Gαq splice variants mediate phototransduction, rhodopsin synthesis, and retinal integrity in *Drosophila*

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Heterotrimeric G proteins mediate a variety of signaling processes by coupling G protein–coupled receptors to intracellular effector molecules. In *Drosophila,* the *Gαq* gene encodes several Gαq splice variants, with the Gαq1 isoform protein playing a major role in fly phototransduction. However, *Gαq1* null mutant flies still exhibit a residual light response, indicating that other Gαq splice variants or additional Gq α subunits are involved in phototransduction. Here, we isolated a mutant fly with no detectable light responses, decreased rhodopsin (Rh) levels, and rapid retinal degeneration. Using electrophysiological and genetic studies, biochemical assays, immunoblotting, real-time RT-PCR, and EM analysis, we found that mutations in the *Gαq* gene disrupt light responses and demonstrate that the Gαq3 isoform protein is responsible for the residual light response in *Gαq1* null mutants. Moreover, we report that Gαq3 mediates rhodopsin synthesis. Depletion of all Gαq splice variants led to rapid light-dependent retinal degeneration, due to the formation stable Rh1-arrestin 2 (Arr2) complexes. Our findings clarify essential roles of several different Gαq splice variants in phototransduction and retinal integrity in *Drosophila* and reveal that Gαq3 functions in rhodopsin synthesis.

Heterotrimeric G proteins and G protein–coupled receptors play pivotal roles in mediating a variety of extracellular signals to intracellular signaling pathways, such as hormones, neurotransmitters, peptides, and sensory stimuli (1, 2). In the *Drosophila* visual system, light stimulation activates the major rhodopsin (Rh1) to form metarhodopsin, which in turn activates heterotrimeric G proteins and *norpA* gene-encoded phospholipase C (PLCβ)[4] (3). Activated PLC catalyzes phosphatidylinositol 4,5-bisphosphate to generate diacylglycerol (DAG) and inositol 1,4,5-triphosphate (IP3) (4). IP3 induces the release of Ca^{2+} from intracellular Ca^{2+} stores, whereas both DAG and IP3 may trigger extracellular Ca^{2+} influx by opening transient receptor potential (Trp) and transient receptor potential-like (TrpL) channels on the cell membrane (5–8). The *Gαq* gene encodes several Gαq splice variants, among which the Gαq-RD variant generates the Gαq1 isoform protein, and other splice variants generate the Gαq3 isoform protein (9). Although both strong alleles of *norpA* and *trpl;trp* double mutants show completely abolished photoresponses (4, 10, 11), the *Gαq1* isoform null mutant allele ($Gαq^{961}$) still displays a residual light response (12). These data indicate that other Gαq splice variants, or the Gq α subunits encoded by additional genes, contribute to the residual light responses in *Gαq1* null mutants.

Intracellular Ca^{2+} homeostasis controlled by Gq signaling is also essential for photoreceptor cell survival (13). Mutations in phototransduction cascade components, such as those in *trp* and *norpA*, prevent normal light-induced Ca^{2+} influx, resulting in stable Rh1/Arr2 complex formation and severe rapid light-dependent retinal degeneration (14, 15). Disruption of stable Rh1/Arr2 complexes by genetic removal of Arr2 or suppression of Rh1 endocytosis can suppress the retinal degeneration either in *norpA* or *trp* mutant flies (15, 16). Rh1/Arr2 complex formation is thought to attribute to impaired Ca^{2+} influx-activated CaM kinase II, which usually phosphorylates Arr2 to release Arr2 from Rh1 (17, 18). However, neither $Gαq^{1}$ nor $Gαq^{961}$ mutants undergo rapid retinal degeneration (12, 19), exhibiting only slight retinal degeneration after keeping them in 12-h light/12-h dark cycles for 21 days (12). The disagreeing retinal degeneration phenotype between *Gαq* and *norpA* mutant is therefore unclear.

Here, we isolate a mutant fly with no detectable light responses and reveal that mutations in the *Gαq* gene cause the defective light responses. We demonstrate that Gαq3 is responsible for the residual light response in *Gαq1* null mutants and show that depletion of all Gαq splice variants results in rapid light-dependent retinal degeneration due to formation of stable Rh1/Arr2 complexes. In addition, we reveal that Gαq3 plays essential roles in Rh1 synthesis. Our study clarify the essential role of different Gαq splice variants in fly phototransduction, retinal degeneration, and rhodopsin synthesis.

Results

Isolation of a fly mutant with no detectable responses to light stimulation

To characterize the components of the phototransduction machinery, we obtained a collection of transgenic transposon

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[4] The abbreviations used are: PLC, phospholipase C; IP3, inositol 1,4,5-triphosphate; Trp, transient receptor potential; TrpL, transient receptor potential-like; DAG, diacylglycerol; ERG, electroretinogram.

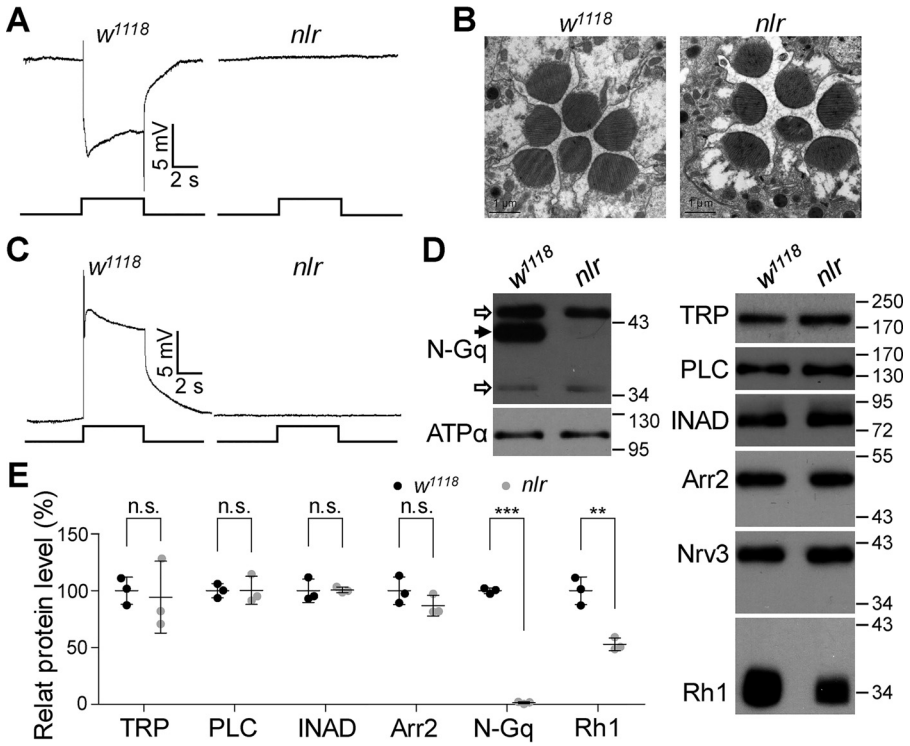

Figure 1. *nlr* mutants exhibit no response to light stimulation. *A,* representative ERG traces of WT and *nlr* mutant flies. For all ERG traces, event markers represent 5-s light pulses, and *scale bars* are 5 mV. *B,* EM images show a normal rhabdomeral structure in 1-day-old *nlr* mutant flies. *C,* intracellular recordings of light responses in photoreceptors. The *scale bar* and light pulse is 5 mV and 5 s, respectively. *D,* Western blots of Gαq protein levels recognized by anti-N-Gαq antibodies and other visual molecules in *nlr* mutants. Each lane was loaded with half of the isolated retina. The specific Gαq band is indicated with a *black arrow* and nonspecific band with *open arrows*. ATPα and Nrv3 were used as loading controls. *E,* quantification of protein levels of visual molecules in w^{1118} and *nlr* mutants.

p[GawB] homozygous viable strains and performed an electroretinogram (ERG)-based screen for additional genes in fly phototransduction. We isolated a mutant fly, which showed no detectable ERG responses to saturated light stimulations (Fig. 1*A*). Using the inverse PCR technique, we identified that the *p[GawB]* element was inserted into the 18C3 chromosomal region located on the X chromosome. To eliminate extra mutations in the genetic background, we backcrossed the mutant with the WT w^{1118} strain (based on the ERG phenotype) for eight generations and refer to the out-crossed mutant as *nlr* (no detectable light response) mutant. Unexpectedly, *nlr* mutants did not contain any *p[GawB]* element insertions, indicating the abolished ERG response in the *nlr* mutants is due to mutations in the genetic background, and not *p[GawB]* element insertion.

The significantly reduced ERG response in *nlr* mutants could be due to a defective rhabdomere structure or reflect deficits in the phototransduction cascade. To distinguish between these possibilities, we first performed an electron microscopy (EM) study to examine the rhabdomere structure of newly enclosed adult flies. However, EM images did not reveal any morphological defects in *nlr* mutant rhabdomeres (Fig. 1*B*). Intracellular recording found that light stimulation was unable to evoke any detectable responses in *nlr* mutant photoreceptors (Fig. 1*C*). These data indicated that the defective light response in *nlr* mutants was due to abnormalities in the phototransduction cascade. We performed Western blot analysis to examine the protein levels of components and regulators in the phototransduction cascade, including major rhodopsin (Rh1), Gαq, PLC, TRP, INAD, and Arr2. Interestingly, protein levels for all Gαq isoforms recognized by anti-Gαq-N antibodies were significantly reduced (100 ± 2.4 *versus* 1.4 ± 0.9%, $p < 0.0001$, *t* test; Fig. 1*D–E*). Meanwhile, a partial reduction of Rh1 (100 ± 12.2 *versus* 38.3 ± 5.5%, $p = 0.0014$, *t* test) was found in *nlr* mutants, whereas the other visual molecules examined were comparable with WT flies (Fig. 1*D–E*). These results suggest that the defective light responses in *nlr* mutants might be due to the absence of Gαq protein.

Mutations in Gαq gene are responsible for defective light responses in nlr mutants

To identify the mutations in *nlr* flies that are responsible for the defective light-response phenotype, we first mapped the mutations to the 49B5-49B12 chromosomal region based on the ERG phenotype covered by the deficient chromosome *Df(2R)Exel7121* (missing 49B5 to 49B12, Fig. 2, *A* and *B*). This result further supports the conclusion that the abnormal ERG phenotype in *nlr* mutants is not due to *p[GawB]* element insertion. Next, we further narrowed the mutation to the 49B8-49B10 region based on the ERG phenotype covered by the deficient chromosome *Df(2R)Gαq1.3*, which removes *Gαq*, *CG30054*, *CG17760*, *muskelin*, and part of the *CG33792* genes (Fig. 2, *A* and *B*). Next, we generated clones of *Df(2R)Gαq1.3*-covered gene nulls in the retina through *ey-FLP*-induced *FRT* recombination in the deficiency line, *Df(2R)Gαq1.3*. We found that this fly lacked Gαq protein and showed an abolished light response (Fig. 2, *C* and *D*), indicating that the defective light

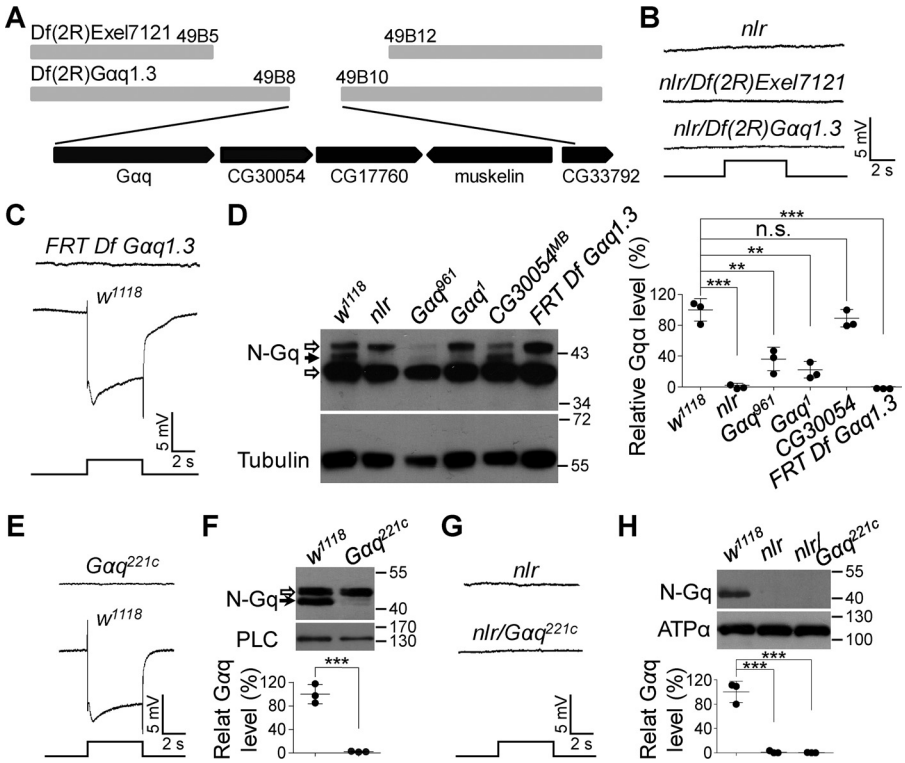

Figure 2. *Gαq* gene mutations cause the abolished light responses in *nlr* mutants. *A,* annotated deletion region of the deficient chromosome *Df(2R)Exel7121* and *Df(2R)Gαq1.3*. Annotated genes covered by *Df(2R)Gαq1.3* are presented in the *bottom panel*. *B, nlr* over either *Df(2R)Exel7121* (*nlr/Df(2R)Exel7121*) or *Df(2R)Gαq1.3* (*nlr/Df(2R)Gαq1.3*) show no photoresponses. Event markers represent 5-s light pulses, and *scale bars* are 5 mV. *C,* ERG response of the clones of *Df(2R)Gαq1.3*-covered gene nulls in the retina. *D,* Western blots of Gαq protein levels in the various mutants. Each lane was loaded with half of the isolated retina. $Gαq^{961}$ and $Gαq^{1}$ mutants were used as negative controls, whereas $CG30054^{MB}$ mutants were used as positive controls. The specific Gαq band and nonspecific bands are marked with a *black arrow* and *open arrows*, respectively. Quantification of Gαq levels for each genotype is presented in the *right panel*. *E,* ERG response of $Gαq^{221c}$ null mutant clones in the retina. Event markers represent 5-s light pulses, and *scale bars* are 5 mV. *F,* Western blots of Gαq protein levels in w^{1118} and $Gαq^{221c}$ null mutant photoreceptor cells. The specific Gαq band and nonspecific bands are marked with a *black arrow* and an *open arrow*, respectively. The eye-specific protein PLC was used as a loading control. Quantification of Gαq levels for each genotype is presented in the *lower panel*. *G, nlr/*$Gαq^{221c}$ flies show no photoresponses. Event markers represent 5-s light pulses, and *scale bars* are 5 mV. *H,* Western blots of Gαq protein levels in the various mutants. Each lane was loaded with half of the isolated retina. Quantification of Gαq levels for each genotype is presented in the *lower panel*.

response observed in *nlr* mutants is contributed to by the mutations located in the deficient chromosome *Df(2R)Gαq1.3*-covered region.

Among the genes covered by the deficient chromosome *Df(2R)Gαq1.3*, the *Gαq* gene plays an essential role in *Drosophila* phototransduction (12, 20). To test whether *Gαq* gene mutations contribute to the significantly reduced light responses in *nlr* mutants, we obtained a $Gαq^{221c}$ mutant allele that removes a 359-bp fragment around the translation start site of all Gαq splice variants (21). Because $Gαq^{221c}$ homozygous mutations are lethal, we generated $Gαq^{221c}$ null mutant clones in the retina through *ey-FLP*-induced *FRT* recombination. This fly was also absent of Gαq protein (100 ± 16.4 *versus* 2.1 ± 0.7%, $p = 0.0005$, *t* test) and displayed an abolished light response (Fig. 2, *E* and *F*). Next, we recombined *nlr* with $Gαq^{221c}$ mutant flies and found that *nlr/*$Gαq^{221c}$ flies showed no detectable responses to light stimulus and an absence of Gαq protein (Fig. 2, *G* and *H*). These data demonstrate that mutations in the *Gαq* gene are responsible for the defective light responses in *nlr* mutants.

Identification of new Gαq1 isoform mutation in nlr mutant

The *Gαq* gene encodes several *Gαq* splice variants (Fig. 3*A*), and the splice variant Gαq1 (also named as Gαq-PD, AAM68631) has been shown to play a major role in *Drosophila* phototransduction (12, 20). Thus, we wondered whether *nlr* mutants contain any mutations in the *Gαq* gene. Indeed, subsequent DNA sequencing revealed a mutation ($5501^{T/A}$) in exon 7 of the *Gαq* gene in *nlr* mutant flies (Fig. 3*A*), which is within the Gαq1 isoform but not included in other Gαq splice variants. This mutation corresponds to a missense mutation ($303^{V/D}$) in the GTPase domain of Gαq1 (amino acids 247–359).

To examine whether this mutation ($5501^{T/A}$) disrupts Gαq1 function, we combined the *nlr* mutant allele with a *Gαq* hypomorphic allele ($Gαq^{1}$), which changes a Gly to Ala in a splice acceptor site causing the use of a cryptic splice site 9 nucleotides downstream and an in-frame deletion of 3 codons encompassing amino acid residues 154–156 (20). Meanwhile, we combined the *nlr* mutant allele with a *Gαq1* isoform null mutant allele ($Gαq^{961}$), which contains a mutation ($961^{C/T}$) in exon 4 and causes a nonsense mutation (Arg-117 to stop codon) of *Gαq1* exclusively (12). Western blotting showed that Gαq protein levels were significantly reduced in both *nlr/*$Gαq^{1}$ and *nlr/*$Gαq^{961}$ flies (Fig. 3*B*). ERG recording further revealed that both *nlr/*$Gαq^{1}$ and *nlr/*$Gαq^{961}$ flies exhibited significantly reduced light responses to saturated light stimulation, simi-

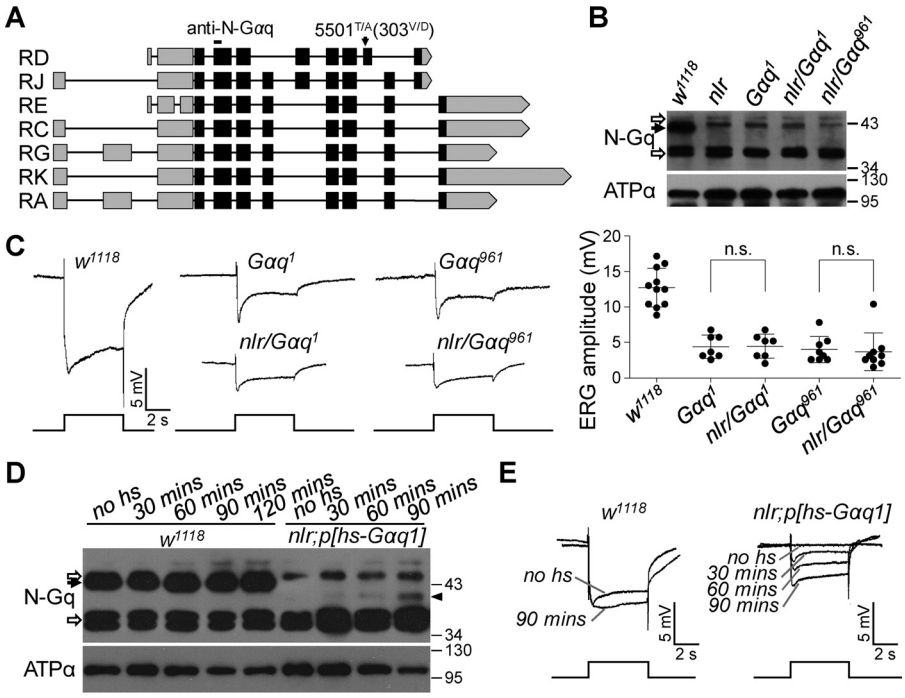

Figure 3. Gαq^{V303D} mutation of the Gαq1 isoform largely contributes to the abolished light response in *nlr* mutants. *A,* annotated transcripts of the *Gαq* gene. Position of the point mutation in the *nlr* allele is marked with a *black arrow*. The anti-N-Gαq antibody-recognized site is labeled at the *top*. *B,* Western blots of Gαq protein levels in each genotype. Each lane was loaded with half of the isolated retina. The specific Gαq band is indicated with a *black arrow* and nonspecific bands were indicated with *open arrows*. *C,* light response in *nlr*/*Gαq*^{1} and *nlr*/*Gαq*^{961} heterozygous flies. For all ERG traces, event markers represent 5-s light pulses, and *scale bars* are 5 mV. Quantification of ERG amplitudes for each genotype is presented in the *right panel*. *D,* Western blots show Gαq protein levels with different treatments. *nlr;P[HS::Gαq]* adults reared at 21 °C were exposed to different heat shock times at 37 °C, and all flies were collected and analyzed 24 h after heat shock. The specific Gαq band and nonspecific bands are marked with a *black arrow* and *open arrows*, respectively. *E,* ERG traces show light responses with different treatments. Gαq protein was induced as described in *D*. For all ERG traces, event markers represent 5-s light pulses, *scale bars* are 5 mV, and the treatment conditions for each trace are marked.

lar to that of either *Gαq*^{1} (Fig. 3*C*; 4.3 ± 1.6 *versus* 4.4 ± 1.7 mV, $p = 0.94$, *t* test) or *Gαq*^{961} (Fig. 3*C*; 4.0 ± 1.8 *versus* 3.7 ± 2.6 mV, $p = 0.77$, *t* test) flies. These results indicated that the 5501$^{T/A}$ *Gαq* gene mutation largely contributed to the abolished light response in the *nlr* mutant. Furthermore, the above data excluded the possibility that abolished light responses in *nlr* mutants were due to the dominant suppression of the Gαq^{V303D} mutant protein.

To further validate that the Gαq^{V303D} mutation in Gαq1 largely contributed to the abolished light response in *nlr* mutants, we obtained *p[HS::Gαq1]* transgenic flies and performed rescue experiments. Convincingly, Gαq1 expression largely restored the abolished ERG responses in *nlr* mutants (Fig. 3, *D* and *E*). Taken together, these data demonstrate that the 5501$^{T/A}$ *Gαq* gene mutation largely contributed to the abolished light response in *nlr* mutants and excludes the possibility that the abolished light responses in *nlr* mutants were due to the dominant suppression of Gαq^{V303D} mutant protein.

Gαq3 is responsible for residual light responses in Gαq1 isoform null mutants

Given that mutations in the *Gαq* gene are responsible for the abolished light responses in *nlr* mutants, and the *Gαq* gene encodes several *Gαq* splice variants, we suspected that other *Gαq* splice variants might contribute to the residual light responses in *Gαq1* null mutant flies. To test this hypothesis, we generated *Gαq*^{1}/*Gαq*^{221c} and *Gαq*^{961}/*Gαq*^{221c} flies, and found that although these flies responded to light stimulus, ERG amplitudes were smaller than that of either *Gαq*^{1} (Fig. 4*A*; 4.3 ± 1.6 *versus* 0.8 ± 0.4 mV, $p < 0.0001$, *t* test) or *Gαq*^{961} (Fig. 4*A*; 4.0 ± 1.8 *versus* 0.7 ± 0.3 mV, $p = 0.0008$, *t* test) flies. In *Drosophila*, splice variants Gαq-RE, Gαq-RC, Gαq-RG, Gαq-RK, and Gαq-RA encode the same Gαq3 isoform (Fig. 3*A*). These data indicate that the residual light response observed in *Gαq1* null mutants was contributed to by Gαq3.

To examine Gαq3 protein levels in *nlr* mutants, we conducted Western blot analysis using anti-Gαq-C antibodies that can recognize Gαq3 specifically. Protein levels of Gαq3 were significantly reduced in *nlr* mutants (Fig. 4*B*; 100 ± 21.8 *versus* 31.9 ± 18.7%, $p = 0.015$, *t* test), suggesting additional mutations in *nlr* mutants affect the expression of Gαq3 isoforms. Next, we conducted DNA sequencing in the whole *Gαq* gene region. We failed to identify additional mutations in Gαq3 isoform-coding regions of *nlr* mutant flies. However, in the promotor region of the *Gαq* gene in *nlr* mutants, we identified an 11-bp sequence insertion (GTTTTTCTAAC) at the −534 to −524 position that was absent in *w*^{1118} flies, as well as a mutation (2199$^{C/A}$) in an 11-bp sequence (2193–2203, CTAATTCGATT) conserved in the promoter region of several photoreceptor cell-specific genes (22, 23) (Fig. 4*C*). Moreover, qRT-PCR analysis validated that the mRNAs of Gαq3 isoforms, as well as Gαq1, were significant reduced in *nlr* mutants (Fig. 4*D*; Gαq1: 100 ± 1.0 *versus* 33.7 ± 2.4%, $p < 0.0001$, *t* test; Gαq3: 100 ± 3.3 *versus* 35.8 ± 7.0%, $p < 0.0001$, *t* test), demonstrating that these mutations in *nlr* mutants affect *Gαq* gene transcription.

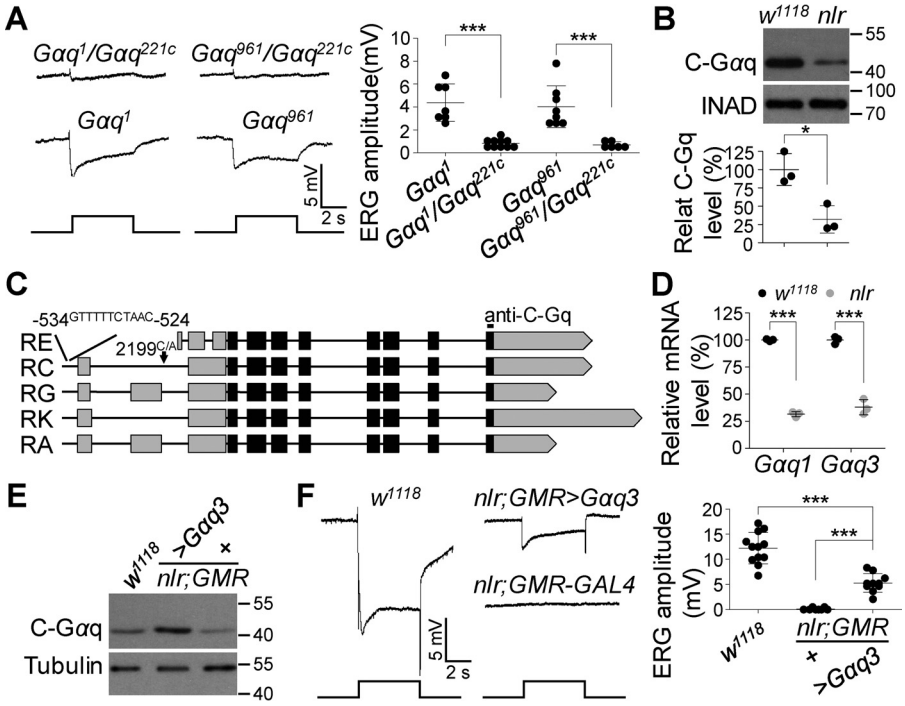

Figure 4. Gαq3 isoform contributes to the residual photoresponse in *Gαq1* isoform null mutants. *A,* representative ERG traces of $Gαq^{1}/Gαq^{221c}$ and $Gαq^{961}/Gαq^{221c}$ flies. For all ERG traces, event markers represent 5-s light pulses, and *scale bars* are 5 mV. Quantification of ERG amplitudes for each genotype is presented in the *right panel*. *B,* Western blots of Gαq3 protein levels in w^{1118} and *nlr* mutant photoreceptor cells. The eye-specific protein INAD was used as a loading control. Quantification of Gαq3 levels for each genotype is presented in the *lower panel*. *C,* additional mutations in the *Gαq* gene. The point mutation in the *nlr* allele is marked with a *black arrow*, and the sequence inserted upstream of the transcription start site is listed at the *top*. The anti-C-Gαq antibody-recognized site is labeled at the *top*. *D,* mRNA levels of *Gαq1* and *Gαq3* in w^{1118} and *nlr* mutant retina. Total RNA was extracted from isolated adult retina. Rp49 was used as an internal control. Data are presented as mean ± S.D. from three independent experiments. *E,* Western blots show Gαq3 protein levels in rescued flies. *F,* representative ERG traces of w^{1118}, *nlr;GMR-GAL4,* and *nlr;GMR-GAL4/UAS::Gαq3* flies. For all ERG traces, event markers represent 5-s light pulses, and *scale bars* are 5 mV. Quantification of ERG amplitudes for each genotype is presented in the *right panel*.

To further confirm that Gαq3 mediates phototransduction, we generated *p[UAS::Gαq3]* transgenic flies and performed rescue experiments. Consistently, Gαq3 expression in *nlr* mutants generated clear ERG responses (0.13 ± 0.2 *versus* 5.3 ± 1.8 mV, $p < 0.0001$, *t* test), and the amplitude of ERG responses was comparable with that in either $Gαq^{1}$ or $Gαq^{961}$ mutant flies (Fig. 4, *E* and *F*). Taken together, these data demonstrate that Gαq3 also mediates phototransduction, and further indicates that the residual light response observed in *Gαq1* null mutants is contributed to by Gαq3.

Depletion of all Gαq splice variants results in severe retinal degeneration

In *Drosophila*, mutations in genes that prevent normal Ca^{2+} influx after light stimulation, such as those in *trp* and *norpA*, usually cause stable Rh1/Arr2 complex formation and light-dependent retinal degeneration (13–15). In contrast, neither 6-day-old $Gαq^{1}$ nor $Gαq^{961}$ mutants show obvious retinal degeneration, and $Gαq^{961}$ mutants show only slight accumulation of stable Rh1/Arr2 complexes after light exposure (12, 19). The residual light responses in either $Gαq^{1}$ or $Gαq^{961}$ mutants might be able to trigger sufficient Ca^{2+} influx to activate CaM kinase II, which subsequently phosphorylates Arr2 to release Arr2 from Rh1 (17, 18). If true, stable Rh1/Arr2 complexes should accumulate with severe retinal degeneration after the depletion of all Gαq splice variants. Indeed, EM images revealed obvious retinal degeneration in 7-day-old *nlr* and *Df(2R)Gαq1.3*-covered gene null mutants raised under regular 12-h light/12-h dark cycles, but not in $Gαq^{1}$ mutants raised in the same conditions (Fig. 5*A*).

Next, we examined whether retinal degeneration in *nlr* mutants was due to the accumulation of stable Rh1/Arr2 complexes. With 480-nm blue light exposure, Rh1 is photoconverted to metarhodopsin and induces binding with Arr2. Metarhodopsin can be photoconverted to inactivated rhodopsin by an additional 580-nm orange light exposure, which leads to the release of Arr2 (15). In WT flies, blue light exposure caused about 74% binding between Arr2 and Rh1, and ~49% release of Arr2 from Rh1 following exposure to orange light (Fig. 5, *B* and *C*). In contrast, blue light exposure triggered approximately 88% binding between Arr2 and Rh1, and less than 32% release of Arr2 from Rh1 following exposure to orange light in *nlr* mutants (Fig. 5, *B* and *C*). These data imply that depleting all Gαq splice variants results in stable Rh1/Arr2 complexes accumulation.

To provide further support that stable Rh1/Arr2 complex formation triggers retinal degeneration in *nlr* mutants, we conducted an EM study in 7-day-old dark-reared *nlr* mutants. Interestingly, 7-day-old dark-reared *nlr* mutants displayed intact rhabdomere structures (Fig. 5*D*). Furthermore, genetic removal of *arr2* in the *nlr* mutant background suppressed retinal degeneration in *nlr* mutant flies (Fig. 5*E*). These data demonstrate that depleting all Gαq splice variants stabilizes Rh1/

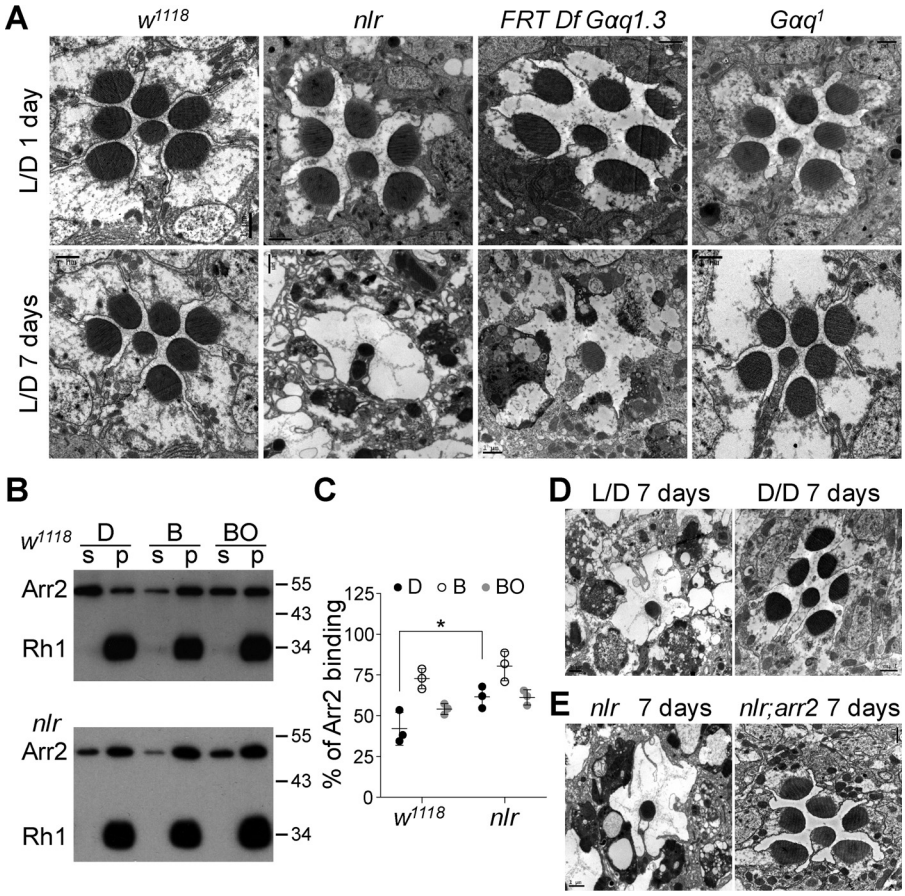

Figure 5. Depletion of all Gαq splicing variants leads to rapid retinal degeneration. *A,* EM analysis reveals *nlr* mutants and the clones of *Df(2R)Gαq1.3*-covered gene nulls underwent rapid retinal degeneration. Flies were raised in 12-h light/12-h dark conditions for the indicated time. Each picture shows a single ommatidium. *Scale bar*, 1 μm. *B,* Western blotting of fractions of w^{1118} and *nlr* mutant heads. Arr2-Rh1– binding assays were performed as described under "Experimental procedures." *C,* 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*). *D,* EM images show normal rhabdomeral structure in 7-day-old dark-reared *nlr* mutant flies. Flies were raised in constant darkness. *Scale bar*, 1 μm. *E,* EM images show normal rhabdomeral structure in 7-day-old *nlr;arr2* double mutants. Flies were raised in 12-h light/12-h dark conditions for 7 days. *Scale bar*, 1 μm.

Arr2 complex formation, triggering severe light-dependent retinal degeneration.

Gαq3 mediates Rh1 synthesis

Except for undetectable responses to light stimuli, 1-day-old *nlr* mutants also exhibited reduced Rh1 levels (Fig. 1, *D* and *E*; Fig. 6*A*). Convincingly, $Gαq^{221c}$ null mutant clones in the retina also showed a significant reduction in Rh1 levels (Fig. 6*B*; 100 ± 13.1 *versus* 42.4 ± 16.6%, $p = 0.0092$, *t* test). Furthermore, Western blot analysis showed that $nlr/Gαq^{221c}$ recombined flies displayed a significant reduction in Rh1 levels, whereas $Gαq^{1}$, $Gαq^{1}/Gαq^{221c}$, and $Gαq^{961}/Gαq^{221c}$ flies displayed a partial Rh1 reduction (Fig. 6*A*). These results indicate that other Gαq splice variants, but not Gαq1, largely contribute to Rh1 synthesis. Next, we carried out rescue experiments and found that Gαq3 expression in *nlr* mutants largely restored Rh1 levels (Fig. 6*C*; 73.1 ± 10.3 *versus* 39.1 ± 3.6%, $p = 0.0057$, *t* test). These data demonstrate that Gαq3 plays essential roles in Rh1 synthesis.

To explore the role of Gαq3 in regulating Rh1 synthesis, we monitored the distribution of Rh1 in developing photoreceptors. During pupal development, nascent Rh1 in WT pupae was gradually loaded into rhabdomeres, and most Rh1 was successfully loaded into the rhabdomeres by fly eclosion (Figs. 6*D*). In contrast, a large fraction of Rh1 remained in the cytoplasm in *nlr* mutant pupae (Fig. 6, *D* and *E*). These results indicate that proper loading of Rh1 into rhabdomeres requires Gαq3.

Discussion

Gαq3 isoforms mediate residual light responses in Gαq1 null mutants

In *Drosophila* photoreceptors, G proteins are essential to activate the phototransduction cascade (20, 24). The *Gαq* gene encodes several *Gαq* splice variants, and Gαq1 has been shown to function as the predominant G protein in fly phototransduction (12, 20). In this study, we identified a mutation ($5501^{T/A}$) in the *Gαq* gene, which specifically mutates Val to Asp at residue 303 in Gαq1 but not Gαq3 isoforms. Although Val is replaced with Ile at residue 303 in vertebrate Gαq proteins, the hydrophobicity at this position is evolutionally conserved. Structural analyses have shown that the Val-303 region localizes to the interface between Gα proteins and its downstream effector PLC (25–27). The change of a hydrophobic residue to a polar one may affect the interaction between these two proteins.

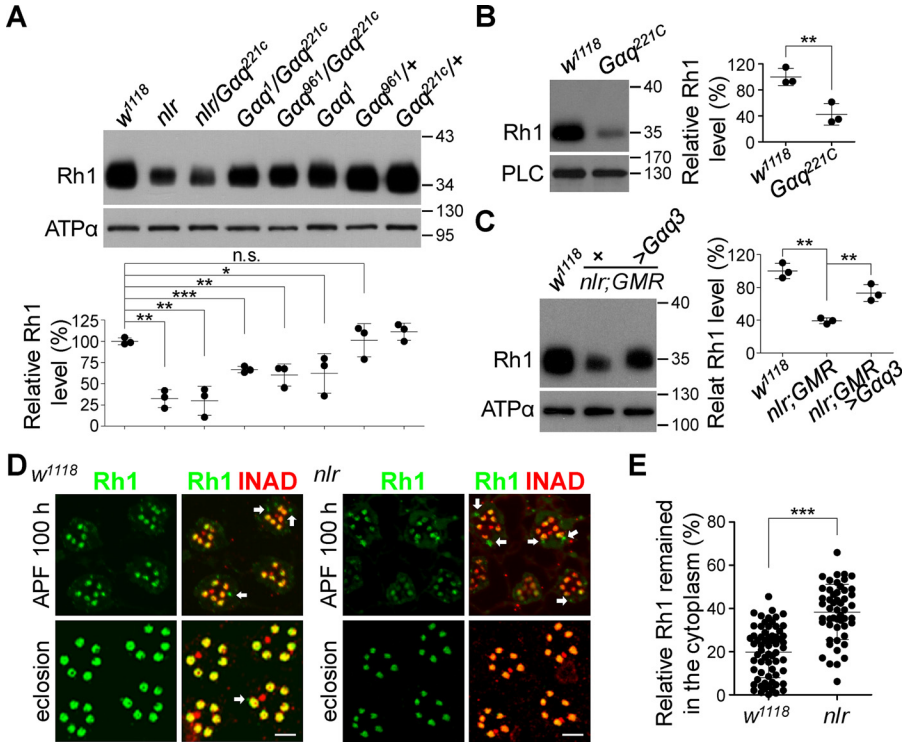

Figure 6. Gαq3 isoform regulates rhodopsin synthesis. *A,* Western blots of Rh1 levels in each genotype. Rh1 quantification for each genotype is presented in the *lower panel*. *B,* Western blots of Rh1 levels in w^{1118} and $Gαq^{221c}$ mutant flies. Rh1 quantification for each genotype is presented in the *right panel*. *C,* Western blots of Rh1 levels in w^{1118}, *nlr;GMR-GAL4,* and *nlr;GMR-GAL4/UAS::Gαq3* flies. Rh1 quantification for each genotype is presented in the *right panel*. *D,* Rh1 distribution in developing w^{1118} (*left*) and *nlr* mutant retina (*right*). Fly heads were collected at 100 h after pupal formation and eclosion, and sections were prepared and stained with anti-Rh1 (*green*) and anti-INAD (*red*) antibodies. The Rh1 signal remaining in the cytoplasm is indicated with *arrows*. *Scale bar*, 5 μm. *E,* quantification of Rh1 remaining in the cytoplasm at 100 h after pupal formation for each genotype.

A recent study has shown that the $Gαq^{V303D}$ mutant protein is unable to activate PLC *in vivo* (28).

Although the $5501^{T/A}$ *Gαq* gene mutation largely contributes to abolished light responses, this mutation is not fully responsible for the abolished light responses in *nlr* mutants because both *nlr/*$Gαq^{1}$ and *nlr/*$Gαq^{961}$ flies still exhibited a residual light response similar to $Gαq^{1}$ and $Gαq^{961}$ mutants (12). These data also excluded the possibility that the $Gαq^{V303D}$ mutant protein dominantly suppresses the function of Gαq protein. Gαq1 expression in *nlr* mutants largely recovers the light response, and further excluded the possibility that abolished light responses in *nlr* mutants are due to the dominant suppression of $Gαq^{V303D}$ mutant protein.

The *Gαq* gene encodes several *Gαq* splice variants, and $Gαq^{221c}$ mutants disrupt the expression of all Gαq splice variants (21). Our ERG recording revealed that $Gαq^{221c}$ null mutant clones showed no light responses. Previous whole-cell voltage-clamp recordings showed that the photoresponse of $Gαq^{1}$ homozygous cells is larger than that of $Gαq^{1}$ heterozygous cells (20). These results indicate that other *Gαq* splice variants might contribute to the residual light response in *Gαq1* null mutants. In this study, we demonstrate that Gαq3 contributes to the residual light response in *Gαq1* null mutants.

The *Gαq* gene encodes several *Gαq* splice variants. Originally, two cDNAs resulting from different *Gαq* gene splicing were isolated (23). These two cDNAs encode Gαq1 and Gαq2 isoform proteins, respectively. Functional studies demonstrated that Gαq1 mediates the light response, whereas Gαq2 has no effect on phototransduction (20). Subsequently, two additional *Gαq* splice variants were isolated (9). Now, seven total *Gαq* splice variants have been annotated in flybase, and these splice variants encode three different isoform proteins, including Gαq1, Gαq3, and Gαq4. In this study, we demonstrated that Gαq3 also mediate phototransduction. Overexpression of Gαq3 in *nlr* mutants induced detectable light responses but failed to fully restore the light response. Interestingly, the rescue flies exhibited comparable ERG trace amplitude and dynamics with that of $Gαq^{1}$ and $Gαq^{961}$ flies. These results indicate that different Gαq isoform proteins play different roles in phototransduction.

Gαq mediates retinal degeneration

Mutations in most genes encoding components of the phototransduction cascade result in rapid retinal degeneration, except for *Gαq* hypomorphic allele $Gαq^{1}$ and *Gαq1* isoform null mutant allele $Gαq^{961}$ (12, 13, 19, 29). Previous studies have shown that both $Gαq^{1}$ and $Gαq^{961}$ mutants underwent slow light-dependent retinal degeneration due to slow accumulation of stable Rh1/Arr2 complexes (12, 29). In these *Gαq* mutants, the small residual photoresponse may reduce Ca^{2+} influx, which partially activates CaM kinase II and leads to the slow release of Arr2 from Rh1. In this study, we showed that *nlr* mutants underwent rapid light-dependent retinal degeneration similar to that observed in *norpA* mutants (15, 30). We showed that disruption of stable Rh1/Arr2 complex formation prevented retinal degeneration in the mutants. Under normal con-

ditions, the interaction between Arr2 and Rh1 is transient, because light-triggered Ca^{2+} influx may activate CaM kinase II, which subsequently phosphorylates Arr2 to release Arr2 from Rh1 (17, 18). In *nlr* mutants, photoresponses were completely abolished so that the normal rise in Ca^{2+} after light stimulation was blocked, causing stable Rh1/Arr2 complex formation and retinal degeneration. These observations and explanations are consistent with mutations such as *trp* and *norpA*.

Gαq3 isoforms regulate Rh1 synthesis

In this study, we showed the first evidence that Gαq3 regulates Rh1 synthesis. Rh1 is transported to the plasma membrane by vesicular transport mechanisms regulated by a large number of trafficking proteins (31–35). Previous studies have shown that Gαq homologue CG30054 regulates inositol 1,4,5,-trisphosphate receptor (IP3R) to mediate calcium mobilization from intracellular stores and promote calcium-regulated secretory vesicle exocytosis (36). Given that Gαq3 shows high sequence identity to CG30054, they may regulate Rh1 synthesis through promoting calcium-regulated secretory vesicle exocytosis.

Experimental procedures

Fly genetics

p[GawB] strain collections obtained from Yi Rao's lab were originally generated by Ulrike Heberlein's lab (37) and *nlr* mutant flies were out-crossed for eight generations with the w^{1118} strain to eliminate the genetic background. The $G\alpha q^{1}$ mutant and *p[HS::dGαq]* transgenic fly were obtained from Charles S. Zuker's laboratory. All other flies used in this work were from Bloomington stock center. Genetic mosaics of the deficiency line *P{neoFRT}42D,Df(2R)Gαq1.3* and $G\alpha q^{221c}$ 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 (38). The genotype of WT flies is w^{1118} and the mutant alleles used for each gene in this work are $G\alpha q^{961}$, $G\alpha q^{1}$, and $arr2^{5}$. To avoid light-dependent retinal degeneration, all flies were reared at 22 °C in dark and examined at 1–2 days old.

Generation of transgenic flies

To generate *p[UAS::Gαq3]* transgenic flies, *Gαq3* cDNA was subcloned into the pUAST-attB vector and injected into y^{1},*w67c23;P{CaryattP2}* flies. The transgene was subsequently crossed into the *nlr* mutant background and proteins were specifically expressed in the eye using binary expression systems (39).

Antibodies

The Nrv3 antibody generated by GenScript (Nanjing, China) was raised in rabbits against GST-Nrv3 (96–176 amino acids) recombinant protein. The antibody was purified through an affinity column coupled with His-Nrv3 fusion protein. The sources of other antibodies were Developmental Studies Hybridoma Bank (Rh1, RRID: AB528451; ATPα, RRID: AB2166869), Millipore (C-Gαq, RRID: AB310221), Abcam (N-Gαq, ab190082), Sigma (tubulin, RRID: AB477593), Hongsheng Li (TRP), and Craig Montell (PLC).

Inverse PCR

Inverse PCR was performed as previously described (40). Briefly, genomic DNA was first digested with HpaII, and the resulting fragments were circulated using DNA ligase. The circular products were used as templates in PCR amplifications with primers flanking the 5′ end or 3′ end of the known *p*-element sequence. The PCR products were sequenced and aligned to the fly reference genome to locate *p*-element insertion sites.

Electrophysiological recordings

ERG recordings were performed as previously described (41). Briefly, recording and reference electrodes filled with Ringer's solution were placed on the surfaces of the fly eye and shoulder. Fly eyes were stimulated with 5-s light pulses (4000 Lux). For each genotype and condition, more than eight flies were examined. To quantitate the light response, the amplitude of ERG response was measured and the standard deviation was calculated.

Intracellular recordings were performed as previously described (42). 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 placed at the retina layer. The microelectrode placed on the eye was gradually inserted into the opening until light-induced membrane depolarization was observed. The signals were amplified and recorded using a Warner IE210 Intracellular Electrometer.

Western blots

After immersing fly heads in precooled phosphate-buffered saline (PBS), retinas were separated from brains with fine tweezers and homogenized in SDS-sample buffer. Proteins were fractionated by SDS-PAGE and transferred to polyvinylidene difluoride membranes (Pall) in Tris glycine buffer. Blots were probed with anti-Rh1 (1:3,000), anti-C-Gαq (1:1,000), anti-N-Gαq (1:1,000), anti-Arr2 (1:1,000), anti-INAD (1:1,000), anti-PLC (1:1,000), anti-TRP (1:1,000), anti-ATPα (1:2,000), anti-Nrv3 (1:2,500), and anti-tubulin (1:10,000) antibodies. Blots were subsequently probed with either anti-rabbit or anti-mouse IgG conjugated with peroxidase (GE Healthcare), and signals were detected using ECL reagents (Amersham Biosciences).

Real-time RT-PCR

Total RNA was extracted from isolated retina using TRIzol reagent (Invitrogen). Real-time RT-PCR was conducted using One-step SYBR Primer Script RT-PCR kit (TAKARA) with primer pairs for *Gαq1* (5′-GATCACGCTGCGGCCAAACA-3′/5′-GTTTTCGGTATCTGTGGC-3′), *Gαq3* (5′-CAGATTTAGAATGGTAGACG-3′/5′-CCTCCATTCGATTCTCATTATC-3′), and *rp49* (5′-AACGTTTACAAATGTGTATTCCGACC-3′/5′-ATGACCATCCGCCCAGCATACAGG-3′). Rp49 was used as an internal control, and relative mRNA levels were calculated by setting WT *Gαq1* and *Gαq3* mRNA levels as 100%.

EM

Electron microscopy (EM) was performed as described previously (43). Fly heads were immerged in 2.5% glutaraldehyde, 0.1 M sodium cacodylate (pH 7.2) at 4 °C for 12 h. After rinsing with 0.1 M sodium cacodylate three times, fixed fly heads were stained with 1% osmium tetroxide for 1 h at room temperature.

A series of standard ethanol dehydrations were conducted, and fly heads were immersed in two 10-min washes of propylene oxide. Fly heads were then embedded with standard procedures. For EM, 100-nm thin sections were cut and collected on copper support grids. After staining with uranyl acetate, sections were stained with lead citrate. Micrographs were taken at 80 KV on Hitachi-7650.

Arr2-binding assays

Arr2-binding assays were carried out as described previously (16). For each group and condition, 10 adult flies were collected and adapted in complete darkness for 2 h. After exposure with 60 s of pure blue light (480 ± 10 nm), fly heads were isolated by liquid nitrogen and homogenized in the dark. After centrifugation at 14,600 × *g* for 5 min, the pellet and supernatant were separated for Western blot analysis. Arr2-release assays were performed in the same manner, except that flies were exposed to pure blue light for 60 s, followed by pure orange light exposure for 2 min (580 ± 10 nm). All operations were conducted under very dim red light.

Experimental design and statistical analysis

Genetic studies and electrophysiological recordings on fly eyes were conducted to explore the function of Gαq splice variants and Gαq homology in phototransduction and Rh1 endocytosis. Biochemical studies and genetic studies on fly eyes, etc. were conducted to explore the detailed mechanism.

Western blotting was analyzed by ImageJ software (National Institute of Health, USA), and data from three independent experiments were averaged. Statistical analysis was performed using Prism 6.0 (GraphPad software). All data are presented as mean ± S.D. For quantification of ERG amplitudes, more than eight flies were recorded for each genotype and ERG amplitudes were averaged to obtain a mean. All data are presented as mean ± S.D. Two-tailed Student's *t* tests were used to compare between genotypes. Statistical significance was set as $p < 0.05$ (*), $p < 0.01$ (**), $p < 0.001$ (***), and no significance (n.s.).

Data availability

All data are contained within the manuscript.

Author contributions—Q. G. and J. W. data curation; Q. G. formal analysis; Q. G. and J. W. investigation; Y. T., S. C., Z. C. Z., and J. H. funding acquisition; Y. T., S. C., and Z. C. Z. methodology; Y. T., S. C., and Z. C. Z. writing-review and editing; J. H. conceptualization; J. H. supervision; J. H. writing-original draft; J. H. project administration.

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