) but is essential for its endocytosis (11). In contrast, the dephosphorylation of rhodopsin by retinal degeneration C (RDGC) is essential for receptor deactivation (14). Ca2+ also plays critical roles in regulating the termination of the photopresponse in Drosophila (8, 15, 16). Several proteins that mediate this Ca2+-regulated termination have been identified, such as eye-specific protein kinase C (INAC), calmodulin, INAD, and myosin III (NINAC) (8, 17–20).

It has been shown that the loss of DGq leads to the slow termination of the light response (4). Here, we provide evidence that the metarhodopsin/Gq interaction affects subsequent Arr2-Rh1 binding, which mediates the deactivation of metarhodopsin. Our study has characterized the role of Gq in photoresponse termination. This pathway represents a general rapid feedback regulation in GPCR signaling.

EXPERIMENTAL PROCEDURES

Fly Genetics—The genotype of wild-type flies is cn,bw. sast mutants were generated with the chemical mutagen ethyl methanesulfonate (EMS) in cn,bw background, and all flies were put into cn,bw background to eliminate the effects of eye color. The mutant alleles used for each gene in this work are ninaE8, Gαq1, and norpA42. To avoid age-dependent retinal degeneration, all flies were reared at 22 °C in dark and examined when they were 2–3 days old. The Gαq mutant and p[hs: dGαq] transgenic fly were obtained from the C. Zuker laboratory, and all other flies used in this work were from the Bloomington Stock Center. The deficiency line Df(2R) Exel7121 was obtained from the Bloomington Stock Center and recombined into FRT 42D background. Genetic mosaics were induced by the FLP-FRT pl[GMR-hid] technique with an ey-FLP driver to generate mitotic clones of a single genotype in the eye (21).

Generation of Transgenic Flies—To generate Gαq1212A transgenic flies, a mutate dGαq cDNA, which mutate His1212 to Ala,
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was subcloned into pUAST vector and then injected into w^{1118} flies. The transgene was subsequently crossed into the G_{q\_sast}^{1} and G_{O_{q\_sast}}^{961} mutant background, respectively. G_{q\_sast}^{11212A} protein was expressed by using eye-specific driver (p{UAS:GAL4}).

**Electrophysiological Recordings**—Electroretinogram (ERG) recordings were performed as described previously (22). Briefly, fly eyes were stimulated with 5-s light pulses (4000 lux). For each genotype and condition, >10 flies were examined. To quantitate the speed of response termination, the time required for half-recovery was measured, and the S.E. was calculated. t\_{1/2} was defined as the time required for a half-recovery from the responses upon stimulation cessation. Prolonged depolarization after potentials were examined using the same setup except with different color filters and light intensities.

Relative light sensitivity measurement was carried out as described previously (23). For each genotype and condition, 10 flies were examined, and the relative sensitivities were averaged to obtain a mean. The S.E. were calculated and presented as error bars in the figures.

Intracellular recordings were performed as described previously (24). Briefly, a low resistance glass microelectrode filled with 2 M KCl was placed into a small hole on the compound eye. A reference electrode was filled with Ringer’s solution and inside the eye at the retina layer. The microelectrode placed on the eye was gradually inserted into the opening until light-induced membrane depolarization was observed. The signal was amplified and recorded using a Warner IE210 Intracellular Electrometer.

**Western Blotting**—Fly heads were homogenized in SDS-sample buffer, and the proteins were fractionated by SDS-PAGE and transferred to PVDF membranes (Pall) in Tris-glycine buffer, and the proteins were fractionated by SDS-PAGE and transferred to PVDF membranes (Pall) in Tris-glycine buffer. The blots were probed with mouse anti-Rh1 antibodies (1:3000 dilution, Developmental Studies Hybridoma Bank), rabbit anti-G\_B\_\_E antibodies (1:2000 dilution) (25), rabbit anti-G\_\_O\_\_q antibodies (1:2000 dilution, Calbiochem), rabbit anti-N-G\_\_O\_\_q antibodies (1:1000 dilution; Abcam), rabbit anti-Arr2 antibodies (1:1000 dilution), rabbit anti-INAD antibodies (1:1000 dilution). The blots were subsequently probed with either anti-rabbit, mouse IgG-peroxidase conjugate (GE Healthcare), and the signals were detected using ECL reagents (Amersham Biosciences).

**EM and Immunolocalizations**—Fly heads were immersed in 2.5% glutaraldehyde, 0.1 M sodium cacodylate (pH 7.2) at 4 °C for 12 h. After rinsing three times with 0.1 M sodium cacodylate, the fixed tissue was stained with 1% osmium tetroxide for 1 h at room temperature. A standard ethanol dehydration series was performed, and tissue was immersed in two 10-min washes of propylene oxide. The tissue was then embedded using standard procedures. For electron microscopy, thin sections (100 nm) were cut, collected on copper support grids, and stained with uranyl acetate followed by lead citrate staining. Micrographs were taken at 80 kV on Hitachi-7650.

For immunofluorescence staining of Rh1 and Arr2, fly heads were fixed with 4% paraformaldehyde in PBS, dehydrated with acetone, and embedded in LR White resin. One-micrometer sections were cut and double stained with anti-Rh1 antibody, 4CS (1:100, DSHB), and anti-Arr2 antibody (1:400).

**Arr2 Binding Assays**—Arr2 binding assays were performed as described previously with a minor modification (26). For each group and condition, eight adults were collected and adapted with food in complete dark for >2 h. After exposure for 60 s to pure blue light (480 ± 10 nm), fly heads were isolated by liquid nitrogen and homogenized in the dark. After centrifuging at 14,600 × g for 5 min, pellet and supernatant fractions were separated under very dim red light for Western blotting analysis. Arr2 release assays were performed in the same manner, except that the flies were exposed to 60 s of pure blue light followed by 2 min of pure orange light (580 ± 10 nm).

**RESULTS**

**Isolation and Identification of a novel G\_\_O\_\_q Mutant Allele**—To characterize the components of the phototransduction machinery, we performed an ERG-based chemical mutagenesis screen for additional genes in the fly. We isolated a mutant fly, sast (small amplification and slow termination), which shows small ERG responses (3.7 ± 0.3 mV versus 11.2 ± 0.4 mV) and slow on-transpose termination (t\_{1/2} = 1.02 ± 0.22 s versus t\_{1/2} = 0.06 ± 0.01 s) compared with wild-type flies (Fig. 1, A–C). In addition, sast mutant displays the reduced ON transient and OFF transient (Fig. 1, A and D). The light sensitivity of this mutant is also significantly reduced (Fig. 1E). Intracellular recording further revealed that the defective light response in sast mutants was due to an abnormality in photoreceptor cells (Fig. 1F).

To identify the specific mutation in sast flies, we first mapped the mutation to the 49B5–49B12 chromosomal region based on the ERG phenotype covered by the deficient chromosome Df(2R) Exel7121 (missing 49B5–10 to 49B12) (Fig. 2A). This genomic region contains 26 genes, including G\_\_O\_\_q\_B, which encodes the G\_\_O\_\_q protein. Because the mutant flies showed an ERG phenotype similar to that of the G\_\_O\_\_q\_sast mutants, the sast mutant might harbor a G\_\_O\_\_q mutant allele. The sast/G\_\_O\_\_q\_sast flies displayed an ERG phenotype similar to that of either the sast or G\_\_O\_\_q\_sast mutant (Fig. 2B), supporting this conclusion. A previous study showed that the G\_\_O\_\_q\_B gene encodes several DG\_\_q splice variants (27) and that DG\_\_q\_1 plays a predominant role in phototransduction in Drosophila (4). Using an antibody that recognizes DG\_\_q\_1, we revealed that the DG\_\_q\_1 protein is absent in sast mutants (Fig. 2C and supplemental Fig. 1). In contrast, other phototransduction proteins were present at normal levels (Fig. 2D). Subsequent DNA sequencing revealed that the sast mutant contains a C961T mutation in the G\_\_O\_\_q\_B gene (Fig. 2E). This mutation corresponds to a nonsense mutation (Arg117 to stop codon) in exon 4 (amino acids 153–196 of DG\_\_q\_1, Fig. 2E), which is not included in the DG\_\_q\_3 and DG\_\_q\_4 variants (27). To examine whether the sast mutant retains any truncated DG\_\_q\_1 protein, we performed Western blotting with an antibody against the N terminus of DG\_\_q\_1. We could not detect any truncated DG\_\_q\_1 protein, suggesting that the sast mutant is likely to possess one G\_\_O\_\_q\_1-null allele (Fig. 2F). Therefore, we named the sast mutant G\_\_O\_\_q\_sast.
Because \(G_{q}\) is a \(G_{q}\)-null mutant and \(DG_{q}^{1}\) plays a predominant role in phototransduction in \textit{Drosophila}, we worried whether the residual light response observed in the \(G_{q}\) mutant was evoked by either \(DG_{q}^{3}\) or \(DG_{q}^{4}\) variants. To detect \(DG_{q}^{3}\) and \(DG_{q}^{4}\) protein in both \(G_{q}\) and \(G_{q}\) mutants, we performed Western blotting by using the anti-\(G_{q}\) subunit antibody (371754; Calbiochem) that also recognizes \(DG_{q}^{3}\) and \(DG_{q}^{4}\) variants. Unfortunately, we did not detect any \(DG_{q}^{3}\) or \(DG_{q}^{4}\) in either \(G_{q}\) or \(G_{q}\) mutants even loading with 32 isolated retinas (data not shown). This observation implied that the expressions of \(DG_{q}^{3}\) or \(DG_{q}^{4}\) in the retina were extremely low and the residual light response in the \(G_{q}\) mutant might be attributable to other \(G\) protein genes, but not the \(G_{q}\)-null mutant. To further support this hypothesis, we generated clones of \(G_{q}\)-nulls in the retina through \textit{ey-FLP}\textit{-induced FRT} recombination in deficiency line, \textit{Df(2R)/Exel7121}, which deletes the whole \(G_{q}\) gene. This fly also showed small light response and slow termination ERG phenotype (supplemental Fig. 2). This observation indicates that the residual light response observed in the \(G_{q}\) mutant is contributed by other \(G\) protein, but not \(DG_{q}^{3}\) or \(DG_{q}^{4}\).

To further confirm that the absence of the \(DG_{q}^{1}\) protein is indeed responsible for the \(G_{q}\) mutant phenotype, we performed rescue experiments by expressing \(DG_{q}^{1}\) protein in a \(G_{q}\) mutant background. After induction of heat shock, the levels of the \(DG_{q}^{1}\) protein were recovered (Fig. 2G), and ERG responses were restored in \(G_{q}\)-null \(DG_{q}\) flies (Fig. 2H). These results illuminate that the loss of \(DG_{q}^{1}\) leads to light response defects in \(G_{q}\) flies.

\(DG_{q}\) is Essential for Deactivation of Photoreceptor—In the rescue experiments, we observed that the deactivation kinetics of the photoreceptor correlated with the available amount of \(DG_{q}\) protein. In \(G_{q}\)-null \(DG_{q}\) flies, \textit{in vivo} \(DG_{q}\) protein levels could be manipulated by controlling the time of heat shock. Within 30 min of heat shock at 37 °C, \(DG_{q}\) protein levels were partial recovered (14.2 ± 1.3%, Fig. 3A), accompanied by an increase in the termination speed (\(t_{1/2} = 0.47 ± 0.08\) s, Fig. 3B). Extended heat shock induced further increases in the levels of \(DG_{q}\) protein (22.4 ± 1.7%, Fig. 3A) and triggered rapid termination speed (\(t_{1/2} = 0.11 ± 0.01\) s, Fig. 3B). We excluded the possibility that the increased termination speed was due to the heat-shock treatment, because the heat-shock treatment did not change the protein level of \(DG_{q}\) and the termination speed in wild-type flies (Fig. 3B, right, and supplemental Fig. 3). These data suggest that termination speed depends on the available amount of the \(DG_{q}\) protein.

Previous work has shown that \(G_{q}\) mutates generate quantum bumps with prolonged latency (4). It is possible that the slow termination phenotype reflects a slow activation process. To test this hypothesis, we generated a variant \(DG_{q}\) transgenic fly with a mutated His212 to Ala, resulting in a loss of capacity of \(G_{q}\) to activate PLC (28). In \textit{cn},\(G_{q}\)-null \(DG_{q}\) flies, the levels of variant \(DG_{q}\) protein were comparable with the levels of \(G_{q}\) protein in wild-type flies (supplemental Fig. 4). Interestingly, deactivation kinetics, but not ERG amplitude, was nearly restored in this fly (Fig. 3C). These results indicate that the slow termination phenotype in \(G_{q}\) mutants is not due to delayed bump generation.

\(DG_{q}\) Mediates Deactivation of Metarhodopsin—During fly photoreceptor termination, metarhodopsin deactivation is the most important step (29). Thus, we first investigated whether \(DG_{q}\) was required for the deactivation of metarhodopsin. Prolonged depolarization afterpotential (PDA) is induced when the number of activated rhodopsin molecules exceeds the number of available regulators (29). In mutants lacking rhodopsin regulators, because lower amounts of rhodopsin need to be activated, the light intensity required to induce PDA is much lower than that in wild-type flies (14). The minimum light intensity...
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FIGURE 2. *sast* is a novel *Gq* mutant allele. A, *sast* over Df(2R)Euml7121 heterozygote (*sast/Def*) showing ERG responses similar to those of the *sast* homozygote. Event markers represent 5-s orange light pulses. B, *sast* over *Gq^t^*, heterozygote (*Gq^t^/sast*) showing ERG responses similar to those of the *sast* homozygote. C, Western blots of DGq protein levels in the *sast* mutant. Each lane was loaded with one-eighth head. Note that the upper weak bands are nonspecific. D, Western blots of protein levels of other visual molecules in the *sast* mutant. Each lane was loaded with one-quarter head. E, annotated splice variants of the *Gq49B* gene. The position of the point mutation in the *sast* allele is shown. The translation starts in each of the splice variants are marked with arrows. *, stop codon. F, Western blots of truncated DGq protein in the *sast* mutants. Each lane was loaded with three isolated retinas and detected with an antibody that specifically recognizes the N terminus of DGq. The flies were heat shocked at the late pupal stage by immersing fly vials in a 37 °C water bath for 1 h every day. Note that the upper band pointed with arrow is a nonspecific band. H, ERG recordings of light responses in *sast; p[hs:: dGq] flies*. Flies were treated as described in G.

needed to induce PDA was approximately 420 lux in *Gq^961q^* versus approximately 2,600 lux in wild-type and 2300 lux in *cn,Gq^961q^,bw;P[hs::Gq] flies* (Fig. 4, A and B). Therefore, this result suggests that the deactivation of metarhodopsin is impaired and that there is a shortage of rhodopsin regulator molecules in *Gq^961q^* mutant.

Because the deactivation of metarhodopsin is impaired in *Gq^961q^* mutants, we attempted to increase the termination speed of *Gq^961q^* photoresponses by reducing the level of Rh1, the major rhodopsin in all outer (R1–R6) photoreceptor cells. We genetically reduced Rh1 levels via the introduction of *ninaE^8^*, which contains <1% Rh1 protein (30), into a *Gq^961q^* background. Because much less Rh1 need to be deactivated, the impaired regulating machinery might be sufficient for the deactivation of rhodopsin, and the termination speed of the photoresponse should be faster in *Gq^961q^,ninaE^8^* double mutants compared with that in the *Gq^961q^* single mutant. Indeed, in *Gq^961q^,ninaE^8^* double mutants, the deactivation of the photoresponse was much faster than that in the *Gq^961q^* single mutant (Fig. 4, C and D), suggesting that DGq is required for the deactivation of metarhodopsin.

**Metarhodopsin/G_q Interaction Affects Arr2-Rh1 Binding**—Because Arr2 is the primary deactivator of rhodopsin through its binding with rhodopsin (11, 12), we next assessed whether G_q mediates the deactivation of metarhodopsin by affecting Arr2-Rh1 binding. To characterize the Arr2/Rh1 interaction, we used an arrestin pelleting assay (31, 32). Upon 30 s of blue light stimulation, >80% Arr2 binds to metarhodopsin in wild-type flies, whereas only 52% Arr2 binds to metarhodopsin in the *Gq^961q^* mutant (80.1 ± 3.2% versus 52.3 ± 4.3%, Fig. 5A, middle panel). Immunostaining results were consistent with those of

**FIGURE 3. DGq is required for termination of light response.** A, *Gq* protein levels in different treatments. *cn,Gq^961q^,bw;P[hs::Gq] adults reared at 21 °C were exposed to 30 min of heat shock at 37 °C (30 min) or 1 h of heat shock at 37 °C (60 min) or no heat shock (no hs). All flies were collected and analyzed at 24 h after heat shock. INAD was used as the loading control. Note that the upper bands are nonspecific bands. Quantification of relative DGq protein levels is shown on the right. B, ERG recordings showing termination kinetics of light responses in each treatment. *Gq* protein was induced as described in A. For all ERG traces, event markers represent 5-s orange light pulses, and the treatment conditions for each trace are marked. Quantification of the time required for half-recovery in each treatment, including wild-type controls, is shown on the right. C, representative ERG traces of *cn,Gq^961q^,bw and cn,Gq^961q^,bw;gmr^>^Gq^212A^ flies*. Quantification of the time required for half-recovery in each treatment is shown on the right. Error bars indicate S.E.
the arrestin pelleting assay (Fig. 5B). These observations suggest that DGq indeed affects Arr2-Rh1 binding.

Because Gq functions as a molecular switch on phototransduction cascade, we next investigated whether Gq affects Arr2-Rh1 binding by activating the phototransduction cascades. In cn,Ga961,bwgrm>Ga9612A flies, phototransduction cascade cannot be activated by the variant DGqH212A (Fig. 3C). Interestingly, the minimum light intensity needed to induce PDA in this fly (2200 ± 300 lux) was comparable with that in wild-type flies (2600 ± 200 lux) (Fig. 6A), reflecting the normal Arr2-Rh1 binding. An Arr2-binding assay further showed that Arr2-Rh1 binding is normal in these flies (Fig. 6B). Furthermore, Arr2-Rh1 binding is normal in norpA mutants, which also show defects in the activation of phototransduction (Fig. 6C) (33).

Taken together, these observations suggest that the metarhodopsin/Gq interaction, but not the activation of the phototransduction cascade, affects Arr2-Rh1 binding.

PLCβ, encoded by norpA, is the downstream effector of Gq (33). A loss of PLCβ results in the formation of stable Arr2-Rh1 complexes, which trigger retinal degeneration (32). Hence, we examined whether the same mechanism causes retinal degeneration in the Ga961 mutant. With 480-nm blue light exposure, Rh1 is photoconverted to metarhodopsin and induces binding with Arr2. Metarhodopsin can be photoconverted to inacti-

FIGURE 4. DGq mediates deactivation of metarhodopsin. A, representative recorded traces of PDA induced by 600 lux (low intensity) blue light. All flies were in white-eye background and reared in the dark. o, orange light; b, blue light. B, quantification of minimum light intensities required for PDA production in each genotype. C, representative ERG traces of cn,Ga961,bw and cn,Ga961,bw,ninaE double mutant flies. D, quantification of the time required for half-recovery in each genotype. Error bars, S.E.

FIGURE 5. Lack of Gq leads to the impaired Arr2-Rh1 binding. A, assessment of deficits in Arr2-Rh1 binding. Dark-adapted flies were exposed to blue light for 30, 60, or 90 s, respectively. Supernatant (S) and membrane pellet (P) fractions of fly heads were subjected to Western blotting. D, kept in dark; B, blue light exposure. Quantification of Arr2-Rh1 binding is shown on the right. B, immunostaining of Arr2 and Rh1 in different genotypes and under different conditions. Sections were prepared as described under “Experimental Procedures.” Quantification of Arr2 in rhabdomeres is shown on the right. The averaged data from three independent sections are shown. Error bars, S.E.
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vated rhodopsin, leading to the release of Arr2 (32). In wild-type flies, blue light exposure caused >80% binding between Arr2 and Rh1 and >80% release of Arr2 from Rh1 following exposure to orange light (Fig. 7, J and K). In contrast, in the mutant, blue light exposure only triggered approximate 48.5% binding between Arr2 and Rh1, and <60% release of Arr2 from Rh1 following exposure to orange light (Fig. 7, J and K). These data imply that the formation of stable Arr2-Rh1 complexes might trigger retinal degeneration in the Gαq mutant.

**DISCUSSION**

Our work isolated and identified a novel Gαq mutant allele and demonstrated that metarhodopsin/Gαq interaction affects subsequent Arr2-Rh1 binding, which might mediate the deactivation of metarhodopsin. Our study revealed the involvement of a general rapid feedback regulation in GPCR signaling.

**Gq Mediates Termination of Light Response**—Rapid termination of the light response is critical for fly vision to achieve high temporal resolution and to prevent excessive calcium-induced cell toxicity (9, 10). Using an ERG-based chemical mutagenesis screen, we isolated a novel Gαq mutant allele, Gαq 961, which shows defects in the termination of the light response. A similar phenotype has been reported in another Gαq mutant allele, Gαq 808 (4). Compared with the Gαq mutant, Gαq 961 showed even more severe defects in phototransduction deactivation. One explanation is that Gαq 961 mutants contain an even lower amount of Gq protein than that found in Gαq 808 mutants. Western blotting revealed that the Gαq 961 mutant is likely a null mutant. In contrast, Gαq 808 mutants produce approximately 1% of wild-type levels of the DGq protein (4). This explanation is further supported by rescue experiments, which showed that the termination kinetics depend on the amount of available Gq protein.

A previous study showed that the absence of Gq leads to the generation of quantum bumps with prolonged latency (4) because it takes longer for rare residual Gq to encounter metarhodopsin. In this study, we provided evidence that the slow termination phenotype of the Gαq mutants was not due to slow activation. Expression of DGq 1212A, which can bind metarhodopsin but cannot activate PLC, is nearly able to restore the
defects of photoresponse termination in a Gαq<sup>961</sup> mutant background. In Gαq<sup>961</sup>,G<sub>mr</sub>↑>G<sub>H122A</sub> flies, the termination speed of the photoresponse is slower than that in wild-type flies, which might be attributed to slow activation and impaired calcium-dependent receptor deactivation mechanisms (35).

Metarhodopsin/G<sub>q</sub> Interaction Affects Arr2-Rh1 Binding——Combined with genetic, biochemical, and electrophysiological approaches, we showed that metarhodopsin/G<sub>q</sub> interaction affects subsequent Arr2-Rh1 binding. Using the arrestin pelletting assay, we showed that more Arr2 binds to metarhodopsin in wild-type flies than that in the Gαq<sup>961</sup> mutant upon blue light stimulation (80.1 ± 3.2% versus 52.3 ± 4.3%). Although some of the Arr2 can still bind with Rh1 in the Gαq<sup>961</sup> mutant, the metarhodopsin/G<sub>q</sub> interaction clearly affects subsequent Arr2-Rh1 binding, which mediates the deactivation of photoreceptor. This conclusion is further supported by the observations in cn,Gαq<sup>961</sup>,bgwgrm>g>H<sub>222A</sub> flies. We showed the rapid deactivation kinetics of ERG, normal Arr2-Rh1 binding, and comparable minimum light intensity needed to induce PDA in this fly. This rapid feedback regulation does not need to activate the entire phototransduction cascade and allows the termination of photoreceptors with extremely fast kinetics, permitting adaptation in a huge dynamic range of light intensities (10, 34, 36). Such regulation also prevents excessive Ca<sup>2+</sup> influx and Ca<sup>2+</sup>-dependent excitotoxicity during intensive stimulation (37–40).

It is possible that the metarhodopsin/G<sub>q</sub> interaction exposes the binding site in metarhodopsin or shifts metarhodopsin into an intermediate metarhodopsin status, which facilitates Arr2-Rh1 binding. Using fluorescence pump probe and time-resolved fluorescence depolarization measurement approaches, it has been shown that helix 8 of rhodopsin undergoes dynamic changes at the receptor surface, which modulates arrestin-rhodopsin binding (41). In vertebrates, the phosphorylation of rhodopsin is important for β-arrestin/rhodopsin binding. GPCR kinase 2 has been shown to undergo selective binding with the G<sub>q</sub> α subunit (42). These results indicate that the rhodopsin/G<sub>q</sub> interaction might mediate arrestin-rhodopsin binding via the promotion of rhodopsin phosphorylation. However, this is not the case in the deactivation of the photoresponse in the fly because the phosphorylation of metarhodopsin is not required for Arr2-Rh1 binding in fly photoreceptors (14, 32, 43).

Gαq Mutant Undergoes Slow Retinal Degeneration——Mutations in almost any gene that functions during phototransduction trigger rapid retinal degeneration, except the Gαq<sup>961</sup> mutant, which undergoes slow retinal degeneration (34, 43, 44). In this study, we showed that the Gαq<sup>961</sup> mutant undergoes slow light-dependent retinal degeneration, similar to that observed in the Gαq<sup>961</sup> mutant (44). We provided evidence that mild retinal degeneration in the Gαq<sup>961</sup> mutant was due to the slow accumulation of stable Arr2-Rh1 complexes, which trigger retinal degeneration (32). Light stimulation triggers Ca<sup>2+</sup> influx and activates CaM kinase II, which phosphorylates Arr2 and results in Arr2 release from Rh1 (26, 45). In the Gαq<sup>961</sup> mutant, impaired Ca<sup>2+</sup> influx leads to the slow release of Arr2 from Rh1. On the other hand, lack of G<sub>q</sub> partially inhibits basal Rh1 endocytosis (24) and inhibits the translocation of Arr2 to rhabdomeres (46). These two opposite effects lead to the slow accumulation of stable Arr2-Rh1 complexes and trigger mild retinal degeneration.

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