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$G_{q\alpha}$ Protein Function In Vivo: Genetic Dissection of Its Role in Photoreceptor Cell Physiology

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Summary

Heterotrimeric G proteins mediate a variety of signaling processes by coupling seven-transmembrane receptors to intracellular effector molecules. The Drosophila phototransduction cascade is a G protein-coupled signaling cascade that utilizes a phospholipase C (PLCβ) effector. PLCβ has been shown to be activated by $G_{q\alpha}$ in reconstituted systems. To determine whether a G_q-like protein couples rhodopsin to PLC, and to study its function, we isolated a mutant defective in a photoreceptor-specific G_q protein, DG_q. We now demonstrate that G_q is essential for the activation of the phototransduction cascade in vivo. We also generated transgenic flies expressing DG_q under an inducible promoter and show that it is possible to manipulate the sensitivity of a photoreceptor cell by controlled expression of DG_q. Characterization of quantum bumps in mutants expressing less that 1% of the levels of DG_q revealed that the rhodopsin-G protein interaction does not determine the gain of the single photon responses. Together, these results provide significant insight into the role of G_q in regulating the output of a photoreceptor cell.

Introduction

Heterotrimeric G proteins mediate numerous cell signaling events, including responses to hormones, neurotransmitters, peptides, and sensory stimuli like light, odorants, and tastants, transducing highly specific receptor–ligand interactions into activation of common second messenger pathways (Neer, 1995). Although several hundred G protein–coupled receptors have been identified, less than 30 G_α subunits have been isolated, and these couple to a limited number of signaling pathways.

Based on effector activation properties and sequence similarity, G_α subunits have been grouped into several different classes (reviewed by Neer, 1995). The $G_{s\alpha}$ family primarily stimulates adenylate cyclase; $G_{i\alpha}$ primarily inhibits adenylate cyclase; $G_{\alpha 12/13}$ has been implicated in sodium/potassium exchange; and $G_{q\alpha}$, $G_{\alpha 15}$, and $G_{\alpha 16}$ activate phospholipase C (PLC). Although the function and specificity of G_α subunits is well established in transfected and reconstituted systems (Gilman, 1987; Simon et al., 1991), little is known about the precise role of the different isoforms in well-defined, physiologically relevant pathways. This is important, as the diversity of G protein forms is likely to be matched by a corresponding range of cellular functions. Thus, a dissection of G protein function in vivo will require a system suitable to genetic manipulation.

Drosophila phototransduction is an ideal model system for the study of G protein–coupled transduction cascades. First, phototransduction in Drosophila is a prototypical phosphoinositide-mediated, G protein–coupled signaling pathway (Smith et al., 1991b; Ranganathan et al., 1995). Second, many genes encoding components of this pathway, upstream and downstream of the G protein, have been characterized (Zuker, 1992). Third, the eye is not required for viability, so it is possible to manipulate the photoreceptor cell environment using a combination of classical mutational analysis and transgenic technology (Wolff and Ready, 1993). Finally, photoreceptor cell function can be assayed with exquisite sensitivity and specificity (Ranganathan et al., 1991, 1994; Hardie, 1991). In Drosophila, light activation of rhodopsin activates a PLC (Yoshioka et al., 1983; Bloomquist et al., 1988). PLC catalyzes the hydrolysis of the minor membrane phospholipid phosphatidylinositol-4,5-bisphosphate (PIP_2) into the second messengers inositol trisphosphate and diacylglycerol. The breakdown of PIP_2 leads to the eventual opening of cation-selective membrane channels and a depolarization of the photoreceptor cell, resulting in the generation of a receptor potential and neurotransmitter release.

The major rhodopsin in Drosophila is encoded by the *ninaE* gene (O'Tousa et al., 1985; Zuker et al., 1985), and the PLC is encoded by the *norpA* locus (Bloomquist et al., 1988). The identity of the G_α subunit coupling rhodopsin to the effector molecule has not been clearly defined. Drosophila genes encoding a photoreceptor cell–specific G_β subunit, $G_{\beta e}$ (Yarfitz et al., 1988, 1991), and a photoreceptor cell–specific G_α subunit, DG_q (Lee et al., 1990, 1994), have previously been isolated. The $G_{\beta e}$ gene encodes a 39 kDa protein sharing 45% sequence identity with other G_β subunits. The recent isolation and characterization of mutants lacking $G_{\beta e}$ demonstrated that this G_β subunit is a component essential for Drosophila phototransduction (Dolph et al., 1994). The *dgq* gene encodes a G_α subunit sharing more than 75% amino acid identity with mammalian $G_{q\alpha}$ (Lee et al., 1990). We now report the isolation and characterization of a DG_q mutant; this is the first known G_q protein mutant in a metazoan organism. The characterization of this mutant not only gives important insight into the in vivo function of G_q in a defined signaling pathway, but also provides fundamental insight into the modulation of photoreceptor cell function.

Results and Discussion

Isolation of a DG_q Mutant

Drosophila phototransduction is a G protein–coupled, phosphoinositide-mediated signaling cascade in which activation of rhodopsin leads to the activation of the *norpA*-

encoded PLC (Zuker, 1992). DG_q is a G_q-like G_α subunit abundantly expressed in the fly retina (Lee et al., 1994). To determine whether in vivo DG_q is the target of rhodopsin and the activator of PLC, we undertook a genetic screen to isolate mutations in this G protein subunit. Since DG_q is expressed specifically in sensory systems (Lee et al., 1990, 1994; Dean Smith, personal communication), mutations in this gene should have no effect on viability. The scheme used to generate mutants at this locus was based on the loss of immunoreactivity on Western blots and has been described in detail previously (Dolph et al., 1993). In essence, fly stocks containing individual mutagenized second chromosomes over a chromosomal deficiency spanning the region 49A–49D were generated (*dgq* maps at position 49B on the second chromosome), and each stock was then screened by immunoblot analysis for the loss of DG_q immunoreactivity using DG_q-specific antibodies (see Experimental Procedures). Analysis of 11,300 chromosomes yielded one strong *dgq* allele, hereafter referred to as $G\alpha q^1$.

Using the polymerase chain reaction (PCR), we isolated the $G\alpha q^1$ genomic region and determined its entire nucleotide sequence. We also determined the sequence of the corresponding mRNA by carrying out a PCR amplification reaction on first-strand cDNA prepared from mutant retinas (see Experimental Procedures). The $G\alpha q^1$ allele has a G to A nucleotide change at residue 1910 in the consensus splice acceptor site at the intron 3–exon 4 boundary (nucleotide numbers are according to Lee et al., 1990). Loss of this site results in the use of a cryptic splice site 9 nucleotides downstream, causing the in-frame deletion of 3 codons encompassing amino acid residues 154–156. As a consequence of this change, $G\alpha q^1$ mutants produce ~1% of the wild-type levels of the DG_q protein (Figure 1A).

To determine whether the dramatic reduction in the levels of this G protein subunit affects the expression of proteins thought to interact with DG_q in the phototransduction pathway, we compared the steady-state levels of rhodopsin, G_β, and PLC in wild-type and $G\alpha q^1$ mutant retinas. We also examined the levels of DG_q in mutants lacking rhodopsin (O'Tousa et al., 1985; Zuker et al., 1985), G_β (Dolph et al., 1994), or PLC (Bloomquist et al., 1988). Figure 1 demonstrates that loss of DG_q has little effect on the expression of these proteins and that DG_q itself is normally expressed in mutants lacking receptor, the G_β subunit, or the effector.

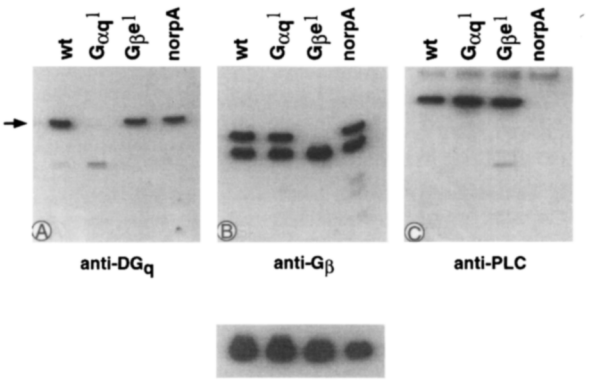

Figure 1. $G\alpha q^1$ Mutants Have a Dramatic Loss of DG_q but Have Normal Levels of G_β, PLC, and Rhodopsin

Shown are Western blots of wild-type, $G\alpha q^1$, $G_{\beta e1}$, and *norpA* mutants probed with anti-DG_q (A), anti-$G_{\beta e}$ (B), anti-norpA (C), and anti-Rh1 (D) antibodies. Each lane contains extracts from two heads. The smaller G_β polypeptide seen in (B) represents a brain-specific G_β isoform that cross-reacts with this antibody (Yarfitz et al., 1988). Note wild-type levels of G_β, PLC, and rhodopsin in the $G\alpha q^1$ mutants. Conversely, all other mutants express normal levels of DG_q. Protein extracts were prepared by sonication in 20 μl of 100 mM Tris (pH 6.8), 3% SDS, 0.7 M β-mercaptoethanol, 10% glycerol and electrophoresed on a 10% SDS–polyacrylamide gel (Stamnes et al., 1991).

DG_q Is Essential for Photoreceptor Cell Function

If *dgq* encodes the G_α subunit that mediates Drosophila phototransduction, then the light response of $G\alpha q^1$ mutants should be severely defective. Using whole-cell voltage-clamp recordings (Hardie, 1991; Ranganathan et al., 1991), we examined the electrophysiological responses of mutant photoreceptor cells to light. Figures 2A and 2B show sample traces of the light-induced currents in wild type and in a $G\alpha q^1$ mutant at different light intensities. Characteristically, wild-type photoreceptors respond over a wide range of light intensities and show light-activated currents that reach several nanoamps in amplitude (Figure 2) (Ranganathan et al., 1991). In contrast, $G\alpha q^1$ mutant photoreceptors have a dramatic loss of light responsiveness, displaying more than a 1000-fold loss in light sensitivity (Figure 2D). These results show that DG_q is essential for phototransduction.

The small response remaining in the $G\alpha q^1$ mutants could result from activation of a parallel signaling pathway independent of DG_q or could be due to expression of very low levels of functional DG_q protein. To examine this, we compared the photoresponse of cells homozygous for the $G\alpha q^1$ allele (two copies of the mutant gene) to the response of cells heterozygous for $G\alpha q^1$ and a deletion that removes the locus (only one copy of the mutant gene). If the response seen in $G\alpha q^1$ mutants is due to activation of a parallel pathway, then a reduction in the levels of the mutant protein should have no effect on the residual response. Figures 2C and 2D show that cells that contain one copy of $G\alpha q^1$ are now almost totally insensitive to light. Together, these findings demonstrate that DG_q is absolutely required in the activation of the phototransduction cascade.

DG_{q1} Mediates the Light Response; DG_{q2} Has No Effect on Phototransduction

Previous studies, relying on cDNA isolations and genomic sequencing, indicated that *dgq* encodes two alternatively spliced, eye-specific transcripts (Lee et al., 1990, 1994). One of these, DG_{q1}, contains the full coding region of the gene, encompassing exons 1–8 (Figure 3), and encodes a predicted protein of 41 kDa. The second, DG_{q2}, is missing exon 7 (amino acids 290–326 of DG_{q1}) and encodes a predicted polypeptide of 37 kDa. Interestingly, exon 7 contains a domain important for the interaction between G_α subunits and effector molecules (for review, see Conklin and Bourne, 1993). For instance, a peptide of residues 293–314 of the bovine photoreceptor G_α subunit, trans-

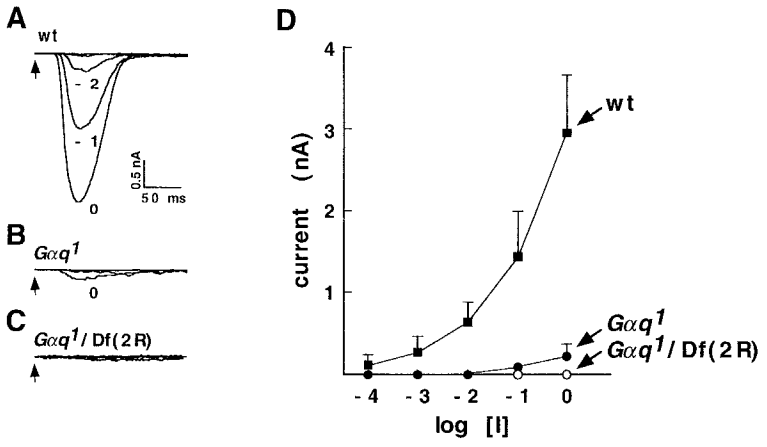

Figure 2. Loss of Light Sensitivity in $G\alpha q^1$ Mutants

(A–C) Representative whole-cell voltage-clamp recordings of light-activated currents from wild-type controls (A), $G\alpha q^1$ mutants (B), and $G\alpha q^1$/Df(2R)vg^{135} heterozygous flies (C). Recordings were carried out as described in Experimental Procedures. Cells were stimulated with 10 ms flashes of increasing light intensity (580 nm), given at the onset of the trace (arrows). Numbers refer to the log order of light intensity associated with that light response (e.g., −1 is 10 times less light than 0). Note the dramatic loss of responsiveness in the $G\alpha q^1$ mutant.

(D) Graph of response amplitude versus light intensity for the same three stocks. $G\alpha q^1$ cells are 3 log orders of magnitude less responsive than wild-type cells, and $G\alpha q^1$/Df(2R)vg^{135} heterozygotes do not generate measurable responses (see text for details). The curves represent the averaged responses ± SD (wild type, n = 10; $G\alpha q^1$, n = 10; $G\alpha q^1$/Df(2R)vg^{135}, n = 5).

ducin, is sufficient to stimulate cGMP phosphodiesterase (Rarick et al., 1992), and mutations of the corresponding residues in the $G_{s\alpha}$ subunit render it unable to activate adenylate cyclase in reconstituted systems (Itoh and Gilman, 1991; Berlot and Bourne, 1992). These results suggest that DG_{q2}, which lacks these critical residues, might serve to regulate phototransduction negatively, reducing the gain of the light response by being able to bind rhodopsin but unable to activate PLC.

To examine the role of DG_{q1} and DG_{q2} in phototransduction, we generated transgenic animals expressing each splice form, P[hs:DG_{q1}] and P[hs:DG_{q2}], in a $G\alpha q^1$ mutant background. A 1.1 kb DNA fragment encompassing exons 1–8, P[hs:DG_{q1}], and a 1.0 kb fragment containing exons 1–6 and 8, P[hs:DG_{q2}], were cloned into P element vectors and introduced into mutant hosts by P element–mediated germline transformation. To control the timing and levels of each protein, each gene was placed under the control of an inducible heat-shock promoter. To demonstrate expression of each splice variant in the transgenic flies, we generated antibodies that distinguish between the two isoforms (see Figure 3). Antibodies specific to DG_{q1} were raised against a peptide of exon 7, a region unique to this splice form. Antibodies specific to DG_{q2} were generated by immunizing with a peptide encompassing the splice junction of exons 6 and 8; this is the only region unique to this splice form.

To assay for inducible expression of each isoform, the transgenic flies were given a heat pulse (see Figure 3, legend), and protein extracts were prepared from isolated heads. Figure 3 shows immunoblots of extracts from wild-type controls and P[hs:DG_{q1}] and P[hs:DG_{q2}] flies. As expected, anti-DG_{q1} antiserum recognizes a 41 kDa protein present in the P[hs:DG_{q1}] transgenic animals and wild-type controls but not in P[hs:DG_{q2}] (Figure 3A). Anti-DG_{q2} recognizes a 37 kDa protein present in the P[hs:DG_{q2}] transgenic animals (Figure 3B) but not in the P[hs:DG_{q1}] transgenic flies or in wild-type controls. If DG_{q2} were indeed a bona fide product of the *dgq* gene, it should be found in the control flies. To examine this discrepancy further, we used a different antibody, one that should recognize both splice forms (raised against a 15 amino acid peptide of exon 8). As a positive control for these studies, we generated heterozygous flies carrying one copy of P[hs:DG_{q1}] and one copy of P[hs:DG_{q2}]. The results shown in Figure 3C demonstrate that, while both proteins are seen in the heterozygous transgenic controls, DG_{q2} is not detected in wild-type heads. Identical results are obtained when using a much larger amount of protein extract from either heads or retinas (data not shown). Together, these results argue that DG_{q2} cannot normally function in vivo as a negative regulator of phototransduction and raise strong doubts about the existence of this isoform (see below and Discussion).

Recently, a constitutively activated form of DG_{q1} has been shown to modulate Drosophila phototransduction, suggesting that this splice variant functions in light activation (Lee et al., 1994). However, those studies were carried out in the presence of a wild-type copy of DG_q in the background, thus preventing a definitive analysis of its function. To assess rigorously the function of DG_{q1} (and DG_{q2}) in phototransduction, we examined the light response of P[hs:DG_{q1}] and P[hs:DG_{q2}] animals using whole-cell voltage-clamp recordings. Figure 4 shows an analysis of the electrophysiological responses to light in each of the transgenic lines after heat shock–induced expression of the transgenes. Figure 4C shows that DG_{q1} expression rescues the defect of the $G\alpha q^1$ mutant host, restoring near wild-type responses. This demonstrates that DG_{q1} mediates phototransduction. In contrast, the light response of P[hs:DG_{q2}] flies is indistinguishable from that of the $G\alpha q^1$ mutants (compare Figures 4A and 4B), demonstrating that DG_{q2} does not promote photoactivation (see also Lee et al., 1994).

Since DG_{q2} lacks part of the effector interacting domain (exon 7), we wondered whether this protein, if expressed, could be used as a dominant negative construct in vivo. Thus, we analyzed the light response of $G\alpha q^1$ flies express-

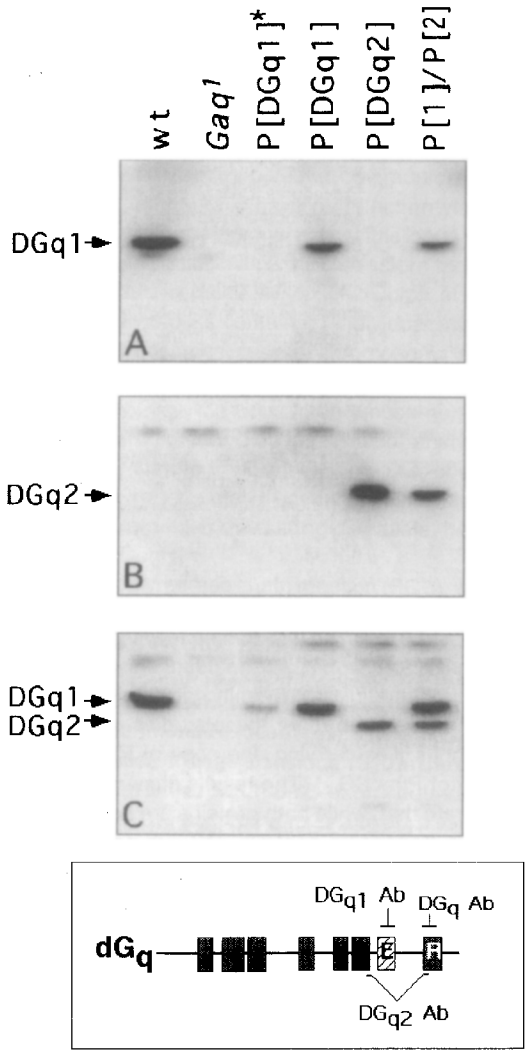

Figure 3. Expression of Specific DG_q Isoforms in Transgenic Flies

The diagram at the bottom shows the predicted structure of *dgq*. DG_{q1} contains exons 1–8, encoding a predicted protein of 41 kDa. The reported structure of DG_{q2} contains exons 1–6 and 8, encoding a predicted protein of 37 kDa. Shaded boxes depict exons. R refers to the putative receptor-binding domain, and E denotes the putative effector-binding domain. This region is missing in the DG_{q2} isoform. Also shown are the regions used to generate anti-peptide antibodies. DG_q expression in wild-type, $G\alpha q^1$, P[hs:DG_{q1}], P[hs:DG_{q2}], and P[hs:DG_{q1}]/P[hs:DG_{q2}] flies was examined by Western blot analysis. The blots were probed with antibodies specific for DG_{q1} (A), DG_{q2} (B), or antibodies that recognize both isoforms (C). Expression in the transgenic lines was induced by the following heat-shock regime: 2 hr at 37°C, 30 min at 22°C, 2 hr at 37°C, and 3 hr at 22°C. Each lane contains extracts from two heads. Expectedly, each splice form is specifically expressed in the appropriate transgenic lines; however, the DG_{q2} splice form is not found in wild-type heads. P[hs:DG_{q1}]* contains protein extracts isolated from P[hs:DG_{q1}] flies that were not heat shocked.

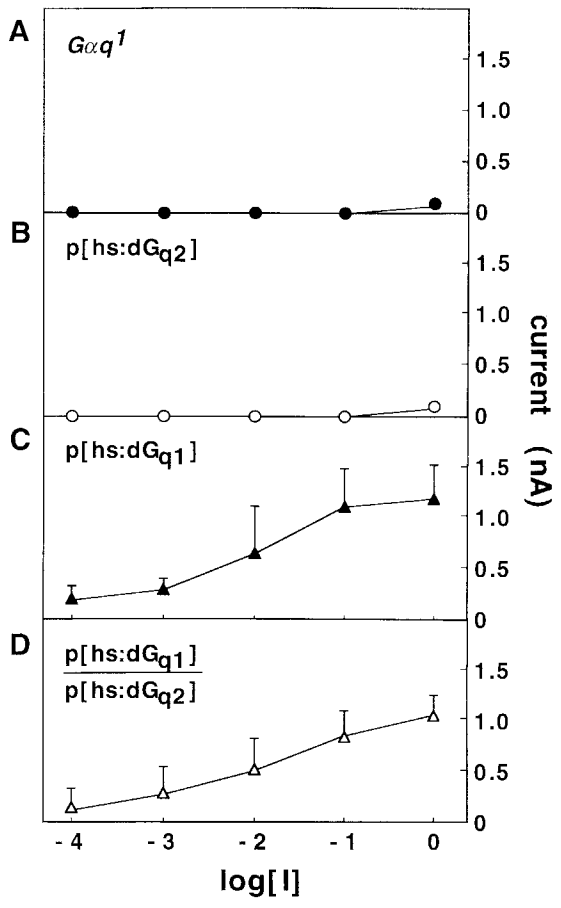

Figure 4. DG_{q1} Mediates Activation of the Phototransduction Cascade

The light responses of $G\alpha q^1$ (A), P[hs:DG_{q2}] (B), P[hs:DG_{q1}] (C), and P[hs:DG_{q1}]/P[hs:DG_{q2}] (D) photoreceptors are displayed as response (± SD) versus intensity plots. Expression of DG_{q1} rescues the visual defect of the $G\alpha q^1$ mutant (compare [C] and [A]), while expression of DG_{q2} does not restore light responsiveness (compare [B] and [A]). Also, expression of DG_{q2} does not negatively regulate phototransduction, since cells expressing DG_{q1} and DG_{q2} show the same light sensitivity as cells expressing DG_{q1} alone (compare [C] and [D]). Each curve represents the averaged responses from 4–6 cells to 10 ms flashes of 580 nm light. All lines were heat shocked 2 hr at 37°C, 30 min at 22°C, 2 hr at 37°C, and 3 hr at 22°C, at which time patch-clamp analysis was performed.

ing both the DG_{q1} and the DG_{q2} transgenes. Figure 4D shows that the visual physiology of these flies does not differ from the response of flies expressing the DG_{q1} transgene alone, demonstrating that DG_{q2} is incapable of negatively regulating phototransduction. Strong confirmation of this finding comes from the analysis of P[hs:DG_{q2}] in $G\alpha q^1$ mutant hosts (Figure 4B). Even under conditions in which the levels of DG_{q2} far exceed the levels of residual DG_{q1} (see Figure 3C), DG_{q2} fails to modulate the light response. Together, these results demonstrate that DG_{q1} is the G protein activator of this signaling pathway and that DG_{q2} does not function in the phototransduction cascade.

Activation of the Light Response in $G\alpha q^1$ Mutants

Since $G\alpha q^1$ cells contain less than 1% of the wild-type levels of DG_q, one may expect defects in coupling due to the small amount of G protein available to couple to activated receptors and to effectors. In particular, one may expect increases in the latency of the response, as the interaction between rhodopsin and the G protein may be diffusion limited.

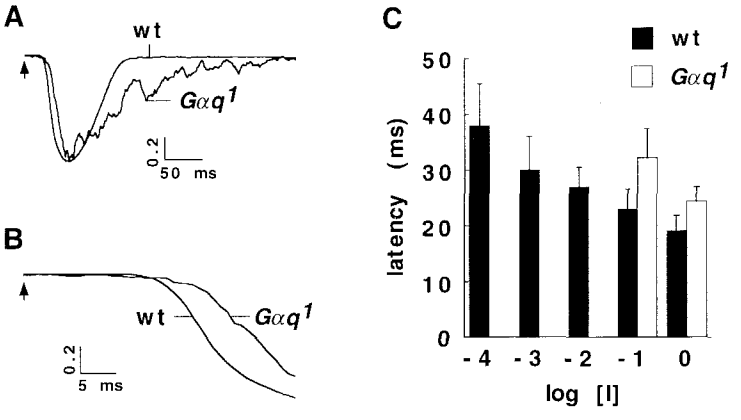

Figure 5. Response Kinetics of $G\alpha q^1$ Mutants

(A) Shown are superimposed current traces of wild-type and $G\alpha q^1$ photoreceptors matched for stimulus intensity (log I = 0). Since the response of $G\alpha q^1$ mutants is considerably smaller (see Figure 2), both traces were normalized to peak amplitude for comparison.

(B) Expanded time scale of the first 40 ms after light stimulation, showing differences in latency between wild-type and $G\alpha q^1$ photoreceptors.

(C) Quantitative analysis of latency times for wild-type (closed bars) and $G\alpha q^1$ (open bars) photoreceptors at different light intensities. At log I = −1, wild type (n = 10): 23.2 ± 3.5 ms, $G\alpha q^1$ (n = 6): 33.3 ± 5.3 (p = .002); at log I = 0, wild type (n = 10): 19.1 ± 2.7 ms, $G\alpha q^1$ (n = 10): 24.2 ± 2.7 (p = .0008). Latency was determined by measuring time from the onset of the stimulus to the initiation of the response (the initial deflection from baseline).

In wild-type photoreceptors, the latency of the light response decreases as a function of light intensity (Figure 5C). This is not surprising since a larger number of rhodopsin molecules are activated at higher light intensities, and thus a G protein would be expected to encounter an activated receptor with greater probability. Analysis of the light responses of $G\alpha q^1$ mutants demonstrated that latency also decreases as a function of light intensity. However, when we compared the latency of the response in wild-type and mutant cells stimulated with the same light intensity, so as to produce the same number of activated receptor molecules, we found there is a significant increase in the latency of the response in the $G\alpha q^1$ cells (Figures 5B and 5C). These results demonstrate that in $G\alpha q^1$ photoreceptors the interaction of rhodopsin with the G protein is a key determinant in the latency of the light response.

The deactivation kinetics of the residual light response in the $G\alpha q^1$ photoreceptors is quite different from that of wild-type cells. This is seen whether we compare light responses of cells stimulated with the same number of photons (Figure 5A) or cells matched for similar response amplitude (in which case the mutant cells are stimulated with several orders of magnitude of higher light intensity; data not shown). For quantitative evaluation of the data, the tail of the deactivation phase of the light-activated current was fitted to a single exponential function, and the time constant (τ) was measured for responses at log I = 0. This analysis showed that wild-type photoreceptors have deactivation time constants of 19.0 ± 5 ms, whereas $G\alpha q^1$ mutants have time constants of 127 ± 24 ms. The deactivation phenotype of $G\alpha q^1$ cells is the result of having small amounts of G protein and not of having a mutant protein, since similar results were obtained in transgenic flies expressing small amounts of wild-type DG_q protein (by recording under conditions in which the $G\alpha q^1$ background was not responsive: i.e., $G\alpha q^1$; P[hs:DG_{q1}] flies, no heat shock at log I = −2; data not shown). This deactivation defect could be explained in terms of calcium-dependent receptor deactivation mechanisms (Lagnado and Baylor, 1994; Ranganathan et al., 1994) since these cells display a large imbalance between the number of activated receptors and the resulting light-activated currents and calcium influx (Ranganathan et al., 1994; see section below on quantum bumps).

DG_q Functions as a Molecular Switch

Because phototransduction involves a large signal amplification, we wondered how the levels of G protein affect the gain of the light response. We generated flies expressing different amounts of DG_{q1} protein, either by varying gene dosage or by using the transgenic flies expressing DG_{q1} under the control of the inducible heat-shock promoter. By experimentally manipulating the heat-shock regime, we varied protein levels. Figure 6 shows the physiological results of these studies; also shown are the corresponding Western blots assessing protein amounts in the different flies. Our data demonstrate that there is a strong correlation between G protein levels and photoreceptor cell sensitivity, such that as we increase the amount of G protein, a corresponding increase in sensitivity is achieved. Maximal responses are obtained with ~50% of wild-type levels of DG_q.

In wild-type photoreceptors, single photon responses give rise to unitary events known as quantum bumps (Figure 7A). Quantum bumps are the result of the activation of a single rhodopsin molecule and reflect the amplification of the entire signaling pathway, culminating in the opening (or closing) of the light-activated channels. Given that the macroscopic current is quite abnormal in $G\alpha q^1$ mutants, we wanted to determine whether changes in the level of G protein affect quantum bump responses. We examined quantal responses in wild-type photoreceptors and $G\alpha q^1$ mutant photoreceptors expressing ~1% of the levels of normal DG_q and determined bump frequencies, amplitudes, and kinetics.

To analyze changes in the frequency of bump formation, we performed a "frequency of seeing" experiment. We determined the probability of bump occurrence at different light intensities using the method of Baylor, Lamb, and Yau (Baylor et al., 1979). In essence, wild-type and $G\alpha q^1$

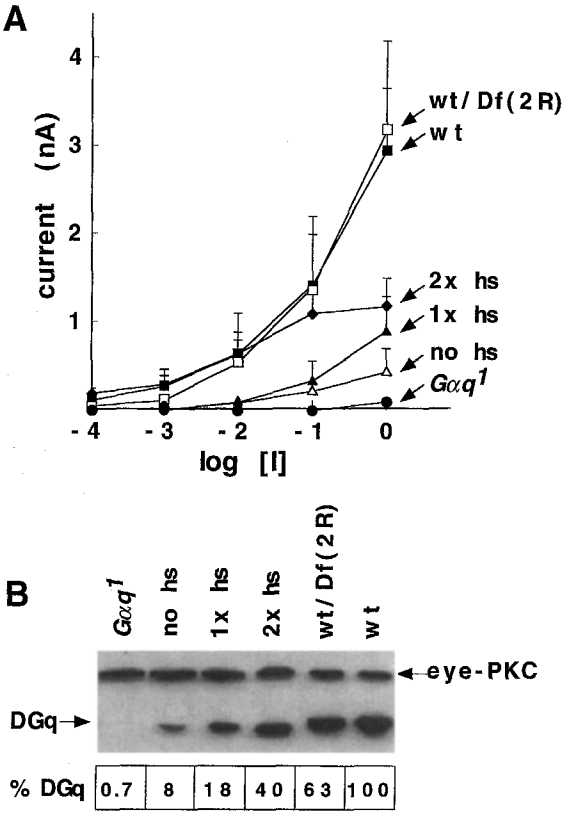

Figure 6. Sensitivity of the Photoreceptor Cells Can Be Manipulated In Vivo by Manipulating DG_{q1} Levels

(A) Response versus intensity curves demonstrate that light sensitivity increases as levels of DG_{q1} are increased in the *Gαq*^{1} mutant hosts. To express desired levels of DG_{q1}, P[hs:DG_{q1}] flies grown at 25°C were given one 2 hr heat shock at 37°C (1× hs), two 2 hr heat shocks at 37°C with 30 min at 22°C in between (2× hs), or no heat shocks (no hs). All cells were analyzed 3 hr post-heat shock. The figure shows that response amplitude and cell sensitivity increase as a function of the amount of DG_q. Each curve represents the averaged responses from 5–10 cells (± SD) to 10 ms flashes of 580 nm light.

(B) Western blot depicting the levels of DG_q protein expressed in each of the assayed lines. The blot was probed with antibodies to DG_{q1}. We also used antibodies to an eye-specific PKC (Smith et al., 1991a) to control for sample loading errors. Scanning densitometry analysis was used to determine the relative levels of DG_q present in each of the lines (normalized to wild-type levels and eye-PKC controls).

photoreceptors were stimulated with 580 nm light at three light intensities, starting at a threshold experimentally determined to generate quantum bumps. Each light intensity was then plotted against the probability of the occurrence of at least one quantum bump (probability of success [P_s] = number of events/number of trials). In wild-type and mutant cells, P_s increases as a function of light intensity. However, *Gαq*^{1} photoreceptors display a dramatic decrease in the probability of bump formation when compared with control cells (Figure 7B). Therefore, in the *Gαq*^{1} cells, a quantum bump does not represent a single photon response, but rather 1000 or so rhodopsin molecules need to be activated to generate a quantum bump successfully. This can be easily rationalized by understanding that the frequency with which activated rhodopsins find the G proteins and generate responses is decreased in the mutant, presumably because the low levels of DG_q limit the transduction of a signal from rhodopsin to downstream components. Consistent with defects in coupling, cells expressing small amounts of DG_q also display significant differences in the latency of bump formation (Figure 7D; $p = .0001$). Interestingly, the defects in quantum bump generation (i.e., the broad distribution of quantum bumps) are likely to underlie the aberrant deactivation kinetics of the macroscopic current of *Gαq*^{1} cells.

Assuming a Poisson distribution of bump amplitudes (Baylor et al., 1979), we calculated the mean bump amplitudes for wild-type and mutant cells using recordings performed under conditions in which $P_s < 1$, such that only a fraction of the trials produced a quantum bump (see Figure 7, legend). Interestingly, although mutant cells have macroscopic (and quantal) responses that are 1000 times less sensitive than those of wild-type controls, wild-type and *Gαq*^{1} cells generate quantum bumps with identical amplitudes (wild type: 24 ± 30 pA; *Gαq*^{1}: 22 ± 23 pA; $p = .980$; Figure 7). These results demonstrate that levels of DG_q do not actively contribute to the gain of the single photon response. Instead, G protein activation must act as an on–off switch simply reporting upstream activity; once a threshold level of G protein activation has been reached, a quantum bump occurs.

Concluding Remarks

In this manuscript we describe the isolation and characterization of the first known G_q protein mutant in a complex multicellular organism. We show that DG_q encodes a $G_{q\alpha}$ subunit, DG_{q1}, essential for phototransduction. We also demonstrate that DG_{q2} is not involved in photoreceptor cell function and likely represents a cloning artifact. Previous work suggested that *DG*$_{q2}$ also encodes a photoreceptor cell protein (Lee et al., 1990, 1994); however, our physiological, immunological, and molecular experiments show this not to be the case.

Phototransduction in Drosophila is the fastest known G protein–coupled signaling cascade. The latency between photon excitation and photoreceptor cell depolarization is only 10–20 ms (Ranganathan et al., 1991). Given that there are more than five biochemical steps between the beginning and end of this signaling pathway (receptor, G protein, effector, intracellular messenger, and ion channels; Zuker, 1992), this is a very fast signaling cascade; this tremendous speed is essential for a sensory modality like vision, which relies on high temporal resolution. We have shown that a dramatic reduction in the levels of G protein in vivo results in a significant increase in the latency of the macroscopic response, as would be expected for a diffusion-limited reaction.

We have used patch-clamp studies to show that the in vivo levels of G protein do not regulate the amplitude of single photon responses, demonstrating that the G protein functions only as a reporter of receptor activity rather than as a single photon amplifier with variable gain control. These results are consistent with the postulate that quantum bump generation occurs downstream of PLC activation (Pak et al., 1976). Using an inducible promoter to ma-

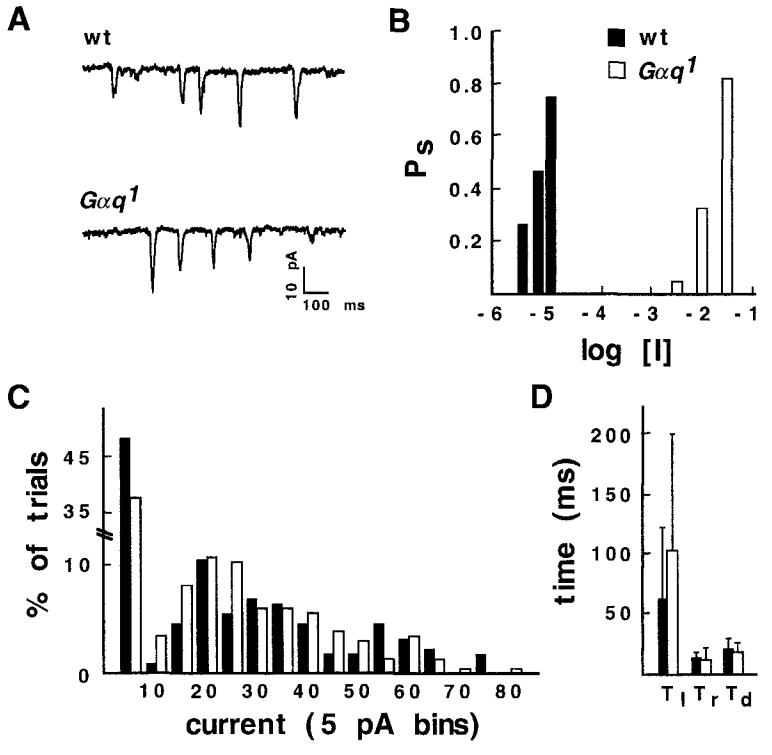

Figure 7. Shape of Single Photon Responses (Quantum Bumps) Is Normal in the $G\alpha q^1$ Mutants

(A) Sample traces of quantum bumps in wild-type flies and $G\alpha q^1$ mutants induced by continuous 580 nm light of log I = −6 for wild-type cells and log I = −3 for $G\alpha q^1$ cells.

(B) The frequency of quantum bump generation is dramatically decreased in $G\alpha q^1$ cells. Shown is a "frequency of seeing" experiment plotting the probability of evoking a quantum bump versus light intensity. Expectedly, P_s increases as a function of light intensity, both in wild-type and $G\alpha q^1$ cells. However, $G\alpha q^1$ photoreceptors are shifted 3 log orders in their light sensitivity.

(C) Amplitude histograms demonstrate that the mean bump amplitude is not notably different in $G\alpha q^1$ cells. Response amplitudes were measured for 239 $G\alpha q^1$ and 220 wild-type trials to a 10 ms flash of 580 nm light. Mean amplitudes were calculated as described by Baylor et al. (1979) by dividing the mean response amplitude by the mean number of events. The mean number of events (m) was calculated by assuming a Poisson distribution of events and solving for $P_o = e^{-m}$.

(D) Kinetics of quantum bumps. Shown are bar graphs comparing latency (T_l), rise time (T_r), and deactivation time (T_d) in wild-type and $G\alpha q^1$ cells. Only latency changes in the $G\alpha q^1$ photoreceptors. The analysis was performed on quantum bumps from 7 cells of each genotype.

nipulate protein levels experimentally, we also analyzed the relationship between G protein expression and photoreceptor cell sensitivity. Our results provide firm evidence for fine control of G protein levels and its importance in regulating the macroscopic current and photoreceptor cell sensitivity.

Finally, the availability of this mutant, together with the recent solution of the crystal structure of a prototypical G protein (Noel et al., 1993; Sondek et al., 1994), now allows for rigorous structure–function studies of G protein function in a model phosphoinositide G protein–coupled transduction pathway suitable to in vivo manipulations. Furthermore, the availability of this and other Drosophila mutants defective in specific aspects of the phototransduction cascade make it possible to design rigorous genetic and physiological experiments to dissect the functioning and regulation of this signaling cascade in its normal cellular and organismal environment.

Experimental Procedures

Mutant Screen and Western Blots

Ethyl methanesulfonate–treated *cn* males were crossed to Df(2R)vg^{135}. These flies carry a deficiency that uncovers the *dgq* locus (Heiman and Beckingham, 1992; Lindsley and Zimm, 1992). Single F1 males were backcrossed to Df(2R)vg^{135}/CyO females, and the non-*Cy* progeny were subjected to Western blot analysis exactly as previously described (Dolph et al., 1993). In essence, single fly heads were removed from flies heterozygous for the mutagenized second chromosome and Df(2R)vg^{135} and sonicated for 3 s in SDS Laemmli buffer. Samples were loaded on 10% SDS–polyacrylamide (one head per mutant per lane) and allowed to enter the gel for 10 min, and then protein extract from a second mutant was loaded so as to minimize the number of gels that need to be processed. A total of 30 individual flies representing 30 treated chromosomes could be screened on a single gel. Blots were incubated with the anti-DG_{q1} antibody and often a control antisera (anti-TRP-I) to check for the amount of extract loaded in the gels. We used an antibody generated against a 15 amino acid peptide from exon 7 of DG_q (see below) in all the screens. This exon is specific to a *dgq* splice form found in the visual system (Dean Smith, personal communication).

Antibodies

To generate antibodies specific to DG_{q1} and DG_{q2} and antibodies that recognize both forms, we synthesized peptides corresponding to DG_{q1}-specific residues 306–320 (KYLACNPDPERQCYS), the DG_{q2}-specific junction of exons 6 and 8 (residues 286–290 and 327–332; FPEYDDTENIK), and a region common to DG_{q1} and DG_{q2}, residues 339–353 (KDTIMQNALKEFNLG). Peptides were coupled to carrier protein and injected into rabbits and rats; antisera was then affinity purified as described previously (Cassill et al., 1991). All antibodies were checked for specificity and affinity using wild-type, mutant, and transgenic controls.

PCR Reactions

The *dgq* genomic region from wild-type and $G\alpha q^1$ mutants was amplified as two overlapping fragments in independent PCR reactions (Smith et al., 1991a). We used oligonucleotide probes corresponding to residues 570–591 and 1701–1725 of DG_q cDNA. PCR products from multiple PCR reactions were sequenced in each case to eliminate possible errors occurring during PCR amplification. We also determined the nucleotide sequence of the corresponding transcripts by reverse transcriptase–PCR (Maniatis et al., 1982). Poly(A)-containing RNA was isolated from mutant heads and wild-type controls as described (Zuker et al., 1985). PCR reactions were carried out using *Taq* polymerase.

Electrophysiological Recordings

Photoreceptors were isolated and whole-cell patch-clamp recordings were performed as previously described (Ranganathan et al., 1991). The bath solution contained 124 mM NaCl, 4 mM KCl, 10 mM HEPES, 5 mM proline, 25 mM sucrose, and 1.5 mM $CaCl_2$ (pH 7.15). The pipette

solution contained 95 mM potassium gluconate, 40 mM KCl, 10 mM HEPES, 2 mM $MgCl_2$, and 0.2 mM EGTA (pH 7.15).

Photoreceptors were clamped at a holding potential of −40 mV. Whole-cell capacitances were >35 pF and seal resistances were >1 GΩ. Junction potentials were nulled just before seal formation, and most (80%) series resistance errors were compensated during recording. In all experiments, light was filtered through a bandpass filter (λ = 580 ± 10 nm) and neutral density filters and focused onto the photoreceptor cells via a 0.5 numerical aperture, 40 × objective. Stimulation was by means of a 75 W Xenon source; unattenuated output at the stage was 10 mW for white light.

For quantum bump analysis, photoreceptors were clamped at a holding potential of −80 mV. Signals were sampled at 1 kHz, amplified 50 ×, and filtered at 500 Hz.

DNA Constructs and Transgenic Flies

A 900 bp DG_q fragment containing exons 1–6 was generated by PCR, using primers from 570–591 and 1472–1493. This fragment was labeled using a random priming kit (Amersham) and used to screen a λZAP Drosophila retinal cDNA library (C. Z., unpublished data), and several independent full-length clones of DG_{q1} cDNA were isolated. Despite repeated attempts, no DG_{q2} cDNA was ever recovered. Identical results were obtained in PCR reactions templated with reverse transcriptase cDNA isolated from wild-type retinas or heads. We constructed a DG_{q2} cDNA by ligating the 900 bp PCR product containing exons 1–6 to a PCR product containing exon 8. To ensure in-frame linkage, we designed PCR primers containing unique restriction sites by introducing a conservative base pair change in the coding sequence of exon 6. All PCR products, cDNAs, and reconstructed genes were sequenced in their entirety.

DG_{q1} and DG_{q2} cDNAs were also cloned into a Drosophila transformation vector under the control of the heat-shock promoter (Baker et al., 1994) and injected into wild-type and $G\alpha q^1$ mutant embryos. P element–mediated germline transformations and all subsequent fly manipulations were performed using standard techniques.

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References

Baker, E., Colley, N., and Zuker, C. (1994). The cyclophilin homolog ninaA functions as a chaperone forming a stable complex *in vivo*, with its protein target, rhodopsin. EMBO J. *13*, 4886–4895.

Baylor, D.A., Lamb, T.D., and Yau, K.-W. (1979). Responses of retinal rods to single photons. J. Physiol. *288*, 613–634.

Berlot, C.H., and Bourne, H.R. (1992). Identification of effector-activating residues of $G_{s\alpha}$. Cell *68*, 911–922.

Bloomquist, B., Shortridge, R., Schneuwly, S., Perdew, M., Montell, C., Steller, H., Rubin, G., and Pak, W. (1988). Isolation of a putative phospholipase C gene of Drosophila, *norpA*, and its role in phototransduction. Cell *54*, 723–733.

Cassill, J., Whitney, M., Joazeiro, C., Becker, A., and Zuker, C. (1991). Isolation of *Drosophila* genes encoding G protein-coupled receptor kinases. Proc. Natl. Acad. Sci. USA *88*, 11067–11070.

Conklin, B.R., and Bourne, H.R. (1993). Structural elements of Gα subunits that interact with Gβγ, receptors, and effectors. Cell *73*, 631–641.

Dolph, P.J., Ranganathan, R., Colley, N.J., Hardy, R.W., Socolich, M., and Zuker, C.S. (1993). Arrestin function in inactivation of G protein-coupled receptor rhodopsin *in vivo*. Science *260*, 1910–1916.

Dolph, P.J., Man-Son-Hing, H., Yarfitz, S., Colley, N.J., Deer, J.R., Spencer, M., Hurley, J.B., and Zuker, C.S. (1994). An eye-specific Gβ subunit essential for termination of the phototransduction cascade. Nature *370*, 59–61.

Gilman, A. (1987). G proteins: transducers of receptor-generated signals. Annu. Rev. Biochem. *56*, 615–649.

Hardie, R. (1991). Whole-cell recordings of the light induced current in dissociated Drosophila photoreceptors—evidence for feedback by calcium permeating the light-sensitive channels. Proc. R. Soc. Lond. (B) *245*, 203–210.

Heiman, R., and Beckingham, K. (1992). Mutation vg^{135} is a deletion and an inversion not a simple deletion. Dros. Inf. Serv. *71*, 154–155.

Itoh, H., and Gilman, A.G. (1991). Expression and analysis of $G_{s\alpha}$ mutants with decreased ability to activate adenylyl cyclase. J. Biol. Chem. *266*, 16226–16231.

Lagnado, L., and Baylor, D.A. (1994). Calcium controls light-triggered formation of catalytically active rhodopsin. Nature *367*, 273–277.

Lee, Y.-J., Dobbs, M.B., Verardi, M.L., and Hyde, D.R. (1990). *dgq*: a Drosophila gene encoding a visual system–specific G_α molecule. Neuron *5*, 889–898.

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

Lindsley, D.L., and Zimm, G.G. (1992). Genome of *Drosophila melanogaster*. (San Diego, California: Academic Press).

Maniatis, T., Fritsch, E.F., and Sambrook, J. (1982). Molecular Cloning: A Laboratory Manual. (Cold Spring Harbor, New York: Cold Spring Harbor Laboratories).

Neer, E.J. (1995). Heterotrimeric G proteins: organizers of transmembrane signals. Cell *80*, 249–257.

Noel, J.P., Hamm, H.E., and Sigler, P.B. (1993). The 2.2 Å crystal structure of transducin-α complexed with GTPγS. Nature *366*, 654–663.

O'Tousa, J.E., Baehr, W., Martin, R.L., Hirsh, J., Pak, W.L., and Applebury, M.L. (1985). The Drosophila *ninaE* gene encodes an opsin. Cell *40*, 839–850.

Pak, W.L., Ostroy, S.E., Deland, M.C., and Wu, C.-F. (1976). Photoreceptor mutant of *Drosophila*: is protein involved in intermediate steps of phototransduction? Science *194*, 956–959.

Ranganathan, R., Harris, G.L., Stevens, C.F., and Zuker, C.S. (1991). A *Drosophila* mutant defective in extracellular calcium dependent photoreceptor inactivation and rapid desensitization. Nature *354*, 230–235.

Ranganathan, R., Bacskai, B.J., Tsien, R.Y., and Zuker, C.S. (1994). Cytosolic calcium transients: spatial localization and role in Drosophila photoreceptor cell function. Neuron *13*, 837–848.

Ranganathan, R., Malicki, D.M., and Zuker, C.S. (1995). Signal transduction in *Drosophila* photoreceptors. Annu. Rev. Neurosci. *18*, in press.

Rarick, H.M., Artemyev, N.O., and Hamm, H.E. (1992). A site on rod G protein α subunit that mediates effector activation. Science *256*, 1031–1033.

Simon, M., Strathmann, M., and Gautam, N. (1991). Diversity of G proteins in signal transduction. Science *252*, 802–808.

Smith, D.P., Ranganathan, R., Hardy, R.W., Marx, J., Tsuchida, T., and Zuker, C.S. (1991a). Photoreceptor deactivation and retinal degeneration mediated by a photoreceptor-specific protein kinase C. Science *254*, 1478–1484.

Smith, D.P., Stamnes, M.A., and Zuker, C.S. (1991b). Signal transduction in the visual system of *Drosophila*. Annu. Rev. Cell Biol. *7*, 161–190.

Sondek, J., Lambright, D.G., Noel, J.P., Hamm, H.E., and Sigler, P.B. (1994). GTPase mechanism of G proteins from the 1.7 Å crystal structure of transducin α-GDP-AlF-4. Nature *372*, 276–279.

Stamnes, M.A., Shieh, B.-H., Chuman, L., Harris, G.L., and Zuker, C.S. (1991). The cyclophilin homolog ninaA is a tissue-specific integral

membrane protein required for the proper synthesis of a subset of Drosophila rhodopsins. Cell *65*, 219–227.

Wolff, T., and Ready, D. (1993). Pattern formation in the *Drosophila* retina. In The Development of *Drosophila melanogaster*, M. Bate and A.M. Arias, ed. (Cold Spring Harbor, New York: Cold Spring Harbor Press), pp. 1277–1326.

Yarfitz, S., Provost, N.M., and Hurley, J.B. (1988). Cloning of a *Drosophila melanogaster* guanine nucleotide regulatory protein β-subunit gene and characterization of its expression during development. Proc. Natl. Acad. Sci. USA *85*, 7134–7138.

Yarfitz, S., Niemi, G.A., McConnell, J.L., Fitch, C.L., and Hurley, J.B. (1991). A G_{β} protein in the Drosophila compound eye is different from that in the brain. Neuron *7*, 429–438.

Yoshioka, T., Inoue, H., and Hotta, Y. (1983). Defective phospholipid metabolism in the retinular cell membrane of *norpA* (no receptor potential) visual transduction mutants of *Drosophila*. Biochem. Biophys. Res. Comm. *111*, 567–573.

Zuker, C.S. (1992). Phototransduction in *Drosophila*: a paradigm for the genetic dissection of sensory transduction cascades. Curr. Opin. Neurobiol. *2*, 622–627.

Zuker, C.S., Cowman, A.F., and Rubin, G.M. (1985). Isolation and structure of a rhodopsin gene from D. melanogaster. Cell *40*, 851–858.