Targeted Mutagenesis of the Farnesylation Site of Drosophila Gye Disrupts Membrane Association of the G Protein βγ Complex and Affects the Light Sensitivity of the Visual System*

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Activation of phototransduction in drosophila is mediated by a heterotrimeric G protein that couples to the effector enzyme phospholipase Cβ. The γ subunit of this G protein (Gye) as well as γ subunits of vertebrate transducins contain a carboxy-terminal CAAX motif (C, cysteine; A, aliphatic amino acid; X, any amino acid; Fig. 1A) with a consensus sequence for protein farnesylation. To examine the function of Gye farnesylation, we mutated the farnesylation site and overexpressed the mutated Gye in Drosophila. Mass spectrometry of overexpressed Gye subunits revealed that nonmutated Gye is modified by farnesylation, whereas the mutated Gye is not farnesylation. In the transgenic flies, mutated Gye forms a dimeric complex with Gβγ, with the consequence that the fraction of non-membrane-bound Gβγ is increased. Thus, farnesylation of Gye facilitates the membrane attachment of the Gβγ complex. We also expressed human Gyrod in Drosophila photoreceptors. Despite similarities in the primary structure between the transducin γ subunit and Drosophila Gye, we observed no interaction of human Gyrod with Drosophila Gβγ. This finding indicates that human Gyrod and Drosophila Gye provide different interfaces for the interaction with Gβγ subunits. Electroretinogram recordings revealed a significant loss of light sensitivity in eyes of transgenic flies that express mutated Gye. This loss in light sensitivity reveals that post-translational farnesylation is a critical step for the formation of membrane-associated Goβγ required for post-translational light activation from rhodopsin to phospholipase Cβ.

Phototransduction in Drosophila and related flies represents the fastest G protein-coupled signaling system known to date (for reviews, see Refs. 1–5). In this signaling pathway a rhodopsin molecule is activated by light absorption and transmits the signal to a heterotrimeric Gq protein consisting of Gαq, Gβγ, and Gye. As a result of G protein activation Goq couples to the norpA-encoded effector enzyme phospholipase Cβ, which is assembled together with an eye-specific protein kinase C and the cation channel transient receptor potential protein into a signaling complex by the scaffolding protein INAD (inactivation of non-afterpotential D protein) (6–9). Activated phospholipase Cβ hydrolyzes the membrane phospholipid phosphatidylinositol bisphosphate to form the second messengers diacylglycerol and 1,4,5-inositol triphosphate. The phototransduction cascade terminates in the opening of cation channels composed of ion channel subunits of the transient receptor potential protein family (10) and thus results in a depolarization of the photoreceptor cell membrane.

Most of the proteins mediating phototransduction in Drosophila have been identified, and their function has been determined with the aid of Drosophila mutants. These mutants exhibit distinct defects in the electrical response to light stimuli which can be recorded by measuring electroretinograms, transepithelial potentials reflecting light-dependent photoreceptor cell depolarization. Thus, the α and β subunits of the visual G protein, referred to as DGq (Goq) and Gβγ, respectively, have been cloned and functionally characterized (11–14). The visual γ subunit (Gye) has also been cloned (15). Characterization of Gye revealed that it exhibits a carboxy-terminal CAAX box (C, cysteine; A, aliphatic amino acid; X, any amino acid, Fig. 1A), which is a common motif of G protein γ subunits. γ Subunits are prenylated at the conserved cysteine residue of this motif (for review, see Refs. 16 and 17). Following prenylation, the carboxy-terminal tripeptide (AAX) is cleaved off, and the newly exposed cysteine residue is usually methylated at its carboxyl terminus (Fig. 1A). With regard to the type of prenylation, G protein γ subunits can be divided in two groups that are either farnesylated (when the last amino acid of the CAAX box is serine, methionine, or glutamine) or geranylated (when the last amino acid is leucine or valine) (18–21). Accordingly, Drosophila Gye as well as the visual Gγ subunits of vertebrate transducins are likely to become farnesylated because they have a methionine or serine in the carboxy-terminal position. In contrast, nonvisual Gγ subunits are typically geranylated. A second Gγ subunit isolated from Drosophila (GY, Ref. 22), which is expressed preferentially in the brain, has a consensus CAAX box for geranylation. The conservation of the farnesylation site of visual Gγ visual G protein; Gβγ, β subunit of Drosophila visual G protein; Gye (Gγ50A), γ subunit of Drosophila visual G protein; Gyrod, γ subunit of human visual G protein; MALDI-TOF matrix-assisted laser desorption/ionization time-of-flight.

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subunits of vertebrates and flies indicates that the farnesyl residue could contribute to a specific role of visual G\textsubscript{y}\textsubscript{e} subunits in phototransduction which is common to both vertebrates and invertebrates, for example binding of the visual G protein to the receptor, they cannot account for effects caused by the complex structural and functional organization provided by the light-transducing compartment of a photoreceptor cell. In the present study we assessed the functional importance of the conserved farnesyl modification of visual G\textsubscript{y}\textsubscript{e} subunits in vivo by generating transgenic flies that express a mutated G\textsubscript{y}\textsubscript{e} without a farnesylation site. Transgenic flies expressing nonfarnesylated G\textsubscript{y}\textsubscript{e} were investigated for alterations in membrane anchoring of the different G protein subunits, for \(\beta\gamma\) complex formation, and for abnormalities in the light response. We show that overexpression of mutated G\textsubscript{y}\textsubscript{e} interferes with membrane attachment of the \(\beta\gamma\) complex and that it renders the photoreceptor cells less sensitive to light.

EXPERIMENTAL PROCEDURES

**Generation of DNA Constructs and Transgenic Drosophila—**In vitro oligonucleotide-mediated mutagenesis was carried out by amplifying the coding region of Drosophila G\textsubscript{y}\textsubscript{e} using the sequence-specific primers 5'-ATTACCGGGAGTGAGACAAAAATCTATTTTCGAAAGAAGCTGC-ATTGGATCCTGCTAACA-3' (Smal restriction site underlined and myc tag italicized) and 5'-GGCGCAAGCTTTATGAATTACCGG (HindIII restriction site underlined; bold type indicates the miss-matching nucleotide) to induce the amino acid mutation C69G. A nonmutated, myc-tagged G\textsubscript{y}\textsubscript{e} amplificate was generated using the same sequence-specific forward primer and the nonmutated reverse primer 5'-GGGCGAAGCTTTATGAATTACCGG (HindIII restriction site underlined). The coding sequence of the human \(\beta\gamma\) rod subunit was amplified from an expressed sequence tag clone (accession number AA015841) and combined with a myc tag by using the forward and reverse primers 5'-ATTACCGGGAGTGAGACAAAAATCTATTTTCGAAAGAAGCTGC-ATTGGATCCTGCTAACA-3' (Smal restriction site underlined and myc tag italicized) and 5'-GGCGCAAGCTTTATGAATTACCGG (HindIII restriction site underlined), respectively. The obtained PCR products G\textsubscript{y}\textsubscript{e}C69G, G\textsubscript{y}\textsubscript{e} and hG\textsubscript{y}\textsubscript{rod} (Fig. 1 B) were cloned into the Smal/HindIII restriction site of the pBluescript II SK vector (Stratagene), \(\beta\gamma\) (31). P-element-mediated transformation of host strain Drosophila yellow \(\text{white}\) was carried out as described (28). The transformants were made homozygous for the \(P\)-element inserts. Flies were raised on a standard corn meal diet under a 12-h light/12-h dark cycle and were used for the experiments at an age of 2–3 days posteclosion.

**Protein Extraction—**For extracting the G protein subunits expressed in the transgenic lines, dissected compound eyes were collected in 1X SDS-buffer (5% SDS, 65 mM Tris-HCl, pH 6.8) and homogenized with a plastic pestle. The proteins were extracted for 20 min at room temperature. Insoluble material was sedimented by centrifugation at 100,000 \(\times\) g for 10 min at 4°C. To obtain soluble proteins, compound eyes or Drosophila heads were collected in 10 mM sodium phosphate buffer, pH 7.0, 1 mM phenylmethylsulfonyl fluoride and homogenized with a plastic pestle, and residual chitinous material was removed (centrifugation at 3,900 \(\times\) g for 1 min at 4°C). The homogenates were then incubated for 20 min at 4°C. Eye membranes were sedimented by centrifugation at 386,000 \(\times\) g for 30 min to separate the soluble and membrane fraction. The supernatant contained the soluble proteins. To isolate the membrane-bound proteins, the pellet was resuspended in 1X SDS-buffer, incubated for 20 min at room temperature, and the extract was centrifuged for 10 min at 116,000 \(\times\) g and 4°C. The supernatant of this centrifugation contained the membrane-bound proteins.

**SDS-PAGE, Western Blot Analysis, and Protein Quantification—**Protein extracts were subjected to SDS-PAGE according to Laemmli (29) on 8–20% gradient gels (Amersham Biosciences, Mupid System). Immunoblotting was performed as described earlier (15). Membranes were incubated with either polyclonal antibodies against Calliphora G\textsubscript{y}\textsubscript{e} (1:50, Ref. 15), Calliphora G\textsubscript{e} (1:500, Ref. 15), Drosophila G\textsubscript{e} (1:500, kindly provided by C. Zuker, Ref. 13), with monoclonal anti-\(\gamma\)-myc antibodies (1:40, Roche Applied Science) or with polyclonal antibodies directed against bovine \(\beta\gamma\) rod (1:200, Santa Cruz). Bound antibodies were detected with protein A conjugated with alkaline phosphatase (1:25,000, Sigma) and visualized through a chromogenic reaction with 5-bromo-4-chloro-3-indolyl phosphate/4-nitro blue tetrazolium chloride or by autoradiography, respectively. To quantify the relative amount of visual G protein subunits present in the soluble and membrane-bound protein fraction, extracts from dissected compound eyes of wild type and P[G\textsubscript{y}\textsubscript{e}C69G] flies were subjected to Western blot analysis with anti-G\textsubscript{e}, anti-G\textsubscript{y}\textsubscript{e}, and anti-\(\gamma\)-G\textsubscript{y}\textsubscript{e} antibodies. For detection of bound antibodies the blot membrane was incubated with \(^{125}\text{I}\)-labeled secondary antibodies. The radioactive sig-
nals were quantified using a bioimaging analyzer (Bas 1000 MacBAS, Fujifilm, program Quant Mode, software MacBAS V1.0). Each of four independently prepared extracts was analyzed twice. For determining the relative amounts of Geq, Gβe, and Gγe the percentage of radioactivity measured in the soluble and in membrane fraction was determined, and mean values and S.D. were calculated.

**Immunocytochemistry**—Labeling of myc-tagged Gγe subunits on sections through Drosophila heads was carried out as described previously for native G protein subunits (15). The primary anti-c-myc antibody (New England Biolabs, dilution 1:20) was detected with a Cy5-conjugated anti-mouse IgG (Sigma, dilution 1:100).

**Immunoprecipitation**—For immunoprecipitation studies of overexpressed Drosophila Gγe subunits and human Gyrod, proteins from Drosophila heads were extracted for 20 min at 4°C with Triton X-100 buffer (1% Triton X-100, 150 mM NaCl, 50 mM Tris-HCl, pH 8.0). Extracted proteins were separated from insoluble material by a centrifugation at 116,000 × g, 4°C, and 10 min. The extracts were added to protein A/G-Sepharose (Pierce) which had been preincubated with monclonal anti-c-myc antibodies (New England Biolabs) for 1–1.5 h. Immunoprecipitation was performed for 1–2 h at 4°C and was followed by five washes with 500 μl of Triton X-100 washing buffer (0.1% Triton X-100, 100 mM NaCl, 50 mM Tris-HCl, pH 8.0). Precipitated proteins were eluted from protein A/G-Sepharose beads with 1× SDS-buffer for 10 min at 80°C and were subjected to SDS-PAGE and Western blot analysis. For detection of immunoprecipitated proteins by Western blot analysis or Coomassie Blue staining of SDS gels, proteins extracted from 90 or 1000 Drosophila heads were used, respectively.

**Mass Spectrometry**—For molecular mass determination of proteins by matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry, immunoprecipitated Gγe subunits of 1,000 Drosophila heads were eluted from the Sepharose beads with 20 μl of 95% formic acid. The eluted proteins were purified and concentrated using ZipTip pipette tips with C18 reversed phase medium (Millipore) and obtained in 4 μl of 0.1% trifluoroacetic acid and 50% acetonitrile. A 0.5-μl aliquot of protein solution was mixed with 0.5 μl of matrix solution (cyano-4-hydroxycinnamic acid in 0.1% trifluoroacetic acid and acetonitrile, 7:3, v/v) on the mass spectrometer sample target and left to crystallize at ambient temperature. Mass analysis was performed in linear mode on a Bruker BIFLEX IV time-of-flight mass spectrometer (Bruker Daltonics). Apomyoglobin (Mr 16952.27), cytochrome c (Mr = 12361.96), and bovine insulin (Mr = 5734.51) (Sigma) were used as calibration standards.

**Electroretinogram Recordings**—Light stimuli were recorded from white-eyed Drosophila raised for 3–5 days in a 12-h dark/12-h light regime. Flies were immobilized with a mixture of Krypton’s wax and thermal conductive paste, and their temperature was maintained at 24°C with a Peltier element. Test stimuli (500-ms duration, 20 nm band width at 520 nm, maximally 2.5×10^10 photons m^-2 s^-1) were generated using a 150-W xenon arc lamp (Osram, Germany), a monochromator (Oriel Instruments, Stanford, CA), and reflective neutral density filters (Melles Griot, Zeevenaar, The Netherlands). To maintain the rhodopsin-metarhodopsin concentration constant, each test stimulus was followed by a bright orange light pulse generated with an additional xenon arc lamp and an OG 580 orange filter (Schott, Germany). Glass electrodes were filled with insect saline (0.67% NaCl, 0.015% KCl, 0.012% CaCl2, 0.015% NaHCO3, pH 7.2) and inserted just below the cornea. An Ag/AgCl wire in the thorax served as a reference electrode. Signals were detected by an AI 401 SmartProbe Differential Amplifier connected to a CyberAmpl 320 signal conditioner (both from Axon Instruments, Inc., Foster City, CA), low pass filtered at 1 kHz, sampled at 7.5 kHz by a Lab-PC-1200 A/D converter (National Instruments), and analyzed by a Whole Cell Analysis program (Strathclyde Electrophysiology software, Whole Cell Analysis Program version 3.0.8, John Dempster, University of Strathclyde, Glasgow, UK).

**RESULTS**

**P-element-mediated Expression of Gy Subunits of Visual G Proteins in the Compound Eye of Drosophila**—By PCR-mediated site-directed mutagenesis the cysteine (Cys-69) of the carboxyl-terminal CAAx box of Drosophila Gγe was exchanged for a glycine. A myc tag, which allowed us to identify the mutated Gγe on Western blots, was attached at the N terminus of the protein. The mutated Gγe (GyeC69G, Fig. 1B) was overexpressed in Drosophila wild type flies under the control of the Drosophila Rh1 promoter, which normally directs the expression of the major Drosophila rhodopsin Rh1 in R1–6 cells of the compound eye. We also generated transgenic Drosophila expressing nonmutated, myc-tagged γ subunits of the Drosophila visual G protein (Gγe) or of human transducin (hGγrod, also referred to as human Gyγ, Ref. 30) under the control of the Rh1 promoter. We obtained four independent transgenic lines for GyeC69G, two lines for Gγe, and one line expressing the human Gy γ subunit of transducin.

The flies were tested for the expression of the individual G protein subunits present in the transgenic lines by Western blot analysis (Fig. 2). Anti-Gye recognizes the native Gye, expressed in the photoreceptor cells of wild type and transgenic flies (Fig. 2A, transparent arrowheads), as well as recombinantly expressed GyeC69G and nonmutated Gγ e (Fig. 2A, black arrowheads). By using anti-c-myc antibodies which specifically detect the myc epitope, it was possible to distinguish the recombinantly expressed, myc-tagged GyeC69G from the native Gγe (Fig. 2B). Because of the myc tag the apparent molecular mass of myc-tagged Gγe subunits is ~1300 kDa higher than that of native Gγe, which has a molecular mass of ~8400 kDa. Except for one line (P[GyeC69G]-4) all transgenic lines showed an overexpression of recombinantly expressed myc-tagged Gγe compared with native Gγe. The myc-tagged human Gyrod was specifically detected by anti-bovine Gyrod antibodies which reacted with hGγrod but not with Drosophila Gγe (Fig. 2C). We failed to detect hGγrod with anti-myc antibodies on Western blots, suggesting that the human Gγ subunit is expressed at a lower level than the myc-tagged Drosophila Gγ e subunit. To obtain information on possible effects of Gγe...
overexpression on the formation of the trimeric G protein complex, the expression level of the visual Gγ and Gβ subunits was investigated (Fig. 2A). Despite the presence of overexpressed forms of Gγ, all transgenic lines expressed an amount of Gαq and Gβ protein similar to that of wild type flies, showing that neither Gαq nor Gβe was up- or down-regulated in the transgenic lines. Lines P[GγeC69G]-1, P[Gγe]-1, and P[hGγrod] were chosen for all following experiments.

To determine whether the localization of the recombinantly expressed Gγ subunits is restricted to photoreceptor cells R1–6 of the compound eye, we carried out immunohistochemical experiments (Fig. 3). Longitudinal sections through heads of transgenic Drosophila were probed with anti-c-myc antibodies. Labeling of the myc-tagged Gγ subunits expressed in P[GγeC69G], P[Gγe], and P[hGγrod] was restricted to the retina and the lamina, i.e. the first optic ganglion in which axons of R1–6 photoreceptors terminate. This labeling pattern is almost identical to the pattern described previously for the native α, β, and γ subunits of the Drosophila visual G protein (15), except that the native subunits can additionally be detected in the medulla, i.e. the second optic ganglion to which R7 and R8 photoreceptor cells project. Thus, in line with the expected expression pattern for genes controlled by the Rh1 rhodopsin promoter, the spatial distribution of the myc-tagged Gγ subunits in head sections reveals a highly specific expression of these proteins in R1–6 photoreceptor cells.

The Point Mutation C69G Disrupts In Vivo Farnesylation of Gγ—Although Drosophila Gγ harbors a bona fide farnesylation site, it has not yet been demonstrated that this Gγ subunit is indeed prenylated. To show that Gγ is modified by post-translational farnesylation, and, equally important, that the mutated GγeC69G does not carry a farnesyl residue, we analyzed the recombinantly expressed Gγ subunits by MALDI-TOF mass spectrometry (Fig. 4, A and B). The molecular mass determined for the c-myc-tagged mutated Gγe (9709.55) was about 42 higher than the theoretical mass calculated for the unmodified amino acid sequence (Mr = 9668.06). This finding suggests a post-translational modification of the protein by acetylation. Indeed the c-myc tag that is attached at the amino terminus of both mutated and nonmutated Gγe has a consen-
sus sequence (methionine followed by a strongly hydrophilic amino acid, Ref. 31) for amino-terminal acetylation. The native Gye also shows such a consensus sequence at its amino terminus. Further support for post-translational acetylation of the myc-tagged Gye was obtained by mass spectrometric analysis of peptides generated by digestion of the mutated or nonmutated Drosophila Gye subunits with endoproteinase Asp-N (data not shown). The molecular mass spectrum obtained for the nonmutated form of Gye revealed two peaks differing in molecular masses by 14.08 (9617.88 and 9631.97, respectively). The higher molecular mass corresponds to the theoretical mass of an acetylated Gye subunit that is fully modified by farnesylation, removal of the last three carboxyl-terminal amino acids, and carboxyl methylation. The more prominent peak at 9617.88 most likely represents a farnesylated state of Gye which lacks carboxyl methylation (14.14). Taken together, the mass spectrometric analysis of the mutated and nonmutated Drosophila Gye subunits showed that both proteins were modified by amino-terminal acetylation, but only the nonmutated Gye subunit was farnesylated. This farnesylation was followed by the removal of the three carboxyl-terminal amino acids. A significant fraction of the farnesylated Gye did not seem to be carboxyl methylated. The myc-tagged human Gyrod expressed in Drosophila photoreceptor cells was also analyzed by mass spectrometry (Fig. 4C). In this mass spectrum a prominent peak at 9773.40 was detected, indicating that the human transducin-γ-subunit, like nonmutated Drosophila Gye, becomes acetylated and fully modified by farnesylation, removal of the carboxyl-terminal tripeptide, and carboxyl methylation in Drosophila photoreceptor cells (calculated M, of acetylated, fully modified hGyrod is 9773.51). Additional peaks observed in this mass spectrum may result from contaminations in the immunoprecipitated probe because their molecular masses do not correspond to nonfarnesylated or to partially modified hGyrod.

**Interaction with Gβε and Membrane Attachment of γ Subunits**—To determine the relevance of the farnesylation site of Gye for dimerization of Gβε and Gye, we performed coimmunoprecipitation studies with anti-c-myc antibodies of protein extracts obtained from Drosophila heads (Fig. 5). SDS-PAGE of the precipitated proteins followed by staining with Coo massie Blue revealed two protein bands with molecular masses of ~42,000 and 10,000, which were present in immunoprecipitated proteins from transgenic flies expressing myc-tagged Gye or GyeC69G (Fig. 5A) but were absent in controls. These two proteins were identified as Gβε and Gye by Western blot analysis (Fig. 5B). Consequently, Gβε coimmunoprecipitated with both farnesylated Gye present in P[Gye] flies as well as with nonfarnesylated Gye expressed in P[GyeC69G] flies. Gαq was detected in neither of the immunoprecipitates, indicating that the interaction between the βγ complex and Gaq is not maintained during the immunoprecipitation procedure applied here. Because Gβε is coprecipitated with nonfarnesylated Gye, it is concluded that the farnesyl modification of the visual Gye subunit of Drosophila is not required for Gβγ complex formation. We also determined whether or not the human Gyrod subunit forms a complex with Drosophila Gβε when it is expressed in Drosophila photoreceptors R1–6. Although our mass spectrometric measurements (see Fig. 4C) showed that human Gyrod becomes farnesylated in Drosophila photoreceptor cells, which suggests functional expression of hGyrod in this system, immunoprecipitation experiments with protein extracts from the heads of P[hGyrod] transgenic flies did not reveal coprecipitation of Gβε with human Gyrod (Fig. 5). This finding argues against the formation of βγ dimers composed of the human and Drosophila G protein subunits, and it suggests that human Gyrod cannot functionally replace Drosophila Gye, despite the conservation of domains that characterize Drosophila Gye and human Gyrod as visual G protein subunits (15).

The farnesyl anchor of the vertebrate transducin γ subunit has been shown to facilitate its membrane attachment (32). Therefore, it is likely that the absence of a farnesylation site in GyeC69G interferes with membrane attachment of this Drosophila Gγ subunit. Furthermore, the absence of such a membrane anchor in Gye could also influence the membrane association of Gβε and Gαq. To examine the effect of the C69G mutation on the subcellular location of the G protein subunits, Western blot analyses of soluble and membrane-bound eye proteins of P[GyeC69G], P[Gye], and wild type flies were carried out (Fig. 6A). In wild type flies Gye is distributed equally between the soluble and the membrane fraction (Fig. 6A, third panel). This finding is in agreement with results showing that farnesylated visual Gβγ dimers can be partially solubilized without detergent, whereas solubilization of geranylgeranylated Gβγ dimers, which are more hydrophobic, requires the presence of a detergent (33). For example, human Gly11 carrying a farnesyl residue also showed an equal distribution between the soluble and membrane fraction, whereas a geranylgeranyl modification of Gy11 resulted in a complete membrane-association of the Gy subunit (34). As in wild type flies, in P[Gye] flies that overexpress nonmutated Gye, about half of the total Gye was detected in the soluble and in the
influences the membrane association of G wild type and P protein subunits in the soluble and membrane-bound protein fractions. To determine the relative amount of the G protein fraction was performed with protein A conjugated with alkaline phosphatase. The mean values of the relative amount of G protein bands was determined with a bioimaging analyzer.

The mean values of the relative amount of G protein, respectively (Fig. 6A, second panel). Mass spectrometric analysis of myc-tagged Gye immunoprecipitated from either the soluble fraction or the membrane fraction revealed that both fractions contained fully modified farnesylated Gye as well as farnesylated Gye lacking carboxyl methylation (data not shown). In contrast to the equal distribution in the soluble and membrane fraction of Gye, the mutated GyeC69G was localized predominantly in the soluble fraction of the photoreceptor cells (Fig. 6A, first panel, and B). Only about 20% of Gye was retained in the membrane fraction in P[GyeC69G] transgenic flies (Fig. 6B). To determine whether the altered membrane association of nonfarnesylated GyeC69G influences the membrane association of Gaq and Gbê in P[GyeC69G] flies, the relative amount of Gbê and Gaq in the soluble and membrane fraction was quantified (Fig. 6, C and D). For Gbê, the distribution between the soluble and membrane fraction was similar to that observed for Gye (Fig. 6, B and C). In P[GyeC69G] flies 70% of Gbê was localized in the soluble fraction compared with 50% in wild type flies. In contrast, there was no change in the membrane association of Gaq compared with the wild type situation (Fig. 6D). In both wild type and P[GyeC69G] flies 70% of Gaq was soluble, and 30% was localized in the membrane fraction. These results show that the membrane association of Gye and Gbê is disrupted by the C69G mutation. They strongly suggest that the membrane attachment of the Gbê complex in the eye of Drosophila is regulated by farnesylation of Gye.

Overexpression of Mutated GyeC69G Reduces the Sensitivity of the Visual System—The analysis of a Drosophila Gbê mutant revealed that lowering the level of Gbê to 5% of the wild type level results in a dramatic loss of light sensitivity (14). Therefore, it is to be expected that alterations in the membrane localization of the Gbê complex have similar physiological consequences. By recording electoretinograms, we compared the light sensitivity of wild type photoreceptors with those that express the mutated GyeC69G, nonmutated Gye, or hGyro. Fig. 7 shows intensity-response functions (V-log I curves) which relate the normalized response amplitude to the relative stimulus intensities. A shift of the V-log I curve to the right, i.e. to higher light intensities, corresponds to lower sensitivity of the examined visual system. Such a shift of the V-log I curve is observed in flies expressing the mutated C69G, whereas the V-log I curves of flies expressing Gye or hGyro are superimposed with the one obtained from wild type flies. A quanti-
tative evaluation of the V-log I curves reveals that the light intensity required to obtain half-maximal responses is shifted by 0.4 log unit to a higher light intensity in flies expressing GyeC69G, which corresponds to a decrease in sensitivity to 0.6 ± 0.5% (Fig. 1B) of the wild type sensitivity. Because light sensitivity of fly photoreceptors is directly proportional to the rhodopsin content (35), by analogy this decrease in sensitivity would approximately correspond to a halving of the photoreceptor quantum yield. On the other hand, expression of the nonmutated Gye and of the human Gyöd had no significant effect on the sensitivity of the visual system.

**DISCUSSION**

*In vitro* studies of isoprenylation of Gγ subunits have assigned a functional relevance of this post-translational modification for activation of the G protein by its receptor, for interaction of the βγ complex with the α subunit, possibly via an S-prenyl binding site in the α subunit (36) and for the membrane attachment of the βγ complex (for review, Refs. 16 and 17). To gain insight into the functional role of the putative farnesyl modification of Gye in the intact visual system of Drosophila photoreceptors, we generated transgenic Drosophila expressing a mutated Gye without a farnesylation site. The study of these flies revealed that farnesylation of Gye facilitates membrane association of the Gβγ complex but is not required for the association of Gye with Gβε. These findings are in agreement with earlier studies on mammalian visual as well as nonvisual Gγ subunits showing that βγ dimerization occurs independently of Gγ isoprenylation (37, 38).

Our mass spectrometric analysis of immunoprecipitated Gye subunits revealed that the myc-tagged Gye analyzed here is acetylated at the amino terminus and, most importantly, that the nonmutated Gγ subunit is indeed modified by farnesylation and cleavage of the last three carboxyl-terminal amino acids. As has been described for the Gγ subunit of bovine transducin (39, 40), a major fraction of Gye expressed in Drosophila photoreceptors does not seem to be carboxyl methylated. Methylidyne of the farnesylated carboxyl-terminal cysteine of transducin Gγ was shown to be a reversible process (39). Together with farnesylation the methylation has been implicated in facilitating the membrane association of Gγ and the interaction of Gγ with Gβγ (40) as well as the coupling of transducin to metarhodopsin II (41). On the other hand, enzymatic demethylation of transducin Gγ did not affect the ability of Gβγ to interact functionally with Gα in detergent and had only a small (2-fold) effect in the presence of rod outer segments, which may be attributed to enhanced membrane association of the carboxyl methylated Gγ (42). Likewise, only minor effects on signal transduction processes were observed after enzymatic demethylation of Gγ (43). Our finding indicating that a significant fraction of the Gye subunits isolated from Drosophila photoreceptors are farnesylated but not carboxyl methylated lends support to the hypothesis that carboxyl methylation is a reversible process in *vivo* with a possible regulatory function.

Besides the altered membrane association of Gβγ we recorded a decrease in the sensitivity of the visual responses in flies which express the mutated Gγ subunit. There are a number of possible explanations for this result. First, the change in the membrane binding properties of the mutated βγ subunit may result in a decreased number of available βγ dimers in the photoreceptive membrane for forming functional heterotrimeric G proteins. Although native Gγ is still present in the eyes of these flies, the majority of β subunits will interact with the overexpressed nonfarnesylated γ subunits. In addition to a functional role of the farnesylation site of Gγ in membrane attachment of the Gβγ complex it is possible that farnesylation of Gγ is required for the binding of Gβγ to Gαq. From studies using, for example, recombinantly expressed human γ1 and γ2 subunits, there is clear evidence that only those βγ complexes containing a prenylated Gγ are able to interact with Gαq (24, 37, 38). Consequently, less heterotrimeric G proteins, which can be activated by the receptor, will be present in the photoreceptive membrane of transgenic flies which overexpress the mutated Gγ. Thus, overexpression of the mutated Gγ may partially phenocopy a hypomorphic mutation in the visual Gγ subunit which leads to a severe defect in receptor G protein coupling and hence to a reduced light sensitivity (14). Second, the affinity between activated receptor and G protein in vertebrate photoreceptors is in part determined by the presence of a farnesyl residue at the Gγ subunit (23, 24, 41, 44, 45). Mutations in the farnesylation site of Drosophila Gye could therefore lead to a reduced affinity of Gq for rhodopsin and consequently to a defect in G protein activation, even if heterotrimeric G proteins containing the mutated Gγ were formed. A third possibility is that the mutation of the farnesylation site of Gγ interferes with the interaction of downstream effectors of the visual G protein. Indeed, there is evidence that the prenyl moiety of vertebrate Gγ subunits has a function in the activation of phospholipase Cβ (46–48). However, cumulative evidence for G protein function in the phototransduction cascade of Drosophila suggests that Gαq alone and not the βγ subunit activates the downstream effector phospholipase Cβ (see, e.g. Ref. 5). A direct interaction with phospholipase Cβ has only been shown for Gαq but not for Gβγ (49). This renders it unlikely that the decreased light sensitivity of flies expressing mutated Gγ results from less effective activation of phospholipase Cβ by the Gβγ subunit. Overexpression of mutated Gye does not abolish the visual response but only leads to a moderate decrease in light sensitivity. This is explained by the presence of nonmutated, native Gγ in the eyes of the transgenic flies, which allows the formation of fully intact heterotrimeric G proteins. By using mutants that express different amounts of Gαq it has been shown that a reduction in the amount of G protein to less than 50% is required to record a reduction in light sensitivity and that flies that express only 1/3 Gαq still show residual light responses (13). Thus, the overexpression of a Gγ subunit lacking the farnesylation site in Drosophila photoreceptor cells must result in a decrease of functional heterotrimeric Gq protein to less than 50% of the wild type level.

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Targeted Mutagenesis of the Farnesylation Site of Drosophila Gγe Disrupts Membrane Association of the G Protein βγ Complex and Affects the Light Sensitivity of the Visual System

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