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Protein G_q 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 Ca^{2+}-induced cell toxicity.
Results: We isolated a novel $G\alpha_q$ mutant allele and revealed that metarhodopsin/G_q interaction affects Arr2-Rh1 binding.
Conclusion: G_q modulates the termination of phototransduction and prevents retinal degeneration.
Significance: Our study revealed the novel role of G_q 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 Ca^{2+}-induced cell toxicity. Using a genetic screen to identify defective photoresponse mutants in *Drosophila*, we isolated and identified a novel $G\alpha_q$ mutant allele, which has defects in both activation and deactivation. We revealed that G_q modulates the termination of the light response and that metarhodopsin/G_q interaction affects subsequent arrestin-rhodopsin (Arr2-Rh1) binding, which mediates the deactivation of metarhodopsin. We further showed that the $G\alpha_q$ mutant undergoes light-dependent retinal degeneration, which is due to the slow accumulation of stable Arr2-Rh1 complexes. Our study revealed the roles of G_q 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.[2] The activation of PLC leads to transient receptor potential and transient receptor potential-like channels opening and extracellular Ca^{2+} 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 G_q α 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). Ca^{2+} also plays critical roles in regulating the termination of the photoresponse in *Drosophila* (8, 15, 16). Several proteins that mediate this Ca^{2+}-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 DG_q leads to the slow termination of the light response (4). Here, we provide evidence that the metarhodopsin/G_q interaction affects subsequent Arr2-Rh1 binding, which mediates the deactivation of metarhodopsin. Our study has characterized the role of G_q 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 $ninaE^8$, $G\alpha_q{}^1$, and $norpA^{24}$. 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 $G\alpha_q$ mutant and $p[hs::dG\alpha_q]$ 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 p[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 G_q^{H212A} transgenic flies, a mutate dG_q cDNA, which mutate His^{212} to Ala,

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[2] The abbreviations used are: PLC, phospholipase C; Arr2, arrestin; ERG, electroretinogram; GPCR, G protein-coupled receptor; INAD, inactivation no afterpotential D; PDA, depolarization afterpotential; Rh1, rhodopsin.

was subcloned into pUAST vector and then injected into w^{1118} flies. The transgene was subsequently crossed into the $G\alpha_q{}^{1}$ and $G\alpha_q{}^{961}$ mutant background, respectively. $G_q{}^{H212A}$ protein was expressed by using eye-specific driver (*p[gmr::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 afterpotentials 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. The blots were probed with mouse anti-Rh1 antibodies (1:3000 dilution, Developmental Studies Hybridoma Bank), rabbit anti-Gβe antibodies (1:2000 dilution) (25), rabbit anti-$G\alpha_q$ antibodies (1:2000 dilution, Calbiochem), rabbit anti-N-$G\alpha_q$ 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, 4C5 (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).

Statistics—For each quantitative analysis, the images of Western blots and immunostaining were quantified using ImageJ software. After averaging three sets of data, the mean values ± S.E. (as *error bars*) are presented.

RESULTS

Isolation and Identification of a novel $G\alpha_q$ 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 amplify and slow termination), which shows small ERG responses (3.7 ± 0.3 mV *versus* 11.2 ± 0.4 mV) and slow photoresponse 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. 1*E*). Intracellular recording further revealed that the defective light response in *sast* mutants was due to an abnormality in photoreceptor cells (Fig. 1*F*).

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. 2*A*). This genomic region contains 26 genes, including *Gα49B*, which encodes the G_q protein. Because the mutant flies showed an ERG phenotype similar to that of the $G\alpha_q{}^{1}$ mutants, the *sast* mutant might harbor a $G\alpha_q$ mutant allele. The *sast*/$G\alpha_q{}^{1}$ flies displayed an ERG phenotype similar to that of either the *sast* or $G\alpha_q{}^{1}$ mutant (Fig. 2*B*), supporting this conclusion.

A previous study showed that the *Gα49B* gene encodes several DGq 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. 2*C* and supplemental Fig. 1). In contrast, other phototransduction proteins were present at normal levels (Fig. 2*D*). Subsequent DNA sequencing revealed that the *sast* mutant contains a C961T mutation in the *Gα49B* gene (Fig. 2*E*). This mutation corresponds to a nonsense mutation (Arg^{117} to stop codon) in exon 4 (amino acids 153–196 of $DG_q{}^{1}$; Fig. 2*E*), 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 $G\alpha_q{}^{1}$-null allele (Fig. 2*F*). Therefore, we named the *sast* mutant $G\alpha_q{}^{961}$.

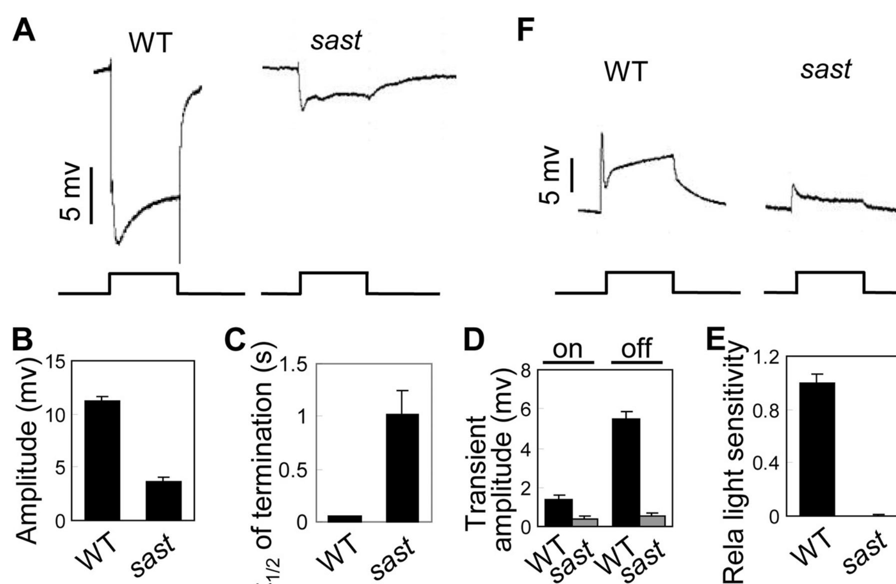

FIGURE 1. ***sast* mutants show abnormal light responses.** *A*, representative ERG traces of wild-type and *sast* mutant flies. For all ERG traces, event markers represent 5-s orange light pulses, and *scale bars* are 5 mV. *B*, quantification of ERG amplitudes for each genotype. *C*, quantification of termination speed. $t_{1/2}$ was calculated as described under "Experimental Procedures." *Error bars* indicate S.E. *D*, quantification of ON/OFF transient amplitudes. For each genotype, 10 flies were examined, and the ON/OFF transient amplitudes were averaged to obtain the mean. *E*, quantification of relative light sensitivities. The shown mean relative sensitivities were calculated as described under "Experimental Procedures." *Error bars* indicate S.E. *F*, intracellular recordings of light responses in photoreceptors. The *scale bar* and light pulse are 5 mV and 5 s, respectively.

Because $G\alpha_q^{961}$ is a $G\alpha_q^{1}$-null mutant and DG_q^{1} plays a predominant role in phototransduction in *Drosophila*, we worried whether the residual light response observed in the $G\alpha_q^{961}$ 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^{1} and $G\alpha_q^{961}$ mutants, we performed Western blotting by using the anti-G_q α 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^{1} and $G\alpha_q^{961}$ 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 $G\alpha_q^{961}$ mutant might be attributable to other G protein genes, but not the *Gq49B* gene. To further support this hypothesis, we generated clones of *Gα49B*-nulls in the retina through *ey-FLP*-induced *FRT* recombination in deficiency line, *Df(2R)Exel7121*, which deletes the whole *Gα49B* 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 $G\alpha_q^{961}$ 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 $G\alpha_q^{961}$ mutant phenotype, we performed rescue experiments by expressing DG_q^{1} protein in a $G\alpha_q^{961}$ mutant background. After induction of heat shock, the levels of the DG_q^{1} protein were recovered (Fig. 2*G*), and ERG responses were restored in $G\alpha_q^{961}$*;p[hs-dG*α_q*]* flies (Fig. 2*H*). These results illuminate that the loss of DG_q^{1} leads to light response defects in $G\alpha_q^{961}$ flies.

DG_q Is Essential for Deactivation of Photoresponse—In the rescue experiments, we observed that the deactivation kinetics of the photoresponse correlated with the available amount of DG_q protein. In $G\alpha_q^{961}$*;p[hs-dG*α_q*]* flies, *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. 3*A*), accompanied by an increase in the termination speed ($t_{1/2}$ = 0.47 ± 0.08, Fig. 3*B*). Extended heat shock induced further increases in the levels of DG_q protein (22.4 ± 1.7%, Fig. 3*A*) and triggered rapid termination ($t_{1/2}$ = 0.11 ± 0.01, Fig. 3*B*). 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. 3*B*, *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 $G\alpha_q^{1}$ mutants 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^{H212A} transgenic fly with a mutated His^{212} to Ala, resulting in a loss of capacity of G_q to activate PLC (28). In *cn,*$G\alpha_q^{961}$*,bw; gmr*>DG_q^{H212A} flies, the levels of variant DG_q^{H212A} 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. 3*C*). These results indicate that the slow termination phenotype in $G\alpha_q$ mutants is not due to delayed bump generation.

DG_q Mediates Deactivation of Metarhodopsin—During fly photoresponse 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

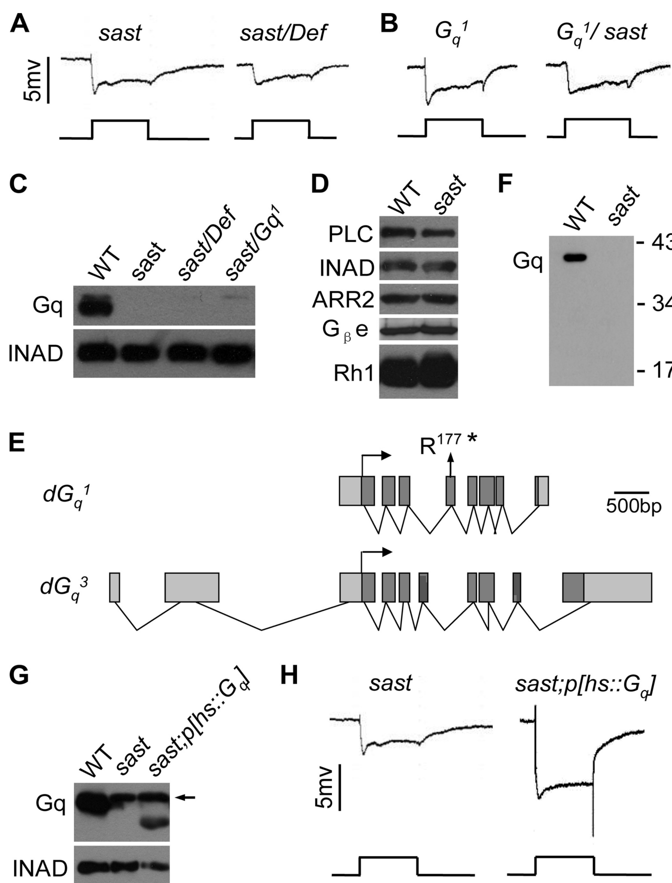

FIGURE 2. ***sast* is a novel $G\alpha_q$ mutant allele.** *A*, *sast* over Df(2R)Exel7121 heterozygote (*sast/Def*) showing ERG responses similar to those of the *sast* homozygote. Event markers represent 5-s orange light pulses. *B*, *sast* over $G\alpha_q{}^1$, heterozygote ($G\alpha_q{}^1$/*sast*) showing ERG responses similar to those of the *sast* homozygote. *C*, Western blots of DG_q 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 *Gα49B* 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 DG_q 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 DG_q. *G*, DG_q protein levels recovered in *sast;p[hs:: $G\alpha_q$]* flies. 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:: $dG\alpha_q$]* flies. Flies were treated as described in *G*.

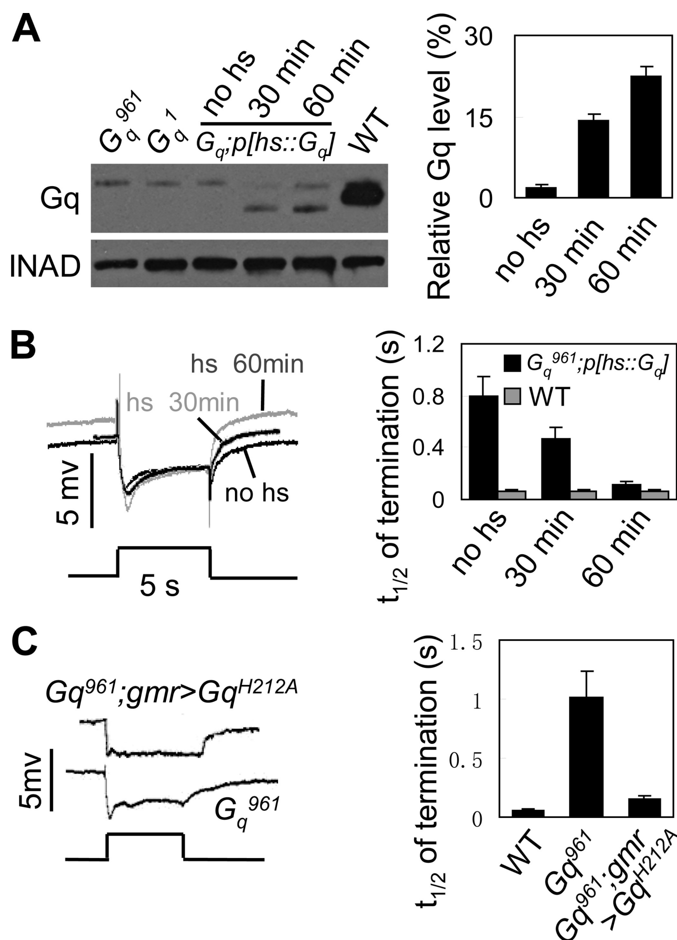

FIGURE 3. **DG_q is required for termination of light response.** *A*, G_q protein levels in different treatments. *cn,$G\alpha_q{}^{961}$,bw; P[hs::$dG\alpha_q$]* adults reared at 21 °C were exposed to 30 min of heat shock at 37 °C (30 min) or 1 h of heat shock at 37 °C (60 min) or no heat shock (*no 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 DG_q protein levels is shown on the *right*. *B*, ERG recordings showing termination kinetics of light responses in each treatment. G_q 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,$G\alpha_q{}^{961}$,bw* and *cn,$G\alpha_q{}^{961}$,bw;gmr>$G_q{}^{H212A}$* flies. Quantification of the time required for half-recovery in each treatment is shown on the *right*. *Error bars* indicate S.E.

needed to induce PDA was approximately 420 lux in $G\alpha_q{}^{961}$ *versus* approximately 2,600 lux in wild-type and 2300 lux in *cn,$G\alpha_q{}^{961}$,bw;P[hs::$G\alpha_q$]* 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 $G\alpha_q{}^{961}$ mutant.

Because the deactivation of metarhodopsin is impaired in $G\alpha_q{}^{961}$ mutants, we attempted to increase the termination speed of $G\alpha_q{}^{961}$ 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 $G\alpha_q{}^{961}$ 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 $G\alpha_q{}^{961}$;*$ninaE^8$* double mutants compared with that in the $G\alpha_q{}^{961}$ single mutant. Indeed, in $G\alpha_q{}^{961}$;*$ninaE^8$* double mutants, the deactivation of the photoresponse was much faster than that in the $G\alpha_q{}^{961}$ single mutant (Fig. 4, *C* and *D*), suggesting that DG_q 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 $G\alpha_q{}^{961}$ mutant (80.1 ± 3.2% *versus* 52.3 ± 4.3%, Fig. 5*A*, *middle panel*). Immunostaining results were consistent with those of

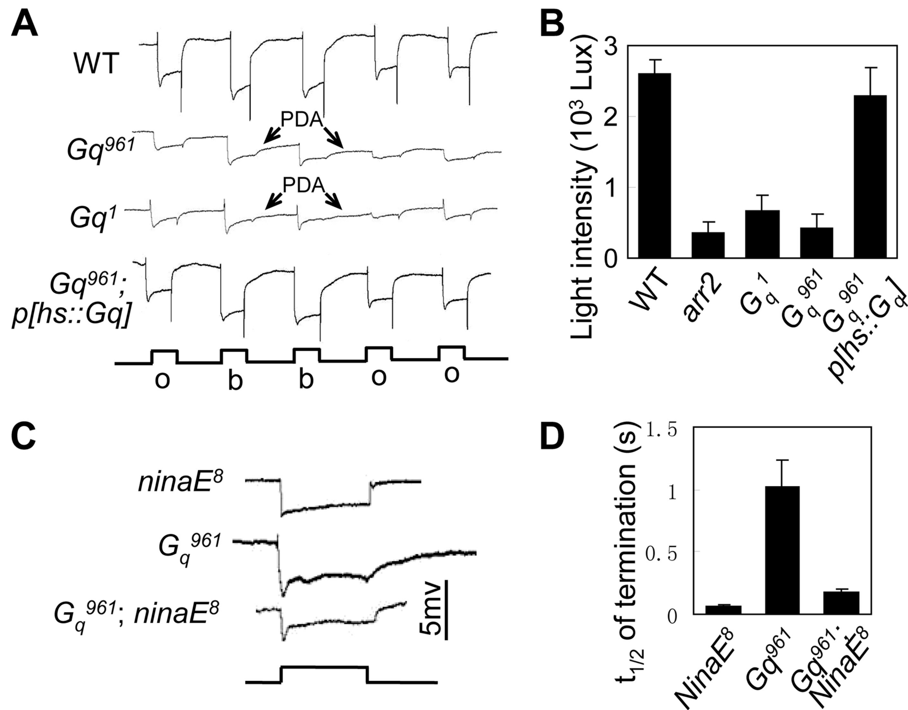

FIGURE 4. **DG_q mediates deactivation of metarhodopsin.** *A*, representative recorded traces of PDA induced by 600 lux (low intensity) blue light. All flies were in white-eye background and reared in the dark. *o*, orange light; *b*, blue light. *B*, quantification of minimum light intensities required for PDA production in each genotype. *C*, representative ERG traces of *cn,Gα$_q$^{961},bw* and *cn,Gα$_q$^{961},bw;ninaE^8* double mutant flies. *D*, quantification of the time required for half-recovery in each genotype. *Error bars*, S.E.

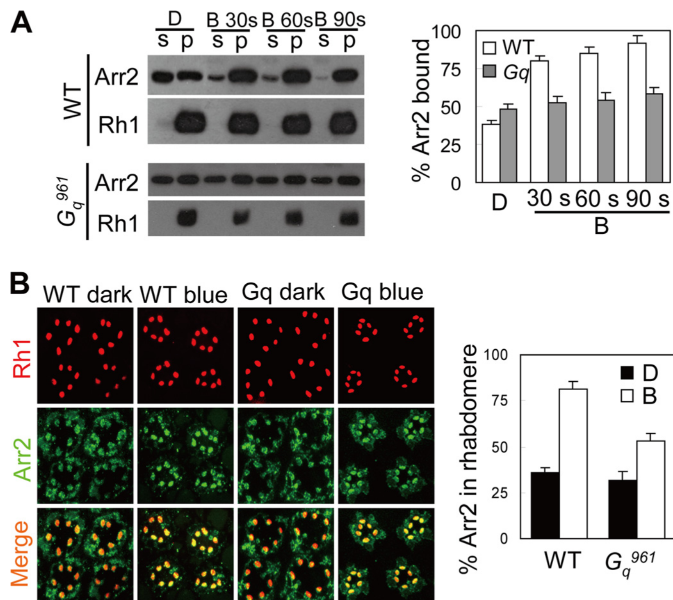

FIGURE 5. **Lack of G_q leads to the impaired Arr2-Rh1 binding.** *A*, assessment of deficits in Arr2-Rh1 binding. Dark-adapted flies were exposed to blue light for 30, 60, or 90 s, respectively. Supernatant (*S*) and membrane pellet (*P*) fractions of fly heads were subjected to Western blotting. *D*, 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.

the arrestin pelleting assay (Fig. 5*B*). These observations suggest that DG_q indeed affects Arr2-Rh1 binding.

Because G_q functions as a molecular switch on phototransduction cascade, we next investigated whether G_q affects Arr2-Rh1 binding by activating the phototransduction cascades. In *cn,Gα$_q$^{961},bw;grm>G$_q$^{H212A}* flies, phototransduction cascade cannot be activated by the variant DG_q^{H212A} (Fig. 3*C*). 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. 6*A*), reflecting the normal Arr2-Rh1 binding. An Arr2-binding assay further showed that Arr2-Rh1 binding is normal in these flies (Fig. 6*B*). Furthermore, Arr2-Rh1 binding is normal in *norpA* mutants, which also show defects in the activation of phototransduction (Fig. 6*C*) (33). Taken together, these observations suggest that the metarhodopsin/G_q interaction, but not the activation of the phototransduction cascade, affects Arr2-Rh1 binding.

Gα$_q$^{961} Mutant Undergoes Light-dependent Retinal Degeneration—In *Drosophila*, mutations in almost any gene involved in the phototransduction cascade lead to light-dependent retinal degeneration (34). Thus, we investigated whether the *Gα$_q$^{961}* mutant and *Gα$_q$^{1}* mutant also undergo retinal degeneration. An EM study showed obvious retinal degeneration in older mutants but not in 6-day-old mutants (Fig. 7, *A*, *B*, *D*, *E*, *G*, and *H*). Additionally, 21-day-old dark-reared mutants did not undergo retinal degeneration (Fig. 7, *C*, *F*, and *I*). These observations indicate that *Gα$_q$^{961}* mutants and the *Gα$_q$^{1}* mutant undergo slow light-dependent retinal degeneration.

PLC_{β}, encoded by *norpA*, is the downstream effector of G_q (33). A loss of PLC 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 *Gα$_q$^{961}* mutant. With 480-nm blue light exposure, Rh1 is photoconverted to metarhodopsin and induces binding with Arr2. Metarhodopsin can be photoconverted to inacti-

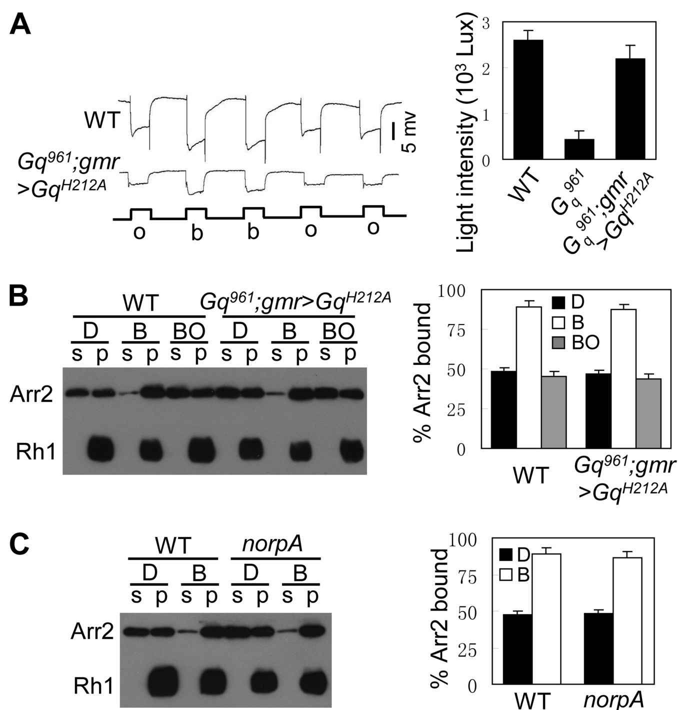

FIGURE 6. **Metarhodopsin/G_q interactions affect Arr2-Rh1 binding.** *A*, minimum light intensities required for PDA production in *cn,Gα_q^{961},bw; gmr>G_q^{H212A}* flies, comparable with that in wild-type flies. Representative recorded traces were induced by 2000 lux blue light. All flies were dark-reared. *o*, orange light; *b*, *blue light*. Quantification of minimum light intensities required for PDA production in each genotype is shown on the *right*. *B*, Arr2-Rh1 binding in *cn,Gα_q^{961},bw;gmr>G_q^{H212A}* flies. Arr2-Rh1 binding and Arr2 release assays were performed as described under "Experimental Procedures." Quantification of relative Arr2-Rh1 binding and release is shown on the *right*. *C*, Arr2-Rh1 binding in *norpA* mutants. Arr2-Rh1 binding assays were performed as described under "Experimental Procedures." Quantification of the relative Arr2-Rh1 binding is shown on the *right*. *Error bars*, S.E.

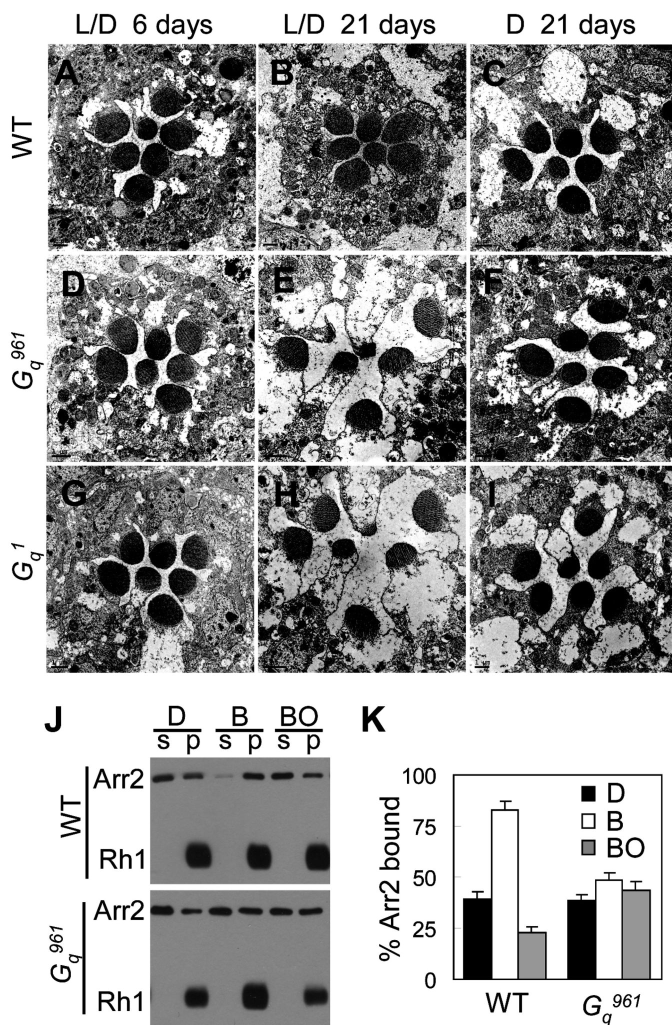

FIGURE 7. ***Gα_q^{961}* mutants undergo slow light-dependent retinal degeneration.** EM sections were prepared to assess retinal degeneration as described under "Experimental Procedures." Wild type (*A–C*), *cn,Gα_q^{961},bw* (*D–F*), and G_q^{1} (*G–I*) flies were raised for 6 (*A*, *D*, and *G*) or 21 days (*B*, *C*, *E*, *F*, *H*, and *I*). L/D flies (*A*, *B*, *D*, *E*, *G*, and *H*) were reared under 12-h light/12-h dark conditions, and D flies (*C*, *F*, and *I*) were kept in constant darkness. *Scale bars* of EM micrographs are 1 μm. *J*, Western blot of fractions of wild-type and *cn,Gα_q^{961},bw* fly heads. Arr2-Rh1 binding assays were performed as described under "Experimental Procedures." *K*, quantification of the percentage of Arr2 bound to rhodopsin-containing membranes in the dark (*D*), after treatment with blue light (*B*), or after treatment with blue light followed by orange light (*BO*). *Error bars*, S.E.

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 $G\alpha_q$ mutant.

DISCUSSION

Our work isolated and identified a novel $G\alpha_q$ mutant allele and demonstrated that metarhodopsin/G_q 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.

G_q 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 $G\alpha_q$ mutant allele, $G\alpha_q^{961}$, which shows defects in the termination of the light response. A similar phenotype has been reported in another $G\alpha_q$ mutant allele, $G\alpha_q^{1}$ (4). Compared with the $G\alpha_q^{1}$ mutant, $G\alpha_q^{961}$ showed even more severe defects in photoresponse deactivation. One explanation is that $G\alpha_q^{961}$ mutants contain an even lower amount of G_q protein than that found in $G\alpha_q^{1}$ mutants. Western blotting revealed that the $G\alpha_q^{961}$ mutant is likely a null mutant. In contrast, $G\alpha_q^{1}$ mutants produce approximately 1% of wild-type levels of the DG_q protein (4). This explanation is further supported by rescue experiments, which showed that the termination kinetics depend on the amount of available G_q protein.

A previous study showed that the absence of G_q leads to the generation of quantum bumps with prolonged latency (4) because it takes longer for rare residual G_q to encounter metarhodopsin. In this study, we provided evidence that the slow termination phenotype of the $G\alpha_q$ mutants was not due to slow activation. Expression of DG_q^{H212A}, which can bind metarhodopsin but cannot activate PLC, is nearly able to restore the

defects of photoresponse termination in a $G\alpha_q^{961}$ mutant background. In $G\alpha_q^{961}$;$gmr>G_q^{H212A}$ 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_q Interaction Affects Arr2-Rh1 Binding—Combined with genetic, biochemical, and electrophysiological approaches, we showed that metarhodopsin/G_q interaction affects subsequent Arr2-Rh1 binding. Using the arrestin pelleting assay, we showed that more Arr2 binds to metarhodopsin in wild-type flies than that in the $G\alpha_q^{961}$ mutant upon blue light stimulation (80.1 $\pm$ 3.2% *versus* 52.3 $\pm$ 4.3%). Although some of the Arr2 can still bind with Rh1 in the $G\alpha_q^{961}$ mutant, the metarhodopsin/G_q interaction clearly affects subsequent Arr2-Rh1 binding, which mediates the deactivation of photoresponse. This conclusion is further supported by the observations in *cn*,$G\alpha_q^{961}$,*bw*;*grm*$>G_q^{H212A}$ 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^{2+} influx and Ca^{2+}-dependent excitotoxicity during intensive stimulation (37–40).

It is possible that the metarhodopsin/G_q 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_q α subunit (42). These results indicate that the rhodopsin/G_q 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).

$G\alpha_q$ Mutant Undergoes Slow Retinal Degeneration—Mutations in almost any gene that functions during phototransduction trigger rapid retinal degeneration, except the $G\alpha_q^{1}$ mutant, which undergoes slow retinal degeneration (34, 43, 44). In this study, we showed that the $G\alpha_q^{961}$ mutant undergoes slow light-dependent retinal degeneration, similar to that observed in the $G\alpha_q^{1}$ mutant (44). We provided evidence that mild retinal degeneration in the $G\alpha_q^{961}$ mutant was due to the slow accumulation of stable Arr2-Rh1 complexes, which trigger retinal degeneration (32). Light stimulation triggers Ca^{2+} influx and activates CaM kinase II, which phosphorylates Arr2 and results in Arr2 release from Rh1 (26, 45). In the $G\alpha_q^{961}$ mutant, impaired Ca^{2+} influx leads to the slow release of Arr2 from Rh1. On the other hand, lack of G_q 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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