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

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ABSTRACT

Heterotrimeric G proteins mediate a variety of signaling processes by coupling G protein-coupled receptors to intracellular effector molecules. In Drosophila, the Gaq gene encodes several Gaq splice variants, with the Gaq1 isoform protein playing a major role in fly phototransduction. However, Gaq1 null mutant flies still exhibit a residual light response, indicating that other Gaq 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 Gaq gene disrupt light responses and demonstrate that the Gaq3 isoform protein is responsible for the residual light response in Gaq1 null mutants. Moreover, we report that Gaq3 mediates rhodopsin synthesis. Depletion of all Gaq 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 Gaq splice variants in phototransduction and retinal integrity in Drosophila and reveal that Gaq3 functions in rhodopsin synthesis.
gene-encoded phospholipase C (PLCβ) (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 Ca²⁺ from intracellular Ca²⁺ stores, whereas both DAG and IP3 may trigger extracellular Ca²⁺ influx by opening transient receptor potential (Trp) and transient receptor potential-like (TrpL) channels on the cell membrane (5-8). The Gaq gene encodes several Gaq splice variants, among which the Gaq-RD variant generates Gaq1 isoform protein, and other splice variants generate Gaq3 isoform protein (9). Although both strong alleles of norpA and trpl;trp double mutants show completely abolished photoresponses (4,10,11), the Gaq1 null mutant allele (Gaq<sup>961</sup>) still displays a residual light response (12). These data indicate that other Gaq splice variants, or the Gq α subunits encoded by additional genes, contribute to the residual light responses in Gaq1 null mutants.

Intracellular Ca²⁺ 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 Ca²⁺ 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 Ca²⁺ influx-activated CaM kinase II, which usually phosphorylates Arr2 to release Arr2 from Rh1 (17,18). However, neither Gaq1 nor Gaq<sup>961</sup> 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 Gaq and norpA mutant is therefore unclear.

Here, we isolate a mutant fly with no detectable light responses and reveal that mutations in the Gaq gene cause the defective light responses. We demonstrate that Gaq3 is responsible for the residual light response in Gaq1 null mutants and show that depletion of all Gaq splice variants results in rapid light-dependent retinal degeneration due to formation of stable Rh1/Arr2 complexes. In addition, we reveal that Gaq3 plays essential roles in Rh1 synthesis. Our study clarifies the essential role of different Gaq 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 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 (Figure 1A). Using the inverse PCR technique, we identified that the p[GawB] element 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 wild-type w<sup>1118</sup> 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 nlr mutants is due to mutations in the genetic background, and not the 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 (Figure 1B). Intracellular recording found that light stimulation was unable to evoke any detectable responses in nlr mutant photoreceptors (Figure 1C). These data indicated that the defective light response in nlr mutants was due to abnormalities in the phototransduction cascade. We performed western blotting analysis to examine the protein levels of components and regulators in the phototransduction cascade, including major rhodopsin (Rh1), Gaq, PLC, TRP, INAD, and Arr2. Interestingly, protein levels for all Gaq isoforms recognized by anti-Gaq-N antibodies were significantly reduced (100 ± 2.4 vs. 1.4 ± 0.9, \( p < 0.0001 \), \( t \)-test; Figure 1D-E). Meanwhile, a partial reduction of Rh1 (100 ± 12.2 vs. 38.3 ± 5.5, \( p = 0.0014 \), \( t \)-test) was found in nlr mutants, while the other visual molecules examined were comparable to wild-type flies (Figure 1D-E). These results suggest that the defective light responses in nlr mutants might be due to the absence of Gaq protein.

**Mutations in Gaq 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, Figure 2A-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)Gaq1.3, which removes Gaq, CG30054, CG17760, muskelin, and part of the CG33792 genes (Figure 2A-B). Next, we generated clones of Df(2R)Gaq1.3-covered gene nulls in the retina through ey-FLP-induced FRT recombination. This fly was also absent of Gaq protein (100 ± 16.4 vs. 2.1 ± 0.7, \( p = 0.0005 \), \( t \)-test) and displayed an abolished light response (Figure 2E-F). Next, we recombined nlr with Gaq221c mutant flies and found that nlr/Gaq221c flies showed no detectable responses to light stimulus and an absence of Gaq protein (Figure 2G-H). These data demonstrate that mutations in Gaq gene are responsible for the defective light responses in nlr mutants.

**Identification of new Gaq1 isoform mutation in nlr mutant**

The Gaq gene encodes several Gaq splice variants (Figure 3A), and the splice variant Gaq1 (also named as Gaq-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 Gaq gene. Indeed, subsequent DNA sequencing revealed a mutation (5501 T/A) in exon 7 of the Gaq gene in nlr mutant flies (Figure 3A), which is within the Gaq1 isoform but not included in other Gaq splice variants. This mutation corresponds to a missense mutation (303V/D) in the GTPase domain of Gaq1 (amino acids 247–359).

To examine whether this mutation (5501 T/A) disrupts Gaq1 function, we combined the nlr mutant...
allele with a Gaq hypomorphic allele (Gaq'), which changes a G to A 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 Gaq1 isoform null mutant allele (Gaq961), which contains a mutation (961CT) in exon 4 and causes a nonsense mutation (Arg117 to stop codon) of Gaq1 exclusively (12). Western blotting showed that Gaq protein levels were significantly reduced in both nlr/Gaq' and nlr/Gaq961 flies (Figure 3B). ERG recording further revealed that both nlr/Gaq' and nlr/Gaq961 flies exhibited significantly reduced light responses to saturated light stimulation, similar to that of either Gaq1 (Figure 3C; 4.3 ± 1.6 vs. 4.4 ± 1.7 mV, p = 0.94, t-test) or Gaq61 (Figure 3C; 4.0 ± 1.8 vs. 3.7 ± 2.6 mV, p = 0.77, t-test) flies. These results indicated that the 5501 T/A allele with a gene mutation largely contributed to the abolished light responses in Gaq1 null mutants (Figure 4A; 4.0 ± 1.8 vs. 0.7 ± 0.3 mV, p = 0.0008, t-test) flies. In Drosophila, splice variants Gaq-RE, Gaq-RC, Gaq-RG, Gaq-RK, and Gaq-RA encode the same Gaq3 isoform (Figure 3A). These data indicate that the residual light response observed in Gaq1 null mutants is contributed to by Gaq3.

To further confirm Gaq3 mediates phototransduction, we generated western blotting analysis using anti-Gaq-C antibodies that can recognize Gaq3 specifically. Protein levels of Gaq3 were significantly reduced in both nlr mutants (Figure 4B; 100 ± 21.8 vs. 31.9 ± 18.7, p = 0.015, t-test), suggesting additional mutations in nlr mutants affect the expression of Gaq3 isoforms. Next, we conducted DNA sequencing in the whole Gaq gene region. We failed to identify additional mutations in Gaq3 isoform coding regions of nlr mutant flies. However, in the promoter region of the Gaq gene in nlr mutants, we identified an 11 bp sequence insertion (GTTTTTCT AAC) at the -534 to -524 position that was absent in w118 flies, as well as a mutation (2199C/A) in an 11 bp sequence (2,193–2,203, CTAAATTCGATT) conserved in the promoter region of several photoreceptor cell-specific genes (22,23) (Figure 4C). Moreover, qRT-PCR analysis validated that the mRNAs of Gaq3 isoforms, as well as Gaq1, were significant reduced in nlr mutants (Figure 4D; Gaq1: 100 ±1.0 vs. 33.7 ± 2.4, p < 0.0001, t-test; Gaq3: 100 ± 3.3 vs. 35.8 ± 7.0, p < 0.0001, t-test), demonstrating that these mutations in nlr mutants affect Gaq gene transcription.

To further confirm Gaq3 mediates phototransduction, we generated p[UAS::Gaq3] transgenic flies and performed rescue experiments.
Consistently, Gaq3 expression in nlr mutants generated clear ERG responses (0.13 ± 0.2 vs. 5.3 ± 1.8 mV, p < 0.0001, t-test), and the amplitude of ERG responses was comparable with that in either Gaq1 or Gaq961 mutant flies (Figure 4E-F). Taken together, these data demonstrate that Gaq3 also mediates phototransduction, and further indicate that the residual light response observed in Gaq1 null mutants is contributed to by Gaq3.

Depletion of all Gaq 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 Gaq1 nor Gaq961 mutants show obvious retinal degeneration, and Gaq961 mutants show only slight accumulation of stable Rh1/Arr2 complexes after light exposure (12,19). The residual light responses in either Gaq1 or Gaq961 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 Gaq splice variants. Indeed, EM images revealed obvious retinal degeneration in 7-day-old nlr and Df(2R)/Gaq1.3-covered gene null mutants raised under regular 12 h light/12 h dark cycles, but not in Gaq1 mutants raised in the same conditions (Figure 5A).

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 wild-type flies, blue light exposure caused about 74% binding between Arr2 and Rh1, and approximately 49% release of Arr2 from Rh1 following exposure to orange light (Figure 5B-C). In contrast, blue light exposure triggered approximate 88% binding between Arr2 and Rh1, and less than 32% release of Arr2 from Rh1 following exposure to orange light in nlr mutants (Figure 5B-C). These data imply that depleting all Gaq 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 (Figure 5D). Furthermore, genetic removal of arr2 in the nlr mutant background suppressed retinal degeneration in nlr mutant flies (Figure 5E). These data demonstrate that depleting all Gaq splice variants stabilizes Rh1/Arr2 complex formation, triggering severe light-dependent retinal degeneration.

Gaq3 mediates Rh1 synthesis

Except for undetectable responses to light stimuli, 1-day-old nlr mutants also exhibited reduced Rh1 levels (Figure 1D-E; Figure 6A). Convincingly, Gaq221c null mutant clones in the retina also showed a significant reduction in Rh1 levels (Figure 6B; 100 ± 13.1 vs. 42.4 ± 16.6 %, p = 0.0092, t-test). Furthermore, western blotting analysis showed that nlr/Gaq221c recombined flies displayed a significant reduction in Rh1 levels, whereas Gaq1, Gaq1/Gaq221c, and Gaq961/Gaq221c flies displayed a partial Rh1 reduction (Figure 6A). These results indicate that other Gaq splice variants, but not Gaq1, largely contribute to Rh1 synthesis. Next, we carried out rescue experiments and found that Gaq3 expression in nlr mutants largely restored Rh1 levels (Figure 6C; 73.1 ± 10.3 vs. 39.1 ± 3.6 mV, p = 0.0057, t-test). These data demonstrate that Gaq3 plays essential roles in Rh1 synthesis.

To explore the role of Gaq3 in regulating Rh1
synthesis, we monitored the distribution of Rh1 in developing photoreceptors. During pupal development, nascent Rh1 in wild-type pupae was gradually loaded into rhabdomeres, and most Rh1 was successfully loaded into the rhabdomeres by fly eclosion (Figures 6D). In contrast, a large fraction of Rh1 remained in the cytoplasm in nlr mutant pupae (Figure 6D-E). These results indicate that proper loading of Rh1 into rhabdomeres requires Gaq3.

**DISCUSSION**

*Gaq3 isoforms mediate residual light responses in Gaq1 null mutants*

In *Drosophila* photoreceptors, G proteins are essential to activate the phototransduction cascade (20,24). The *Gaq* gene encodes several *Gaq* splice variants, and *Gaq1* has been shown to function as the predominant G protein in fly phototransduction (12,20). In this study, we identified a mutation (5501T/A) in the *Gaq* gene, which specifically mutates Val to Asp at residue 303 in *Gaq1* but not *Gaq3* isoforms. Although Val is replaced with Ile at residue 303 in vertebrate *Gaq* proteins, the hydrophobicity at this position is evolutionally conserved. Structural analyses have shown that the V303 region localizes to the interface between G 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. A recent study has shown that *Gaq*V303D mutant protein is unable to activate PLC in vivo (28).

Although the 5501T/A *Gaq* 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/Gaq1 and nlr/Gaq961 flies still exhibited a residual light response similar to *Gaq1* and *Gaq961* mutants (12). These data also excluded the possibility that *Gaq*V303D mutant protein dominantly suppresses the function of *Gaq* protein. *Gaq1* expression in *nlr* mutants largely recovers the light response, further excluded the possibility that abolished light responses in *nlr* mutants are due to the dominant suppression of *Gaq*V303D mutant protein.

The *Gaq* gene encodes several *Gaq* splice variants, and *Gaq221c* mutants disrupt the expression of all *Gaq* splice variants (21). Our ERG recording revealed that *Gaq221c* null mutant clones showed no light responses. Previous whole-cell voltage-clamp recordings showed that the photoresponse of *Gaq1* homozygous cells is larger than that of *Gaq1* heterozygous cells (20). These results indicate that other *Gaq* splice variants might contribute to the residual light response in *Gaq1* null mutants. In this study, we demonstrate that *Gaq3* contributes to the residual light response in *Gaq1* null mutants.

The *Gaq* gene encodes several *Gaq* splice variants. Originally, two cDNAs resulting from different *Gaq* gene splicing were isolated (23). These two cDNAs encode Gaq1 and Gaq2 isoform proteins, respectively. Functional studies demonstrated that Gaq1 mediates the light response, whereas Gaq2 has no effect on phototransduction (20). Subsequently, two additional *Gaq* splice variants were isolated (9). Now, seven total *Gaq* splice variants have been annotated in flybase, and these splice variants encode three different isoform proteins, including Gaq1, Gaq3, and Gaq4. In this study, we demonstrated that Gaq3 also mediate phototransduction. Overexpression of *Gaq3* 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 *Gaq1* and *Gaq961* flies. These results indicate that different *Gaq* isoform proteins play different roles in phototransduction.

*Gaq mediates retinal degeneration*

Mutations in most genes encoding components of the phototransduction cascade result in rapid retinal degeneration, except for *Gaq* hypomorph allele *Gaq1* and *Gaq1* isoform null mutant allele *Gaq961*.
Previous studies have shown that both Gaq1 and Gaq961 mutants underwent slow light-dependent retinal degeneration due to slow accumulation of stable Rh1/Arr2 complexes (12,29). In these Gaq mutants, the small residual photoresponse may reduce Ca2+ influx, which partial 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 complexes formation prevented retinal degeneration in the mutants. Under normal conditions, the interaction between Arr2 and Rh1 is transient, because light-triggered Ca2+ 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 Ca2+ 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.

Gaq3 isoforms regulate Rh1 synthesis

In this study, we showed the first evidence that Gaq3 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 Gaq homologue CG30054 regulates inositol 1,4,5-tris-phosphate receptor (IP3R) to mediate calcium mobilization from intracellular stores and promote calcium-regulated secretory vesicle exocytosis (36). Given that Gaq3 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 w1118 strain to eliminate the genetic background. The Gaq1 mutant and p[HS::dGaq] transgenic fly were obtained from Charles S. Zuker’s lab. All other flies used in this work were from Bloomington stock center. Genetic mosaics of the deficiency line P[neoFRT]42D,Df(2R)Gaql.3 and Gaq221c 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 wild-type flies is w1118 and the mutant alleles used for each gene in this work are Gaq961, Gaq1, and arr25. 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::Gaq3] transgenic flies, Gaq3 cDNA was subcloned into the pUAST-attB vector and injected into y1,w67c23;P{CaryattP2} flies. The transgene was subsequently crossed into the nlr mutant background and proteins 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 (96aa–176aa) 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: AB 528451; ATPα, RRID: AB 2166869), Millipore (C-Gaq, RRID: AB 310221), Abcam (N-Gaq, ab190082), Sigma (tubulin, RRID: AB 477593), Hong-sheng Li (TRP), and Craig Montell (PLC).

Inverse PCR

Inverse PCR was performed as previously
described (40). Briefly, genomic DNA was first digested with Hpa II, 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 (SD) was calculated.

Intracellular recordings were performed as previously described (42). Briefly, a low-resistance glass microelectrode filled with 2M 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 PVDF membranes (Pall) in Tris-glycine buffer. Blots were probed with anti-Rh1 (1:3,000), anti-C-Gaq (1:1,000), anti-N-Gaq (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).

**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 Gaq1 (5’-GATCAGCTGCGGCAAAACA-3’/5’-GTTTTCGGGTATCTGTCGC-3’), Gaq3 (5’-CAGATTATTAGATGGTAGACG-3’/5’-CCTCCAATTCATTATCATT-3’), and rp49 (5’-AACGTTTACAAATGTGTAACCCGAGCAATTCAGG-3’). Rp49 was used as an internal control, and relative mRNA levels were calculated by setting wild-type Gaq1 and Gaq3 mRNA levels as 100%.

**Electron microscopy**

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 electron microscopy, 100-nm thin sections were cut and collected on Cu 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 blotting 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 Image J 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 ± SD. 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 ± SD. 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.

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Conflict of interests

The authors declare that they have no conflicts of interest with the contents of this article.

Author contributions

Q.G. executed genetic and biochemistry experiments and interpreted data. J.W. conducted ERG and intracellular recording. Y.T. and S.C. assisted in data interpretation. ZC.Z. participated in data interpretation and writing. J. H. designed experiments, interpreted data, and wrote the manuscript.

References

1. Pierce, K. L., Premont, R. T., and Lefkowitz, R. J. (2002) Seven-transmembrane receptors. Nature reviews. Molecular cell biology 3, 639-650
2. Neer, E. J. (1995) Heterotrimeric G proteins: organizers of transmembrane signals. Cell 80, 249-257
3. Tian, Y., Hu, W., Tong, H., and Han, J. (2012) Phototransduction in Drosophila. Sci China Life Sci 55, 27-34
4. Bloomquist, B. T., Shortridge, R. D., Schneuwly, S., Perdew, M., Montell, C., Steller, H., Rubin, G., and Pak, W. L. (1988) Isolation of a putative phospholipase C gene of Drosophila, norpA, and its role in phototransduction. Cell 54,
723-733

5. Montell, C., and Rubin, G. M. (1989) Molecular characterization of the Drosophila trp locus: a putative integral membrane protein required for phototransduction. Neuron 2, 1313-1323

6. Hardie, R. C., and Minke, B. (1992) The trp gene is essential for a light-activated Ca2+ channel in Drosophila photoreceptors. Neuron 8, 643-651

7. Phillips, A. M., Bull, A., and Kelly, L. E. (1992) Identification of a Drosophila gene encoding a calmodulin-binding protein with homology to the trp phototransduction gene. Neuron 8, 631-642

8. Ranganathan, R., Harris, G. L., Stevens, C. F., and Zuker, C. S. (1991) A Drosophila mutant defective in extracellular calcium-dependent photoreceptor deactivation and rapid desensitization. Nature 354, 230-232

9. Ratnaparkhi, A., Banerjee, S., and Hasan, G. (2002) Altered levels of Gq activity modulate axonal pathfinding in Drosophila. The Journal of neuroscience : the official journal of the Society for Neuroscience 22, 4499-4508

10. Niemeyer, B. A., Suzuki, E., Scott, K., Jalink, K., and Zuker, C. S. (1996) The Drosophila light-activated conductance is composed of the two channels TRP and TRPL. Cell 85, 651-659

11. Reuss, H., Mojot, M. H., Chyb, S., and Hardie, R. C. (1997) In vivo analysis of the drosophila light-sensitive channels, TRP and TRPL. Neuron 19, 1249-1259

12. Hu, W., Wan, D., Yu, X., Cao, J., Guo, P., Li, H. S., and Han, J. (2012) Protein Gq modulates termination of phototransduction and prevents retinal degeneration. The Journal of biological chemistry 287, 13911-13918

13. Wang, T., and Montell, C. (2007) Phototransduction and retinal degeneration in Drosophila. Pflugers Arch 454, 821-847

14. Wang, T., Jiao, Y., and Montell, C. (2005) Dissecting independent channel and scaffolding roles of the Drosophila transient receptor potential channel. The Journal of cell biology 171, 685-694

15. Alloway, P. G., Howard, L., and Dolph, P. J. (2000) The formation of stable rhodopsin-arrestin complexes induces apoptosis and photoreceptor cell degeneration. Neuron 28, 129-138

16. Mu, Y., Tian, Y., Zhang, Z. C., and Han, J. (2019) Metallophosphoesterase regulates light-induced rhodopsin endocytosis by promoting an association between arrestin and the adaptor protein AP2. The Journal of biological chemistry 294, 12892-12900

17. Alloway, P. G., and Dolph, P. J. (1999) A role for the light-dependent phosphorylation of visual arrestin. Proceedings of the National Academy of Sciences of the United States of America 96, 6072-6077

18. Matsumoto, H., Kurien, B. T., Takagi, Y., Kahn, E. S., Kinumi, T., Komori, N., Yamada, T., Hayashi, F., Isono, K., Pak, W. L., and et al. (1994) Phosrestin I undergoes the earliest light-induced phosphorylation by a calcium/calmodulin-dependent protein kinase in Drosophila photoreceptors. Neuron 12, 997-1010

19. Kiselev, A., Socolich, M., Vinos, J., Hardy, R. W., Zuker, C. S., and Ranganathan, R. (2000) A molecular pathway for light-dependent photoreceptor apoptosis in Drosophila. Neuron 28, 139-152

20. Scott, K., Becker, A., Sun, Y., Hardy, R., and Zuker, C. (1995) Gq alpha protein function in vivo: genetic dissection of its role in photoreceptor cell physiology. Neuron 15, 919-927

21. Banerjee, S., Joshi, R., Venkiteswaran, G., Agrawal, N., Srikanth, S., Alam, F., and Hasan, G. (2006) Compensation of inositol 1,4,5-trisphosphate receptor function by altering sarco-endoplasmic reticulum calcium ATPase activity in the Drosophila flight circuit. The Journal of neuroscience : the official journal of the Society for Neuroscience 26, 8278-8288

22. Mismer, D., and Rubin, G. M. (1989) Definition of cis-acting elements regulating expression of the Drosophila melanogaster ninaE opsin gene by oligonucleotide-directed mutagenesis. Genetics 121, 77-87
23. Lee, Y. J., Dobbs, M. B., Verardi, M. L., and Hyde, D. R. (1990) dgg: a drosophila gene encoding a visual system-specific G alpha molecule. *Neuron* **5**, 889-898

24. Lee, Y. J., Shah, S., Suzuki, E., Zars, T., O'Day, P. M., and Hyde, D. R. (1994) The Drosophila dgg gene encodes a G alpha protein that mediates phototransduction. *Neuron* **13**, 1143-1157

25. Noel, J. P., Hamm, H. E., and Sigler, P. B. (1993) The 2.2 A crystal structure of transducin-alpha complexed with GTP gamma S. *Nature* **366**, 654-663

26. Lambright, D. G., Noel, J. P., Hamm, H. E., and Sigler, P. B. (1994) Structural determinants for activation of the alpha-subunit of a heterotrimeric G protein. *Nature* **369**, 621-628

27. Noel, J. P., Hamm, H. E., and Sigler, P. B. (1993) The 2.2 A crystal structure of transducin-alpha complexed with GTP gamma S. *Nature* **366**, 654-663

28. Cao, J., Bollepalli, M. K., Hu, Y., Zhang, J., Li, Q., Li, H., Chang, H., Xiao, F., Hardie, R. C., Rong, Y. S., and Hu, W. (2018) A Single Residue Mutation in the Galphaq Subunit of the G Protein Complex Causes Blindness in Drosophila. *G3* **8**, 363-371

29. Iakhine, R., Chorna-Ornan, I., Zars, T., Elia, N., Cheng, Y., Selinger, Z., Minke, B., and Hyde, D. R. (2004) Novel dominant rhodopsin mutation triggers two mechanisms of retinal degeneration and photoreceptor desensitization. *The Journal of neuroscience : the official journal of the Society for Neuroscience* **24**, 2516-2526

30. Meyertholen, E. P., Stein, P. J., Williams, M. A., and Ostroy, S. E. (1987) Studies of the Drosophila norpA phototransduction mutant. II. Photoreceptor degeneration and rhodopsin maintenance. *J Comp Physiol A* **161**, 793-798

31. Schopf, K., and Huber, A. (2017) Membrane protein trafficking in Drosophila photoreceptor cells. *Eur J Cell Biol* **96**, 391-401

32. Shetty, K. M., Kurada, P., and O'Tousa, J. E. (1998) Rab6 regulation of rhodopsin transport in Drosophila. *The Journal of biological chemistry* **273**, 20425-20430

33. Satoh, A. K., O'Tousa, J. E., Ozaki, K., and Ready, D. F. (2005) Rab11 mediates post-Golgi trafficking of rhodopsin to the photosensitive apical membrane of Drosophila photoreceptors. *Development* **132**, 1487-1497

34. Xiong, B., and Bellen, H. J. (2013) Rhodopsin homeostasis and retinal degeneration: lessons from the fly. *Trends Neurosci* **36**, 652-660

35. Xiong, B., Bayat, V., Jaiswal, M., Zhang, K., Sandoval, H., Charrng, W. L., Li, T., David, G., Duraine, L., Lin, Y. Q., Neely, G. G., Yamamoto, S., and Bellen, H. J. (2012) Crag is a GEF for Rab11 required for rhodopsin trafficking and maintenance of adult photoreceptor cells. *PLoS biology* **10**, e1001438

36. Yamanaka, N., Marques, G., and O'Connor, M. B. (2015) Vesicle-Mediated Steroid Hormone Secretion in Drosophila melanogaster. *Cell* **163**, 907-919

37. Corl, A. B., Berger, K. H., Ophir-Shohat, G., Gesch, J., Simms, J. A., Bartlett, S. E., and Heberlein, U. (2009) Happyhour, a Ste20 family kinase, implicates EGFR signaling in ethanol-induced behaviors. *Cell* **137**, 949-960

38. Stowers, R. S., and Schwarz, T. L. (1999) A genetic method for generating Drosophila eyes composed exclusively of mitotic clones of a single genotype. *Genetics* **152**, 1631-1639

39. Guo, C., Pan, Y., and Gong, Z. (2019) Recent Advances in the Genetic Dissection of Neural Circuits in Drosophila. *Neuroscience bulletin*

40. Dunne, C. R., Cillo, A. R., Glick, D. R., John, K., Johnson, C., Kanwal, J., Malik, B. T., Mammano, K., Petrovic, S., Pfister, W., Rascoe, A. S., Schrom, D., Shapiro, S., Simkins, J. W., Strauss, D., Talai, R., Tomtishen, J. P., 3rd, Vargas, J., Veloz, T., Vogler, T. O., Clenshaw, M. E., Gordon-Hamm, D. T., Lee, K. L., and Marin, E. C. (2014) Structured
inquiry-based learning: Drosophila GAL4 enhancer trap characterization in an undergraduate laboratory course. *PLoS biology* 12, e1002030

41. Han, J., Gong, P., Reddig, K., Mitra, M., Guo, P., and Li, H.-S. (2006) The Fly CAMTA Transcription Factor Potentiates Deactivation of Rhodopsin, a G Protein-Coupled Light Receptor. *Cell* 127, 847-858

42. Hu, W., Wang, T., Wang, X., and Han, J. (2015) Ih channels control feedback regulation from amacrine cells to photoreceptors. *PLoS biology* 13, e1002115

43. Tian, Y., Li, T., Sun, M., Wan, D., Li, Q., Li, P., Zhang, Z. C., Han, J., and Xie, W. (2013) Neurexin Regulates Visual Function via Mediating Retinoid Transport to Promote Rhodopsin Maturation. *Neuron* 77, 311-322

**FOOTNOTES**

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**Figure legends**

**Figure 1.** *nlr* mutants exhibit no response to light stimulation.

A) Representative ERG traces of wild-type 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 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 black arrow and nonspecific band with open arrow. ATPα and Nrv3 were used as loading controls.

E) Quantification of protein levels of visual molecules in *w*1118 and *nlr* mutants.

**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 *CG30054B* mutants were used as positive controls. The specific Gαq band and nonspecific bands are marked with 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 black arrow and open arrows, respectively. The eye-specific protein PLC
was used as a loading control. Quantification of Gaq levels for each genotype is presented in the lower panel. 

G) nlr/Gaq$^{221c}$ flies show no photoresponses. Event markers represent 5-s light pulses, and scale bars are 5 mV.

H) Western blots of Gaq protein levels in the various mutants. Each lane was loaded with half of the isolated retina. Quantification of Gaq levels for each genotype is presented in the lower panel.

**Figure 3. Gaq$^{V303D}$ mutation of Gaq1 isoform largely contributes to the abolished light response in nlr mutants**

A) Annotated transcripts of the Gaq gene. Positions of the point mutations in the nlr allele are marked with black arrows. The anti-N-Gaq antibody-recognized site is labeled at the top.

B) Western blots of Gaq protein levels in each genotype. Each lane was loaded with half of the isolated retina. The specific Gaq band is indicated with black arrow and nonspecific bands were indicated with open arrows.

C) Light response in nlr/Gaq$^1$ and nlr/Gaq$^{961}$ 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 Gaq protein levels with different treatments. nlr; P[HS::Gaq] 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 Gaq band and nonspecific bands are marked with black arrow and open arrows, respectively.

E) ERG traces show light responses with different treatments. Gaq 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.

**Figure 4. Gaq3 isoform contributes to the residual photoresponse in Gaq1 isoform null mutants**

A) Representative ERG traces of Gaq$^1$/Gaq$^{221c}$ and Gaq$^{961}$/Gaq$^{221c}$ 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 Gaq3 protein levels in w$^{118}$ and nlr mutant photoreceptor cells. The eye-specific protein INAD was used as a loading control. Quantification of Gaq3 levels for each genotype is presented in the lower panel.

C) Additional mutations in the Gaq gene. The point mutation in the nlr allele is marked with black arrow, and the sequence inserted upstream of the transcription start site is listed at the top. The anti-C-Gaq antibody-recognized site is labeled at the top.

D) mRNA levels of Gaq1 and Gaq3 in w$^{118}$ and nlr mutant retina. Total RNA was extracted from isolated adult retina. Rp49 was used as an internal control. Data are presented as mean ± SEM from three independent experiments.

E) Western blots show Gaq3 protein levels in rescued flies.

F) Representative ERG traces of w$^{118}$, nlr;GMR-GAL4, and nlr;GMR-GAL4/UAS:: Gaq3 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.

**Figure 5. Depletion of all Gaq splicing variants leads to rapid retinal degeneration**

A) EM analysis reveals nlr mutants and the clones of Df(2R)Gaq1.3-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 blot of fractions of \textit{w1118} and \textit{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 \textit{nlr} mutant flies. Flies were raised in constant darkness. Scale bar, 1 μm.

E) EM images show normal rhabdomeral structure in 7-day-old \textit{nlr;arr2} double mutants. Flies were raised in 12 h light/12 h dark conditions for 7 days. Scale bar, 1 μm.

Figure 6. \textit{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 \textit{w1118} and \textit{Gαq221C} mutant flies. Rh1 quantification for each genotype is presented in the right panel.

C) Western blots of Rh1 levels in \textit{w1118}, \textit{nlr;GMR-GAL4}, and \textit{nlr;GMR-GAL4/UAS::Gαq3} flies. Rh1 quantification for each genotype is presented in the right panel.

D) Rh1 distribution in developing \textit{w1118} (left) and \textit{nlr} mutant retina (right). Fly heads were collected at different time points after pupal formation and eclosion, and sections were prepared and stained with anti-Rh1 (green) and anti-INAD (red) antibodies. The Rh1 signaling remaining in the cytoplasm is indicated with arrows. Scale bar, 5 μm.

E) Rh1 quantification remaining in the cytoplasm at 100 h after pupal formation for each genotype presented in the right panel.
**Figure 2**

**A**

| Df(2R)Exel7121 49B5 | 49B12 |
|---------------------|-------|
| Df(2R)Gaq1.3       | 49B10 |

Gaquin / CG30054 / CG17760 / muskelin / CG33792

**B**

| nlr | nlr/Df(2R)Exel7121 | 5 mV |
|-----|--------------------|------|
|     |                     | 2 s  |

**C**

_FRT Df Gaq1.3_

**D**

- **W1118**
- **nlr**
- **Gaq**
- **CG30054**
- **FRT Df Gaq1.3**

**E**

- **Gaq**
- **W1118**

**F**

- **N-Gqa**
- **PLC**

**G**

- **nlr**

**H**

- **nlr**
- **nlr/Gaq21c**

**Relative Gqa level (%)**

- **w1118**
- **nlr**
- **Gaq21c**
- **CG30054**
- **FRT Df Gaq1.3**

**Relative Gqa level (%)**

- **N-Gqa**
- **ATPa**
Gαq splice variants mediate phototransduction, rhodopsin synthesis, and retinal integrity in Drosophila
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