Cellular/Molecular

Translocation of $G_q\alpha$ Mediates Long-Term Adaptation in *Drosophila* Photoreceptors

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Light adaptation is a process that enables photoreceptor cells to operate over a wide range of light intensities without saturation. In invertebrate photoreceptors, fast adaptation is mediated by a Ca^{2+}-dependent negative-feedback mechanism, which mainly affects the terminal steps of the cascade. Therefore, the response to each photon is smaller as light intensity increases, accommodating both high sensitivity and a vast dynamic range. Here, we describe a novel type of adaptation, which is mediated by one of the first steps in the phototransduction cascade affecting the sensitivity to absorbed photons. Long exposure to light resulted in dramatic reduction in the probability of each absorbed photon to elicit a response, whereas the size and shape of each single photon response did not change. To dissect the molecular mechanism underlying this form of adaptation we used a series of *Drosophila* mutants. Genetic dissection showed a pivotal role for light-induced translocation of $G_q\alpha$ between the signaling membrane and the cytosol. Biochemical studies revealed that the sensitivity to light depends on membrane $G_q\alpha$ concentration, which was modulated either by light or by mutations that impaired its targeting to the membrane. We conclude that long-term adaptation is mediated by the movement of $G_q\alpha$ from the signaling membrane to the cytosol, thereby reducing the probability of each photon to elicit a response. The slow time scale of this adaptation fits well with day/night light intensity changes, because there is no need to maintain single photon sensitivity during daytime.

Key words: **heterotrimeric G_q protein; quantum bumps; phototransduction; light adaptation; *Drosophila* mutants; protein translocation**

Introduction

Light and dark adaptation in *Drosophila* photoreceptors include fast Ca^{2+}-dependent (<1 min) adaptation and long-term adaptation, which operates on a time scale of many minutes and arises from diverse processes. Examples of long-term adaptation processes are the light-dependent translocation of arrestin 2 (Lee et al., 2003) and of light-sensitive channel transient receptor potential like (TRPL) out of the signaling membrane (Bähner et al., 2002; Cronin et al., 2006).

In invertebrate photoreceptors, heterotrimeric G_q-protein is an essential component of phototransduction, connecting photon absorption by rhodopsin to phospholipase C (PLC) activation and is mainly localized to the plasma membrane domain. The two major determinants of membrane $G_q\alpha$ localization are the dynamic lipid modification of G-proteins by palmitoylation (Wedegaertner et al., 1993) and anchoring of the $G\beta\gamma$ subunits to the membrane (Elia et al., 2005). Light-driven translocation of the vertebrate (Sokolov et al., 2002) and invertebrate G-proteins was found in photoreceptor cells [invertebrates: crayfish (Terakita et al., 1996), squid (Suzuki et al., 1995), *Drosophila* (Kosloff et al., 2003; Cronin et al., 2004)]. In *Drosophila* photoreceptors, the rhodopsin-activated $G_q\alpha$ translocates to the cytosol during illumination and subsequently returns to the membrane (Kosloff et al., 2003; Cronin et al., 2004). Despite previous studies showing that a large reduction in the concentration of $G_q\alpha$ in *Drosophila* mutants reduces the sensitivity to light (Scott et al., 1995), these studies have not experimentally examined whether light-dependent dynamic changes of membrane $G_q\alpha$ concentration mediate long-term adaptation under physiological conditions.

Drosophila photoreceptors exhibit the ultimate sensitivity to light because they are able to detect the absorption of single photons. This property allows measuring of the physiological implication of $G_q\alpha$ movements *in vivo* at the highest resolution. Photon absorption by rhodopsin in fly photoreceptors activates either one (Scott et al., 1995) or few $G_q\alpha$ protein molecules (Minke and Stephenson, 1985; Hardie et al., 2002), which subsequently activates PLC (Devary et al., 1987; Bloomquist et al., 1988) and leads, in a still unclear way, to the generation of a single-photon response called a quantum bump (Yeandle and Spiegler, 1973). The bumps sum to produce the macroscopic response to light (Dodge et al., 1968; Barash and Minke, 1994). Fast light adaptation is known to reduce the amplitude, but not the frequency of the bump production (Wong et al., 1982) by a still unclear Ca^{2+}-dependent mechanism (Lisman and Brown, 1972; Henderson et al., 2000).

In the present study, we identified a novel mechanism of long-term adaptation, which was manifested in a large (approximately

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fivefold) reduction of bump frequency. Genetic dissection using the phototransduction mutants $G\beta_e{}^2$ and myosin III $ninaC^{P235}$ revealed that both long-term adaptation and the targeting of $G_q\alpha$ to the signaling membrane were markedly impaired in these mutants. In addition, a reduction in rhabdomeric $G_q\alpha$ by light or by G-protein mutations was correlated with a reduction in sensitivity to light during long-term adaptation. Therefore, we conclude that dynamic changes of membrane $G_q\alpha$, which translocates in and out of the signaling membrane in a light-dependent manner, constitute a novel mechanism of long-term adaptation.

Materials and Methods

Fly stocks and light protocols. Drosophila melanogaster of the following strains were used: wild-type Oregon-R *w*, $Gq\alpha^1$ and heterozygote $Gq\alpha^1$, $G\beta_e{}^1$ and heterozygote $G\beta_e{}^1$, $G\beta_e{}^2$, $arr2^3$, $ninaC^{P235}$, $Rh1^{\Delta 356}$, and $trpl^{302}$. Unless otherwise stated, flies were raised at 24°C in a 12 h light/dark cycle and the pupae were placed 13 cm from white fluorescent light (L 36, 20 W; OSRAM, Munich, Germany) 4–6 h before eclosion (light adapted), and then the vials were also transferred into darkness for 2 h before eclosion (dark adapted).

Electrophysiology. To measure light responses, red (RG 610 edge filter; Schott, Mainz, Germany), orange (OG 590 Schott edge filter) or white lights were used. The light source was a xenon high-pressure lamp (75 W) and the light stimuli were delivered to isolated ommatidia via the objective lens (40×; Zeiss, Oberkochen, Germany) and attenuated up to approximately six orders of magnitude by neutral density filters as indicated. The relative intensity of red and white lights is expressed in terms of equivalent intensity of orange lights (Schott OG 590 edge filter) that elicited the same frequency of quantum bumps. The maximal luminous intensity, I_0, of the orange light at the level of the ommatidia was 3.2 mW/cm^2. This absolute light intensity was 3.3 log units higher than the light intensity that induced the half-maximal voltage response of dark adapted $trpl^{302}$ mutant flies during current-clamp measurements. Dissociated ommatidia were prepared from newly eclosed adult flies (<1 h posteclosion). In all cases, ~20 min of very dim red light was applied during the dissection of the eye before the beginning of the experiments. Whole-cell voltage-clamp recordings were performed as described previously (Hardie and Minke, 1992; Peretz et al., 1994). In short, recordings were made at 21°C using patch pipettes of 8–12 MΩ pulled from fiber-filled borosilicate glass capillaries. Series resistance was below 25 MΩ and was carefully compensated (>75%) for most of the experiments. Signals were amplified using Axopatch-200B (Molecular Devices, Sunnyvale, CA) patch-clamp amplifier and the current was sampled at 2 kHz and filtered below 1 kHz. For bump analysis, the membrane potential was kept at −70 mV and signals were filtered below 100 Hz. Currents were sampled using the Digidata card and analyzed by the pClamp 9.0 software (Molecular Devices). For recordings in the current-clamp mode, a small negative current of <50 pA was applied to recorded cells to fix the resting potential of the photoreceptor at −60 ± 2 mV. Before application of this current, the resting potential of all of the recorded cells in our solutions (see below) was in the range of 40–55 mV and there was no significant difference in the resting potentials of dark and light raised flies. The bath solution contained (in mM) 120 NaCl, 5 KCl, 10 *N*-Tris-(hydroxymethyl)-methyl-2-amino-ethanesulphonic acid, pH 7.15 (TES buffer), 4 $MgSO_4$, and 1.5 $CaCl_2$. For all experiments, the whole-cell recording pipette contained (in mM) 140 K-gluconate, 2 $MgSO_4$, 10 TES buffer, pH 7.15, 4 MgATP, 0.4 Na_2GTP, and 1 nicotinamide adenine dinucleotide.

Assay of light-dependent $G\alpha_q$ localization. Assay for light-dependent localization of $G_q\alpha$ was performed as described previously (Kosloff et al., 2003). In short, dark adapted flies were subjected to illumination with either blue or white light (18 W white light lamp, 13 cm away from the flies, with or without a blue Schott BG 28 broad-band filter) for various durations at 22°C. Control experiments showed that white and blue illuminations give similar results. Termination was performed by transferring the flies to 4°C in the dark and promptly separating the fly heads. Ten flies were used for each time point. The fly heads were homogenized in 1 ml of hypotonic homogenization buffer (20 mM HEPES, pH 7.6, 20 μg/ml leupeptin, 1 μg/ml pepstatin A, 0.35 mg/ml *o*-phenantroline, 15 mM *N*-ethylmaleamide). Membranes and cytosol were separated by centrifugation (15,800 × *g* for 15 min at 4°C). The pellet was washed, centrifuged again, and the supernatants were combined. Ultracentrifugation at 150,000 × *g* for 30 min did not precipitate additional $G\alpha_q$-proteins or change the distribution between the fractions. The proteins were precipitated by 5% TCA, run on 10% SDS-PAGE, and subjected to quantification. After separation by SDS-PAGE, Western blotting was performed using an anti-$G_q\alpha$ polyclonal antibody (Kosloff et al., 2003). Relative protein amounts on the same gel were determined by quantification of the ECL signal using the Fuji (Tokyo, Japan) LAS-1000 system. To reduce the variance caused by the experimental procedure, the amounts of $G_q\alpha$ in each fraction were calculated as a percentage of the total $G_q\alpha$ in both the pellet and supernatant of each treatment.

Results

Prolonged illumination of dark-raised flies revealed a novel type of adaptation

To investigate the effects of prolonged illumination on the sensitivity to light in the most sensitive manner, we used whole-cell voltage-clamp recordings from isolated *Drosophila* ommatidia and measured the response to absorption of single photons (quantum bumps). Wild-type (WT) flies were raised in a 12 h dark/light cycle and then were either illuminated (light raised) or kept in the dark (dark raised) for ~12 h before eclosion. To examine the effect of previous illumination on the response to light at the quantum bump level, we applied very dim red lights of increasing intensities and measured the effect of previous illumination during the raising period on the properties of the quantum bums. Surprisingly, the only parameter that was affected by the previous illumination conditions was the bump frequency and not the bump shape and amplitude. A relatively high rate of bump frequency was observed in photoreceptors of dark-raised flies relative to the bump frequency of light-raised flies in response to the same light stimuli (Fig. 1*A*, compare $-\log I = 5.5$). Importantly, a lower sensitivity to light, as manifested by lower bump frequency in light-raised flies was not accompanied by changes in bump-shape parameters relative to dark-raised flies (Fig. 1*B*). The measured bump-shape parameters included the average peak amplitude (Fig. 1*B*, amplitude, left columns) and shape. The shape was characterized by average bump duration at half-maximal amplitude (Fig. 1*B*, half width, middle columns) and the total charge of the bump (Fig. 1*B*, area, right columns).

Because prolonged illumination, which reduced membrane $G_q\alpha$ to 30% of maximum (Kosloff et al., 2003), did not change the bump shape and amplitude (Fig. 1*B*), we also examined whether the shape and amplitude of the quantum bumps are modified when the G_q level is further reduced by mutations in $G_q\alpha$. In Figure 1*A* (bottom line), we examined the effects of reduction in membrane $G\alpha_q$ concentration to ~15 and ~1% by prolonged illumination of the heterozygote $Gq\alpha^1/+$ and in homozygote $Gq\alpha^1$ mutants, respectively (for measurements of $G_q\alpha$ level in these mutants, see Figs. 6, 7). Figure 1, *A* (bottom line, left) and *B*, shows that there is no significant difference between the bump shape and amplitude of WT flies (either dark or light raised) and light-raised heterozygote $Gq\alpha1/+$ flies. However, when the $G_q\alpha$ level was drastically reduced by the homozygote $Gq\alpha^1$ mutation to ~1%, which is much below the physiological range of G_q modulations, the bump amplitude was significantly reduced (Fig. 1*A*, bottom line, right) as described previously (Hardie et al., 2002).

To distinguish between the reduction in sensitivity to light after prolonged illumination (Fig. 1) and the known Ca^{2+}-dependent fast light adaptation (Henderson et al., 2000), we elic-

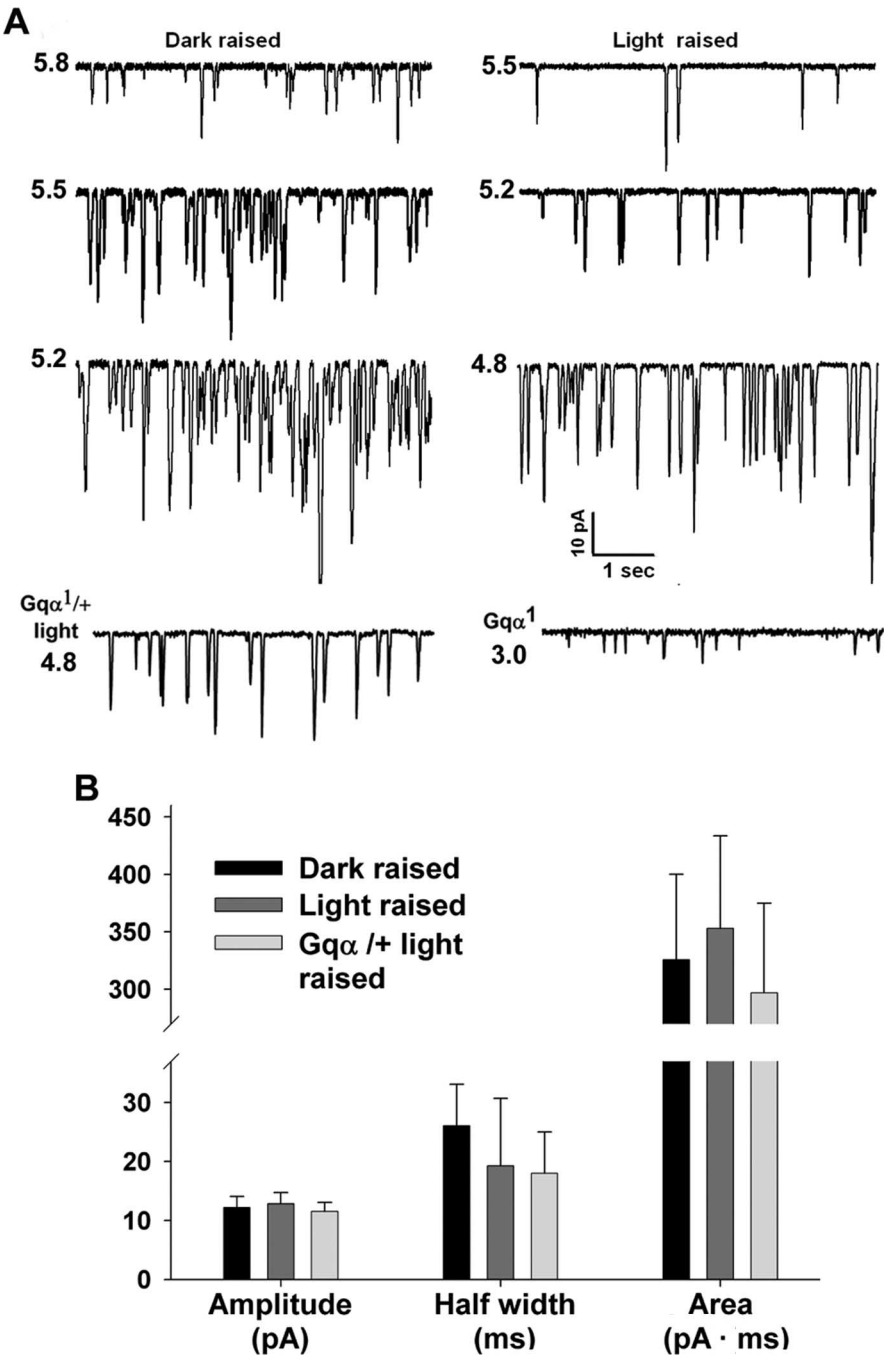


Figure 1. Prolonged illumination reduces quantum bump frequency without affecting bump shape and amplitude. ***A***, Bumps elicited in response to different intensities of dim red lights in dark- and light-raised WT *Drosophila* (3 top lines) and in light-raised $Gq\alpha^1/+$ and dark-raised $Gq\alpha^1$ mutants (bottom line). The left column shows bumps recorded from dark-raised and the right from light-raised WT flies. The relative light intensity *I* is presented in −log units on the left of each trace. The relative intensity of the dim red lights is expressed in terms of equivalent intensity of orange lights (Schott OG 590 edge filter) that elicited the same frequency of quantum bumps in all figures. Notice that light intensity is different for the left and right columns. ***B***, A histogram showing that despite a large difference in bump frequency between the two populations of dark- and light-raised WT flies and in the light-raised $Gq\alpha^1/+$ mutant, no significant difference in bump shape parameters is observed. In contrast, in the homozygote $Gq\alpha^1$ mutant, in which $G_q\alpha$ is drastically reduced, the bump amplitude is highly reduced. Error bars are SEM in all figures. There is no significant difference among the various columns ($p > 0.05$; $n = 5$–9).

ited bumps by application of prolonged dim red background light to dark adapted flies and then superimposed a short (5 s) intense orange adapting light on the background illumination (Fig. 2*A*). During the initial 1 s after the cessation of the adapting light, the bump amplitude was reduced below the noise level (Fig. 2*B*, arrow). The bump amplitude increased quickly with time, reaching ~70% of the averaged dark adapted level within 10 s (Fig. 2*C*). The average reduction in bump amplitude fully recovered to the original dark adapted level in <1 min (Fig. 2*C*). We also found that the bump shape parameters, which were significantly reduced after the adapting light, returned to their previous level in <1 min (data not shown). Together, the data in Figure 2 demonstrate that fast adaptation is characterized by a large reduction in bump amplitude, which recovered in <1 min.

Figure 1 reveals a new mechanism of adaptation solely affecting bump frequency. We designate this mechanism long-term adaptation throughout this work. Together, Figures 1 and 2 indicate that fast and long-term adaptation represent entirely separate yet complementary adaptation processes: fast adaptation affects bump shape and amplitude, whereas long-term adaptation affects only bump frequency.

Both the changes in bump frequency and in peak amplitude of the light-induced current can be used to characterize long-term adaptation

To quantify long-term adaptation in terms of bump frequency, we plotted bump frequency as a function of relative light intensity of prolonged stimuli (*R*-log-*I* curve) for dark- or light-raised WT flies, as indicated (Fig. 1*A*). Figure 3*A* shows an increase in the bump frequency in dark-raised flies relative to light-raised flies as manifested by an ~0.6 log shift of the *R*-log-*I* curve (Fig. 3*A*, dotted line). We designated this shift in sensitivity to light "sensitivity shift" throughout this study.

Bump frequency can be measured reliably only at a limited range of very dim light intensities because the bumps readily superimpose to form a macroscopic light-induced current (LIC) even in dim red lights (Fig. 1*A*). Bump frequency could not be measured reliably in some of the mutants used in this work because the mutant bumps were too small to be reliably detected (e.g., in the *trp* mutant) (Henderson et al., 2000), or because a large frequency of spontaneous bumps masked the detection of light-induced bumps (e.g., the $G\beta_e^1/+$ mutant) (Elia et al., 2005). Thus, the use of various *Drosophila* mutants for analyzing the molecular mechanism underlying long-term adaptation required extending the measurements to a wider range of light intensities. To accomplish this, we derived the *R*-log-*I* curves from measurements of the macroscopic LIC.

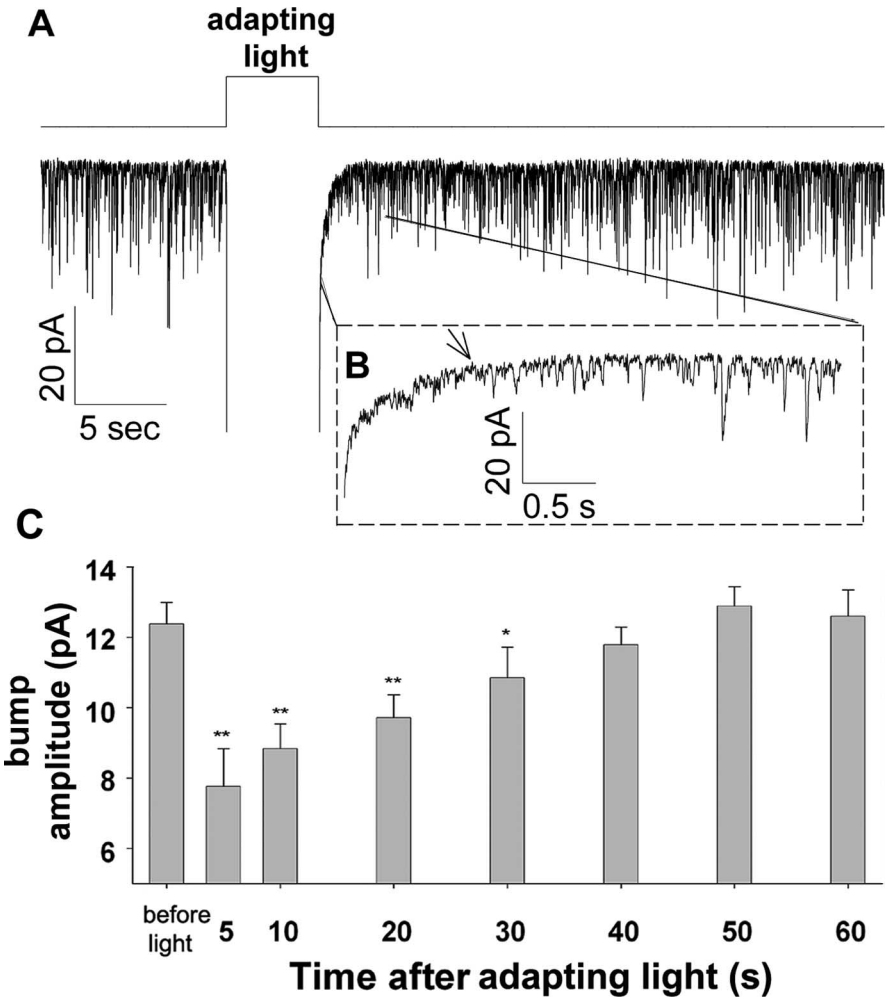


Figure 2. Fast adaptation affects bump amplitude. ***A***, Dim red light ($-\log I = 5$) elicited a continuous production of bumps, which was interrupted by application of intense orange light (adapting light, $-\log I = 1.0$) for 5 s that elicited LIC with amplitude out of scale. ***B***, The initial ~4 s of ***A*** after the cessation of the adapting light is displayed in an expanded time scale as indicated. The arrow indicates 1 s after the cessation of the adapting light. ***C***, Histogram plotting the average peak amplitude of the bumps before and after application of the adapting light as a function of time after the cessation of the adapting light as indicated. $^{**}p < 0.01$, $^{*}p < 0.05$, *t* test ($n = 5$).

To determine the sensitivity shift from the macroscopic LIC, we plotted the peak amplitude of the LIC as a function of increasing light intensities (the *R*-log-*I* curve) in light-raised (Fig. 3*B*, empty circles) and dark-raised (Fig. 3*B*, filled circles) WT flies. The sensitivity shift was determined by shifting the position of the *R*-log-*I* curve of the light-raised flies to achieve the best fit to the *R*-log-*I* curve of dark-raised WT flies that was measured under the same experimental conditions. This resulted in a shift of 0.6 log units (Fig. 3*B*, dotted line), similar to the shift obtained by measuring bump frequency. The *R*-log-*I* curve was measured in the range of dim to medium light intensities (peak LIC, <2000 pA). Increasing the intensity range to include intense lights (approximately six orders of magnitudes) did not lead to saturation of the LIC, nor did it affect the magnitude of the shift of the *R*-log-*I* curve and, thus, did not arise from response compression (Fig. 3*B*, inset). However, at the high-intensity range, the response amplitude already elicited currents larger than 15 nA, which are too large to allow reliable whole-cell patch-clamp recordings (Fig. 3*B* inset). Therefore, we limited our measurements to the dim-medium light intensity range (Fig. 3*B*). Figure 3, *A* and *B*, further shows that a similar sensitivity shift was obtained from the *R*-log-*I* curves measured from either the macroscopic LIC or bump frequency under similar previous illumination conditions.

The effect of illumination during rearing conditions on the voltage response to light is by far more complex than its effect on the LIC. One aspect of this complexity is demonstrated by the large afterhyperpolarization of voltage response in dark-raised flies and its absence in light-raised flies in response to the same saturating stimulus (Fig. 3*C*, inset) (Wolfram and Juusola, 2004). The complexity of the voltage response to light was the main reason to use voltage-clamp rather than current-clamp recordings in our study. Nevertheless, to further support our conclusion that the sensitivity shift that we measured under the voltage-clamp condition did not arise from response compression, we plotted *R*-log-*I* curves using data from the current-clamp recording mode from isolated ommatidia of the $trpl^{302}$ mutant, which expresses only TRP channels (Niemeyer et al., 1996). We used this mutant to maintain a constant composition of the light-sensitive channels in the rhabdomere and, thus, to ensure a single reversal potential in dark- and light-raised flies (Bähner et al., 2002). Figure 3*C* presents *R*-log-*I* curves measured from the peak amplitude of the voltage responses to light of increasing intensities of dark-raised (filled circles) and light-raised $trpl^{302}$ mutant flies. The *R*-log-*I* curves measured under current-clamp conditions reached saturation at intense lights as expected from voltage responses (Fig. 3*C*). Figure 3*C* shows a 0.7 log unit shift of the *R*-log-*I* curve measured from dark-raised flies after prolonged illumination. Voltage responses recorded in current-clamp mode are known to simulate the physiological response to light. Therefore, the similarity between the sensitivity shifts obtained in voltage- and current-clamp modes indicates that the shift obtained in voltage-clamp mode is reliable and represents physiological phenomenon.

An example of the analysis of the effect of a mutation on long-term adaptation is illustrated in Figure 3*D*. First, the *R*-log-*I* curves of illuminated and dark-adapted WT and G-protein mutant flies were measured. Then, the positions of the *R*-log-*I* curve of the dark- and light-adapted mutant and light-adapted WT flies were shifted to achieve best fit to the *R*-log-*I* curve of dark-adapted WT flies that was measured under the same experimental conditions. The shift of the *R*-log-*I* curve (defined as the sensitivity shift) demonstrates how the *R*-log-*I* curve of light-adapted heterozygote $G_q\alpha^1$ ($G_q\alpha^1/+$) flies was best fitted to the *R*-log-*I* curve of dark-adapted WT flies by a shift of 1.05 log units toward less intense light (Fig. 3*D*, dotted line)

Strong inhibition of long-term adaptation induced by mutations that affect the targeting of $G_q\alpha$ to the plasma membrane

The most convenient and reliable way to characterize long-term adaptation in our study was to plot the *R*-log-*I* curve by measuring the

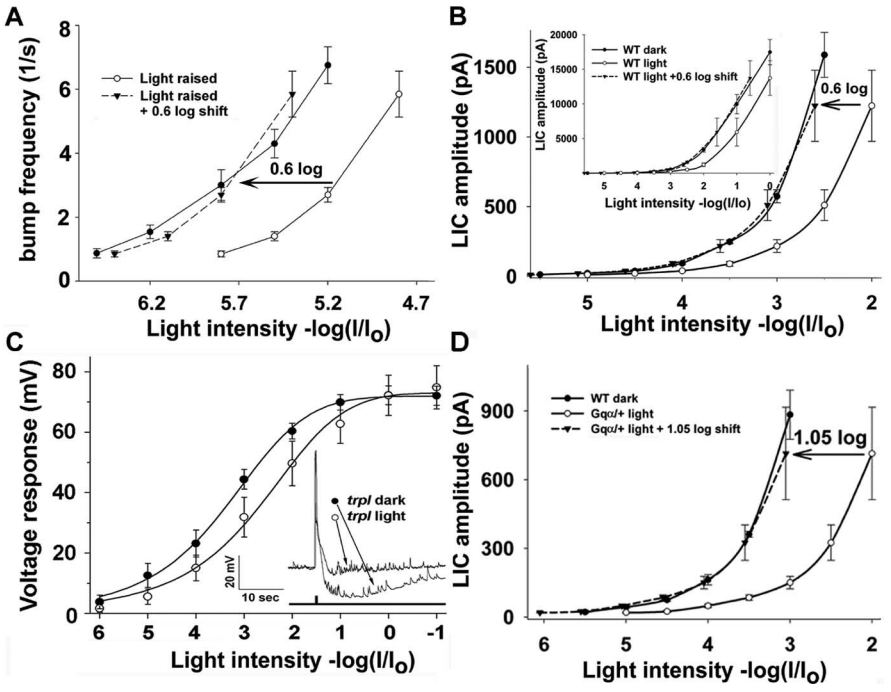


Figure 3. Both the changes in bump frequency and in peak amplitude of the LIC can be used to characterize long-term adaptation. ***A***, Average bump frequency (see Fig. 1) is plotted as a function of relative orange light intensity for light-raised and dark-raised flies ($n = 4$). To measure the sensitivity shift, the *R*-log-*I* curve of the light-raised flies was shifted by 0.6 log units (dashed line, triangles). Note that at higher light intensities, the bump frequency is underestimated because of superposition of the bumps. ***B***, The peak amplitude of the LIC of dark- (filled circles) and light-raised (empty circles) flies is plotted against the relative orange light intensity at dim and medium range. The dashed line (triangles) shows a 0.6 log unit shift of the light-adapted curve, which fits the dark-adapted curve. Inset, The data in ***B*** are presented at the entire range of light intensities. Note that at the maximal light intensity, the peak LIC reached ~20 nA inward current although no sign of saturation is observed. ***C***, The average peak amplitude of the voltage responses, measured in the current-clamp mode from the $trpl^{302}$ mutant, is plotted against the relative orange light intensity in a paradigm identical to that of ***B***. The relative intensity of the intense white lights ($-\log I/I_0 = -1$) at saturated voltage responses is expressed in terms of equivalent intensity of orange lights (Schott OG 590 edge filter) that elicited the same light response. A significant shift toward more intense lights of 0.7 log units ($p < 0.007$; $n = 8$) was observed when dark-raised flies were exposed to prolonged illumination. ***C***, Inset, The inset is sample responses to maximal orange light intensity. The afterhyperpolarization of the voltage responses of dark-raised flies (right arrow) and its absence in the responses of light-raised flies (left arrow) demonstrates the complexity of the voltage response to light. ***D***, The *R*-log-*I* curve of light-adapted heterozygote $Gq\alpha^1$ ($Gq\alpha^1/+$, empty circles) is compared with the *R*-log-*I* curve of dark-adapted WT flies (filled circles). A shift of 1.05 log units of the heterozygote $Gq\alpha^1$ curve (dashed line) is required to fit the *R*-log-*I* curve of the mutant to that of dark adapted WT flies.

peak amplitude of the LIC in response to increasing intensities of short light pulses in illuminated flies (Fig. 4*A*, empty circles) and then measure this curve again in other flies of the same vial after 2 h of dark adaptation (Fig. 4*A*, filled circles). Fig. 4*A* shows that dark-adapted flies (for 2 h) are more sensitive to light (by ~0.45 log units) than light-adapted flies. When the dark adaptation period was extended to 12 h, dark-adapted WT flies were more sensitive to light by ~0.6 log units (Fig. 3*B*).

Previous studies have shown that prolonged illumination of *Drosophila* $G_q\alpha$ mutants induces translocation of $G_q\alpha$ from the signaling membrane to the cytosol, followed by translocation of the cytosolic $G_q\alpha$ back to the rhabdomere in the dark after the cessation of light (Kosloff et al., 2003; Cronin et al., 2004). It was also demonstrated that a large reduction of $G_q\alpha$ concentration in *Drosophila* photoreceptor reduces the sensitivity to light (Scott et al., 1995). If light-dependent modulation of rhabdomeric $G_q\alpha$ underlies long-term adaptation, it is expected that specific mutations that impair the targeting of cytosolic $G_q\alpha$ to the signaling membrane should also impair long-term adaptation.

To critically test this hypothesis, we measured the *R*-log-*I* curves of the macroscopic LIC in illuminated mutants in which the targeting of $G_q\alpha$ to the signaling membrane is impaired. We then measured this curve again in flies from the same vial after 2 h of dark adaptation to determine the effect of specific mutations on long-term adaptation.

Mutations that reduce $G\beta_e$ concentration markedly affect both the targeting of $G_q\alpha$ to the membrane and the recovery of sensitivity in the dark after prolonged illumination

It has been shown previously that in $G\beta_e$ mutants, $G_q\alpha$ is mainly localized to the cytosol (see Fig. 6) (Elia et al., 2005) and its membrane association is defective because it is anchored to the membrane by binding to the $\beta\gamma$ dimmer (Kosloff et al., 2003). Figure 4*B* compares the *R*-log-*I* curve measured in the light adapted $G\beta_e{}^2$ mutant, which has only 5–14% $G\beta_e$ (Dolph et al., 1994; Elia et al., 2005), with the *R*-log-*I* curve of flies from the same vial measured after 2 h in the dark. Strikingly, no significant shift toward dim lights of the *R*-log-*I* curve was observed in the $G\beta_e{}^2$ mutant after 2 h darkness. This result is in sharp contrast to WT flies in which the *R*-log-*I* curve was significantly shifted (Fig. 4*A*). When a longer (12 h) dark period was allowed for the $G\beta_e{}^2$ mutant, a significant shift of the *R*-log-*I* curve was observed (data not shown), indicating that after 2 h darkness, the $G\beta_e$ mutant was still in the light adapted state. To allow comparing the effect of 2 h dark adaptation between illuminated WT flies and the $G\beta_e{}^2$ mutant, the *R*-log-*I* curves of dark- and light-adapted WT flies were shifted to higher levels of light intensities by ~1 log unit (Fig. 4*B*, dotted lines). After the shift, the light-adapted curves of WT and the mutant overlapped whereas their dark-adapted curves revealed a large difference. Thus, Figure 4*B* demonstrates that long-term adaptation is markedly suppressed in a $G\beta_e{}^2$ mutant in which the targeting of $G_q\alpha$ to the membrane is defective.

The $ninaC^{P235}$ myosin III mutation, which slows down the return of $G_q\alpha$ to the signaling membrane, also slows down the recovery of sensitivity in the dark after prolonged illumination

The *ninaC* mutant, which lacks the NINAC myosin III proteins (Montell and Rubin, 1988) displays a significantly reduced rate of $G_q\alpha$ transport from the cell body to the rhabdomere after illumination (Cronin et al., 2004). Accordingly, NINAC determines $G_q\alpha$ concentration in the rhabdomere after illumination. Figure 4*C* compares the *R*-log-*I* curves measured in light- and dark-adapted $ninaC^{P235}$ null mutants. Similar to the $G\beta_e{}^2$ mutant, no significant shift toward dim lights of the *R*-log-*I* curve was observed in the mutant after 2 h of darkness. When a longer (12 h) dark period was allowed, a significant shift of the *R*-log-*I* curve was observed in this mutant, indicating that, after 2 h of darkness,

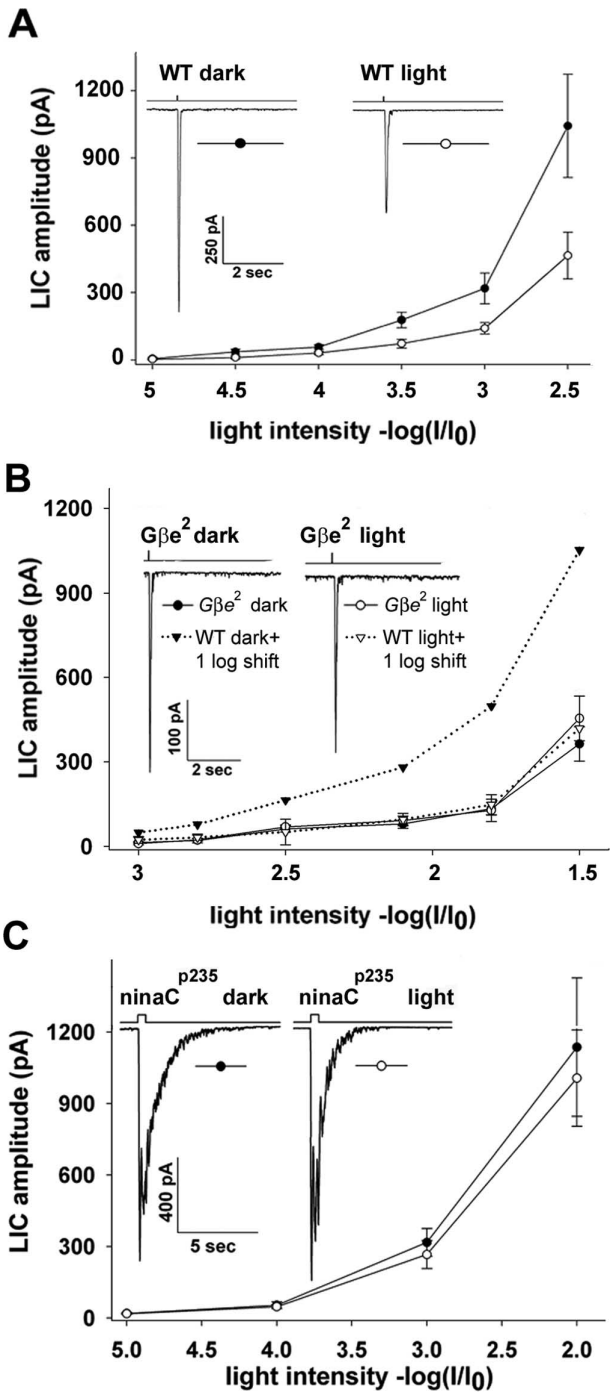


Figure 4. Two different mutations, which impaired the targeting of $G_q\alpha$ to the membrane, strongly inhibit long-term adaptation. The average peak amplitude of the light-induced current is plotted against the relative orange light intensity. ***A***, Intensity–response relationship of WT *Drosophila* photoreceptors measured in illuminated flies (4 h; WT light; empty circles) followed by dark adaptation (2 h; WT dark; filled circles). There is a clear shift toward dimmer light intensities of the *R*-log-*I* curve after 2 h of dark adaptation. The inset shows sample responses to test light of the highest intensity (i.e., log = −2.5) from illuminated (right) and dark-adapted (left) flies ($n = 6$). ***B***, The measurements in ***A*** were repeated in the $G\beta e^2$ mutant in which the shift of the *R*-log-*I* curve after 2 h of dark adaptation was completely blocked. For comparison, the *R*-log-*I* curve of WT flies from ***A*** (dotted lines, triangles) were shifted by ~1 log unit toward more intense lights to fit the *R*-log-*I* curve of the mutant. The figure shows that whereas 2 h of dark adaptation was sufficient to reveal significant shift of the *R*-log-*I* curve in WT flies, no shift was observed in the $G\beta e^2$. ***C***, The measurements in ***A*** and ***B*** were repeated in the $ninaC^{P235}$ mutant in which the shift of the *R*-log-*I* curve measured after 2 h of dark adaptation was completely blocked. The insets are sample responses to the highest light intensity of the presented *R*-log-*I* curves.

the $ninaC^{P235}$ mutant was still in the light-adapted state, similar to the $G\beta_e{}^2$ mutant.

Figure 4, *B* and *C*, demonstrate that the recovery of sensitivity to light during a 2 h dark period after prolonged illumination is markedly and similarly suppressed in mutants that affect the targeting of $G_q\alpha$ to the signaling membrane by two entirely different mechanisms. The result of Figure 4 strongly supports the hypothesis that long-term adaptation is mediated by light-activated $G_q\alpha$ translocation.

Light-regulated translocation of TRPL does not account for the recovery of sensitivity to light in the dark after prolonged illumination

To test the specificity of impairing $G_q\alpha$ targeting to the membrane on long-term adaptation, we repeated the experiments of Figure 4 in a variety of mutants. These mutants are known to reduce the concentration of signaling proteins that in the WT flies translocate between the signaling membrane and the cell body after illumination.

It has been found previously that the *Drosophila* TRPL channel translocates back and forth between the signaling membrane and the cytosol by a light-regulated mechanism (Bähner et al., 2002; Cronin et al., 2006). Because TRPL translocation was accompanied by changes in sensitivity to light during very dim background illumination and this effect was blocked in the null *trpl* mutant, we examined whether TRPL translocation underlies long-term adaptation by measuring the *R*-log-*I* curves in the *trpl* null mutant. Figure 5*A*, which repeats the experiments of Figure 4 using the null *trpl* mutant ($trpl^{302}$) (Niemeyer et al., 1996), shows a clear shift of the *R*-log-*I* curve of the light-raised fly after 2 h of darkness, as found in WT flies.

Figure 5*A* shows that translocation of the TRPL channel plays no significant role in recovery of sensitivity in the dark after prolonged illumination.

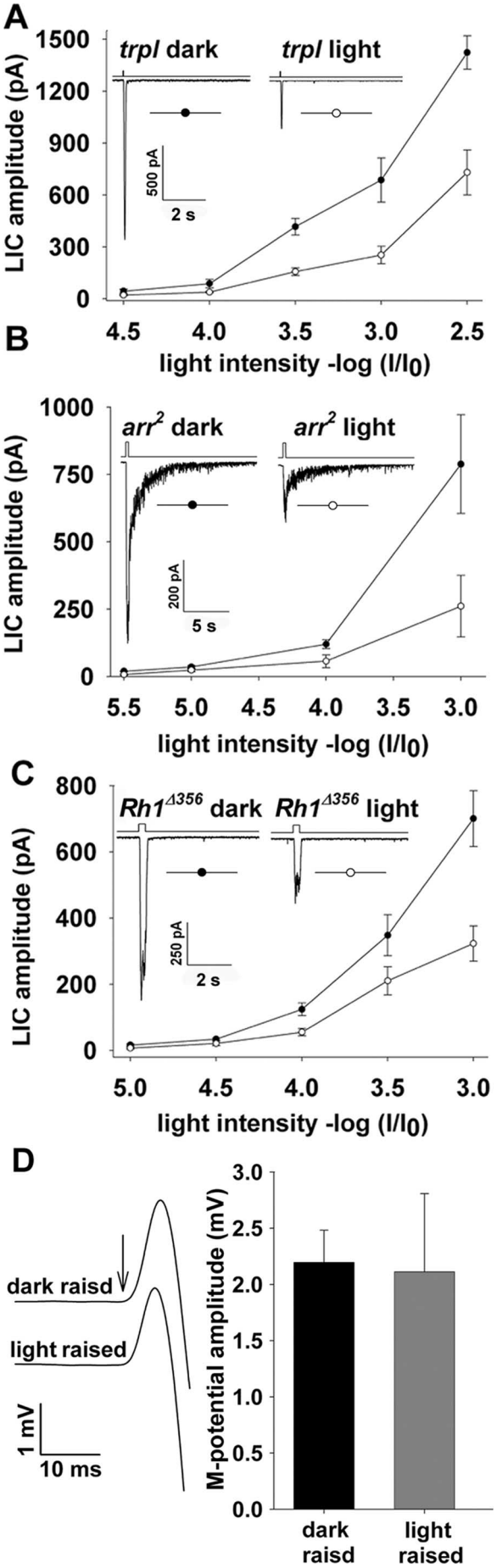
Light regulated translocation of arrestin 2 does not account for long-term adaptation

Inactivation of the physiologically active photopigment is achieved by the binding of the arrestin 2 (Arr2) to the phosphorylated metarhodopsin, which prevents additional association between metarhodopsin and the $G_q\alpha$ protein (Yamada et al., 1990; Byk et al., 1993; Dolph et al., 1993). Therefore, the lifetime of active metarhodopsin is rather short (<100 ms) as measured directly in the *Limulus* median eye (Richard and Lisman, 1992) and depends on the concentration of arrestin (Ranganathan and Stevens, 1995). Several studies have shown that Arr2 translocates into the rhabdomere after illumination (Byk et al., 1993; Lee et al., 2003) and thereby may contribute to long-term adaptation (Lee et al., 2003). To test this hypothesis, we measured the *R*-log-*I* curves in an Arr2 mutant ($arr2^3$), which has a highly reduced level of Arr2 (Alloway et al., 2000).

Figure 5*B* compares the *R*-log-*I* curves measured in the illuminated (4 h) $arr2^3$ mutant and in the same mutant after 2 h of darkness (empty and filled circles, respectively). Figure 5*B* shows a significant shift of the *R*-log-*I* curve toward dim lights after 2 h of darkness similar to that of WT flies, thus indicating that Arr2 is not an essential molecule for the type of long-term adaptation found in this study.

The photopigment cycle is not involved in long-term adaptation

Several studies have shown that, under pathological conditions, light induces a stable association between metarhodopsin and



Arr2, which is followed by phosphorylation-dependent internalization of this complex (Alloway and Dolph, 1999; Alloway et al., 2000; Kiselev et al., 2000) (but see Satoh and Ready, 2005). Furthermore, a previous study has shown that the constitutively active rhodopsin mutant $ninaE^{pp100}$ causes a stable association of the photopigment to Arr2 and thereby reduces the sensitivity to light by >10-fold (Iakhine et al., 2004). To examine the involvement of photopigment internalization in long-term adaptation, we examined whether photopigment phosphorylation plays a role in long-term adaptation. To this end we measured the *R*-log-*I* curve in transgenic flies in which the C-terminal portion of the major rhodopsin (Rh1) was deleted (Vinos et al., 1997) and internalization of the photopigment was prevented (Alloway et al., 2000; Satoh and Ready, 2005). Figure 5*C* compares the *R*-log-*I* curves measured in illuminated (4 h) and dark-adapted $Rh1^{\Delta 356}$ mutants. The Figure shows that the shift of the *R*-log-*I* curve toward dim lights after 2 h of darkness was similar to that of WT flies, indicating that phosphorylation of metarhodopsin and the subsequent internalization of the photopigment are not essential for long-term adaptation. Together, the observation that long-term adaptation is preserved in either the *arr2* (Fig. 5*B*) or in the $Rh1^{\Delta 356}$ mutants (Fig. 5*C*) suggests that the photopigment cycle or the light-induced turnover of the photopigment molecules are not essential for long-term adaptation.

To further eliminate the possibility that light-induced modulations in the photopigment level underlie long-term adaptation under our experimental conditions, we measured the photopigment level in dark- and light-raised WT flies. The photopigment level was measured by eliciting the metarhodopsin potential (M-potential), which is a linear electrical manifestation of the photopigment level in the fly (Pak and Lidington, 1974; Minke and Kirschfeld, 1980). Figure 5*D* compares the amplitude of the M-potential in dark- and light-raised WT flies. The figure shows that there is no significant difference between the average amplitude of the M-potentials of illuminated and dark-adapted flies, thus ruling out the possibility that modulations in the photopigment levels underlie long-term adaptation.

Together, Figures 4 and 5 reveal that suppression of long-term adaptation by mutations, which affect targeting of $G_q\alpha$ to the membrane, is a specific phenomenon and strongly suggest that translocation of $G_q\alpha$ mediates long-term adaptation in *Drosophila* photoreceptors.

The sensitivity to light depends on membrane $G_q\alpha$ concentration in WT and G-protein mutants

It has been well established using transgenic *Drosophila* $G_q\alpha$ mutants, which express variable amounts of normal $G_q\alpha$, that the

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Figure 5. Impairing the targeting of $G_q\alpha$ to the signaling membrane by mutations is a specific effect. The average peak amplitude of the LIC of various mutants is plotted against the relative orange light intensity in a paradigm identical to that in Figure 4. ***A–C***, The insets are sample responses to the highest light intensity of the presented *R*-log-*I* curves. The null *trpl* ($trpl^{302}$) mutant (***A***), the hypomorph Arr2 ($arr2^3$) mutant (***B***), and a mutant rhodopsin having Rh1 without the phosphorylation sites at the C terminus ($Rh1^{\Delta 356}$; ***C***) had no significant effects on long-term adaptation. ***D***, There is no significant reduction in the photopigment level between light-raised and dark-raised WT flies. The photopigment level was measured by eliciting the M-potential, which is a linear electrical manifestation of the photopigment level in the fly eye. Flies <2 d old were illuminated with maximal intensity blue (BG28; Schott broad-band blue filter) for 20 s and after 10 s in the dark a maximal intensity white flash (arrow; stroboscopic photographic flash lamp of 70 Joules) elicited the M-potential (left traces). The histogram compares the average amplitude of the M-potential in dark-raised (left column) and light-raised (right column) flies. No significant difference between the averaged M-potential amplitudes under the two adaptation conditions was observed (*t* test, $p > 0.05$).

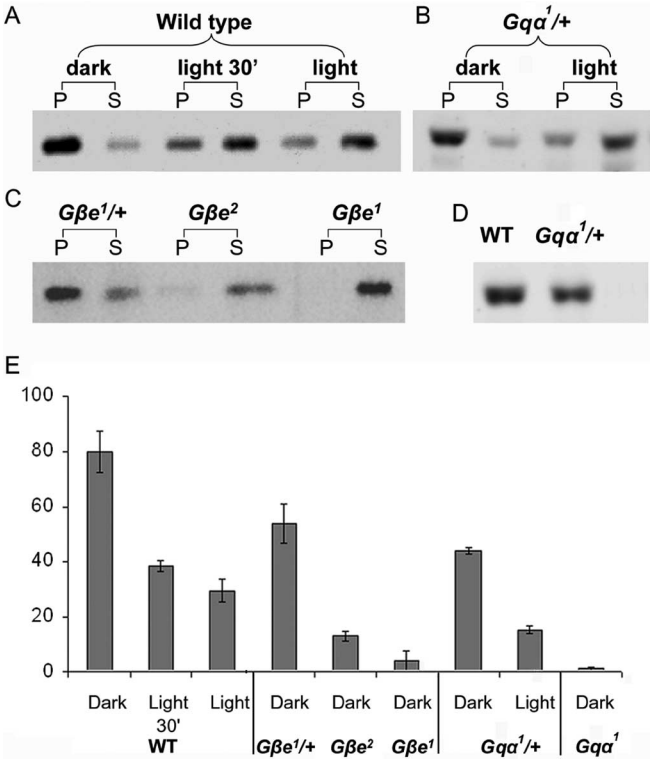

Figure 6. Relative concentration of membrane $G_q\alpha$ in WT and various G_q mutants. ***A–C***, Western blot analysis shows by fractionation analysis the localization of $G_q\alpha$ in the membrane [pellet (P)] and in cytosol [supernatant (S)] in dark- (2 h) and light-adapted (4 h) WT flies (***A***), dark- (2 h) and light-adapted (4 h) heterozygote $Gq\alpha^1$ (***B***), and dark-adapted $G\beta_e$ mutants (***C***). ***D***, Total amounts of $G_q\alpha$ in WT and heterozygote $Gq\alpha^1$. ***E***, $G_q\alpha$ concentration in the membrane presented as percentage of total $G_q\alpha$ of WT flies.

sensitivity of the photoreceptor cell to light critically depends on the concentration of $G_q\alpha$ (Scott et al., 1995). In addition, other studies show that the membrane concentration of $G_q\alpha$ changes with prolonged illumination (Kosloff et al., 2003; Cronin et al., 2004). Although these studies suggested that $G_q\alpha$ translocation contributes to light adaptation they did not actually measure the physiological consequences of $G_q\alpha$ translocation.

To study the effect of changes in membrane $G_q\alpha$ concentration on the magnitude of the sensitivity shift, we analyzed the relationship between the concentration of membrane $G_q\alpha$ in mutants with variable concentration of $G_q\alpha$ and the sensitivity shift. To this end we measured by Western blot analysis the membrane concentration of $G_q\alpha$ in homozygote $Gq\alpha^1$, $G\beta_e^1$, and $G\beta_e^2$ mutants and heterozygote $Gq\alpha^1$ and $G\beta_e^1$ flies (Fig. 6). All of these mutants show reduced $G_q\alpha$ concentration in the signaling membrane relative to WT flies (Fig. 6).

Figure 7*A* plots the sensitivity shift (as measured in Fig. 3*D*) as a function of membrane $G_q\alpha$ concentration of the various mutants relative to dark-raised WT flies. The extreme upper point represents measurements from the strong hypomorph $G\alpha q^1$ mutant, showing maximal shift at minimal membrane $G_q\alpha$ concentration. The extreme lower point shows dark-raised WT flies that by definition have no shift at maximal membrane $G_q\alpha$ concentration. The intermediate points that were obtained from the various mutants show that reduction in membrane $G_q\alpha$ concentration resulted in an increased shift of the *R*-log-*I* curve relative to that of dark-raised WT. The smooth curve is an exponential function that fits the experimental points. Figure 7*A* shows a good correlation ($R^2 = 0.89$) between the sensitivity shift and the membrane $G_q\alpha$ concentration.

The kinetics of long-term adaptation and its relationship to the kinetics of $G_q\alpha$ translocation

To further test the hypothesis that the movement of $G_q\alpha$ in and out of the signaling membrane underlies long-term adaptation, the kinetics of $G_q\alpha$ translocation from the membrane to the cytosol was compared with the kinetics of long-term adaptation.

The kinetics of $G_q\alpha$ movement from the membrane to the cytosol during light has been measured previously (Kosloff et al., 2003). To measure the kinetics of long-term adaptation, we measured the reduction of bump frequency of dark-raised WT flies at various time points during continuous illumination (Fig. 7*B*). The reduction of bump frequency as a function of time fits a first-order reaction described by an exponential function with an apparent time constant of ~196 min (Fig. 7*B*, smooth curve). The reduction in bump frequency as a function of time during illumination can also be expressed as a shift in the sensitivity to light toward more intense lights (sensitivity shift) (Fig. 7*B*, right scale). Indeed, the difference between the sensitivity to light of dark- and light-raised WT flies measured by a shift in the *R*-log-*I* curve was similar to the shift in sensitivity calculated from the reduction in bump frequency as a function of time during prolonged illumination [i.e., ~0.6 log (Fig. 3*A*) and 0.7 log (Fig. 7*B*, right scale)].

Previous data (Kosloff et al., 2003) and Figure 7*B* show that the kinetics of both long-term adaptation and $G_q\alpha$ translocation fit a first-order reaction described by an exponential function, but the time constant of the $G_q\alpha$ translocation is significantly shorter. However, there is a difficulty in correlating the kinetics of these two processes. Fig. 7*A* shows that the relationship between the sensitivity shift and the change in membrane $G_q\alpha$ concentration is highly nonlinear. This nonlinearity is reflected in the almost recessive phenotype of the strong hypomorph $Gq\alpha^1$ (Scott et al., 1995) and most likely arises from the very high expression of $G_q\alpha$ in the photoreceptor cell (see below). Accordingly, ~50% reduction in $G_q\alpha$ concentration in the $Gq\alpha^1/+$ mutant has a very small effect on the sensitivity shift (Fig. 7*A*). To overcome this difficulty and to estimate the correlation between the kinetics of long-term adaptation and $G_q\alpha$ translocation, we used the function obtained from the data of Figure 7*A* (smooth curve) to calculate the membrane $G_q\alpha$ concentration, which is predicted from the sensitivity shift (Fig. 7*B*, right scale). As a first step, we translated the bump frequency at particular times to a shift in sensitivity relative to dark-raised WT flies and plotted the relative shift as a function of time (Fig. 7*B*, right scale). We then used the function described by the smooth curve (Fig. 7*A*) to calculate the predicted membrane $G_q\alpha$ concentration from the measured shift in sensitivity as a function of time during illumination (Fig. 7*C*, smooth curve). The squares in Figure 7*C* are direct biochemical measurements of membrane $G_q\alpha$ concentration (Kosloff et al., 2003). Fig. 7*C* shows a good fit between the direct biochemical measurements of $G_q\alpha$ concentration as a function of time during illumination and the $G_q\alpha$ concentration calculated from the shift in sensitivity. There is a relatively small discrepancy between the measured $G_q\alpha$ membrane concentration and that calculated from the shift in sensitivity at long (>2 h) illumination. This is because the measured $G_q\alpha$ membrane concentration reaches saturation after ~1 h (Kosloff et al., 2003) (see Discussion).

Consistent with the data of Figure 7*C*, measurements of the shift in the *R*-log-*I* curve in WT flies illuminated for 30 and 60 min relative to dark-raised WT flies fit well to the smooth curve of

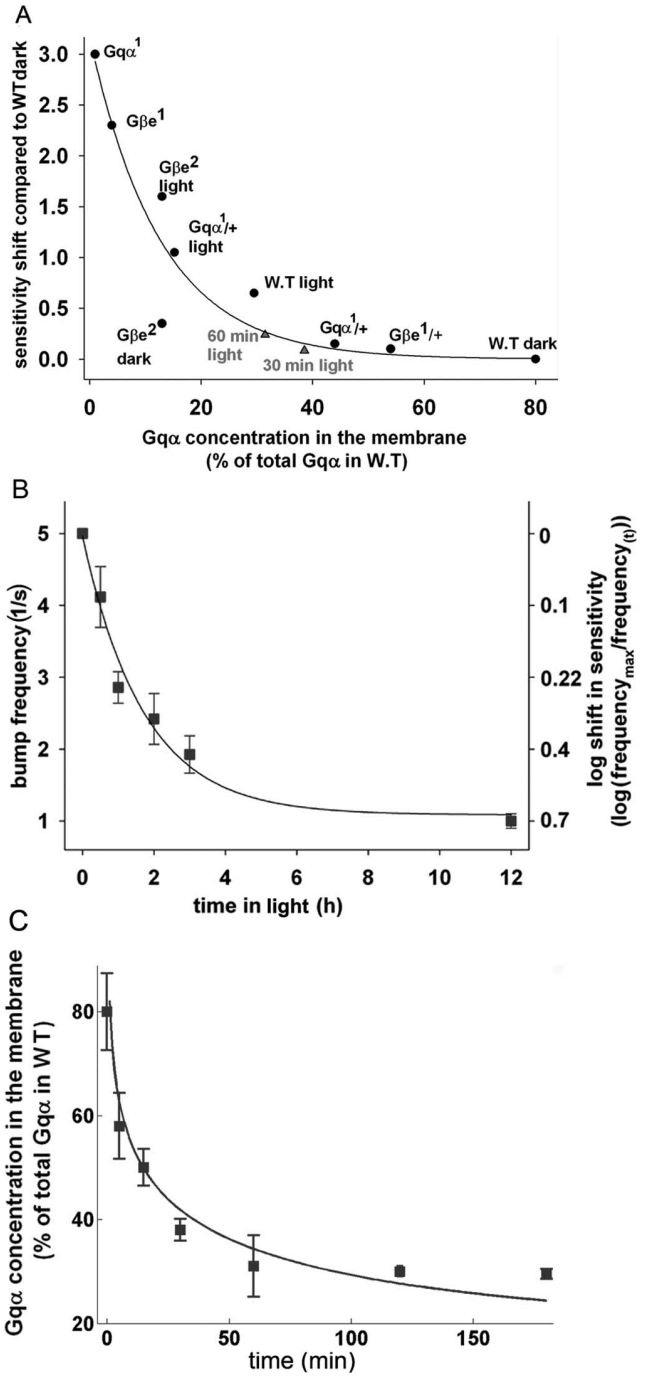

Figure 7. A correlation between long-term adaptation and membrane $G_q\alpha$ concentration in WT and G_q mutants. ***A***, The shift required to fit the *R*-log-*I* curve of the various mutants at steady-state dark/light conditions (as indicated) to that of dark-raised WT flies was plotted against the steady-state concentration of membrane $G_q\alpha$ measured by Western blot analysis in the various G_q protein mutants (Fig. 6). The shift of $Gq\alpha^1$ was taken from Scott et al. (1995). ***B***, The triangles and WT light were derived from kinetic measurements of changes in bump frequency. The smooth curve is an exponential function [sensitivity shift, $3.168 \times \exp(-0.0786 \times G_q\alpha)$] that fits the experimental points, excluding the kinetic data (triangles). This function shows a good correlation ($R^2 = 0.89$) between the shift in sensitivity to light and the membrane concentration of $G_q\alpha$ at steady-state conditions. The kinetic of the change in bump frequency during illumination is shown. Bump frequency was measured in flies raised in darkness and transferred to light for the specified time. The figure shows that the decrease in bump frequency elicited in response to dim orange light ($-\log I = 5.5$) follows a first-order exponential function with an apparent time constant of ~196 min [sensitivity shift, $0.7125(1 - \exp(0.0051 \times t_{(min)})]$. ***C***, A comparison between the direct biochemical measurements of membrane $G_q\alpha$ concentration (squares) (Kosloff et al., 2003) and membrane $G_q\alpha$ concentration calculated from the shift in sensitivity as a function of time during illumination. The smooth curve was calculated from the kinetic of the shift in sensitivity (***B***, right scale) together with the nonlinear function describing the relationship between membrane $G_q\alpha$ concentration and the shift in sensitivity (***A***). The biochemical measurements of light-induced translocation of $G_q\alpha$ fit well with the reduction in membrane $G_q\alpha$ calculated from the shift in sensitivity to light during times <2 h.

Figure 7*A* that was constructed without using any of the kinetic measurements (Fig. 7*A*, triangles).

Together, the data in Figure 7 reveal a good fit between the predicted membrane $G_q\alpha$ concentrations calculated from the reduction in sensitivity to light during prolonged illumination and the membrane $G_q\alpha$ concentration measured directly. The fact that the reduction in membrane $G_q\alpha$ concentration during illumination can be predicted from the shift in sensitivity to light that reflects long term adaptation (Fig. 7*C*) gives additional support to the notion that light-induced translocation of $G_q\alpha$ underlies long-term adaptation.

Prolonged illumination increases the latency of the single photon responses

The results of Figure 7 imply that in *Drosophila* photoreceptors the functional consequences of depleting membrane $G_q\alpha$ only manifest when the reduction in membrane $G_q\alpha$ is severe. To reconcile this observation with the high concentrations of rhodopsin and G_q in the microvilli, we examined theoretically the effect of a reduction in the concentration of $G_q\alpha$ (and hence in the available G_q protein) in the microvillar membrane on its ability to encounter an active rhodopsin molecule.

Drosophila rhabdomeres have very high amount ($\sim 3 \times 10^7$) of rhodopsin molecules and ~10-fold less G_q-protein molecules. The rhabdomere contains ~30,000 microvilli and each microvillus contains ~1000 rhodopsin molecules and ~80 G_q molecules (Hardie and Raghu, 2001). According to the Einstein diffusion equation $D = x^2/2t$, where t is the lifetime of an activated rhodopsin molecule (which is ~0.1 s) (Richard and Lisman, 1992), x^2 reflects the area covered by the Brownian motion of a G_q molecule, and D is the diffusion coefficient of G_q in tissue culture cells (0.10 $\mu m^2/s$) (Perez et al., 2006). The calculated area of the Brownian motion of heterotrimeric G_q in 0.1 s is, therefore, 0.02 μm^2.

The calculated membrane area of a single microvillus, assuming a microvillus diameter of 60 nm and length of 1.5 μm (Hardie and Raghu, 2001) is 0.28 μm^2. Because there are ~1000 rhodopsin molecules in this area, the Brownian motion area of each G_q during the lifetime of activated rhodopsin molecule includes ~70 rhodopsin molecules in dark-adapted WT cells.

During dim lights, only one rhodopsin molecule is activated in a single microvillus. The Brownian motion of the 80 available G_q molecules can theoretically interact with 5600 rhodopsin molecules in an ideal situation, which is much more than the 1000 rhodopsin molecules in a microvillus. This excessive amount of G_q molecules available for excitation should lead to a nonlinear relationship between the reduction in G_q and the ability of an activated rhodopsin to encounter G_q. The data of Figure 7*A* and previous studies (Scott et al., 1995) show that a 50% reduction in the available G_q molecules led to a minor reduction in the sensitivity to light, whereas a reduction to 30% resulted in a significant reduction in sensitivity. These findings suggest that the area covered by the Brownian motion of the available G_q molecules (and hence the diffusion coefficient of G_q) in the microvilli should be smaller than that of the tissue culture cells. Because G_q is the only

signaling molecule that moves in the microvillar membrane (Hardie and Raghu, 2001), its movement must be slowed down by the large concentration of the other membrane proteins of the microvillus such as the rhodopsin and the TRP and TRPL channels.

In Figure 8*A–C* we simulated a planar microvillus area containing 1000 randomly distributed rhodopsin molecules (blue points) and randomly distributed G_q molecules at three different amounts: 80 (representing 80% G_q level at dark-adapted state of WT flies) (Fig. 8*A*), 45 (representing dark-adapted *Gαq*/+ mutant) (*B*), and 30 (representing light-adapted state of WT flies) (*C*). The average area of the Brownian motion of $G\alpha_q$ molecules in 0.1 s is represented by red circles, arbitrarily assuming that diffusion coefficient, *D*, of G_q in the microvilli is threefold smaller than that of the tissue culture cells (i.e., 0.03 $\mu m^2/s$). Figure 8*A–C* shows that for the simulated cases of dark-adapted WT, *Gqα*/+, and light-adapted WT flies, the Brownian motion area of all G_q molecules covers ~80, ~70, and ~35% of the microvillus area, respectively (Fig. 8*A–C*, respectively). The relative area of the microvillus covered by the Brownian motion of G_q can be used to calculate the shift is sensitivity to light [e.g., for the light-raised WT fly, log(80%/35%)], yielding a 0.06 log and 0.35 log shift for the dark-raised $Gq\alpha^1$/+ and light-raised WT fly, respectively. Thus, Figure 8 demonstrates the nonlinearity of the relationship between $G_q\alpha$ concentration and sensitivity to light by showing that a reduction in membrane $G_q\alpha$ significantly reduces the ability of G_q molecules to encounter an activated rhodopsin only when the reduction in membrane $G_q\alpha$ is severe.

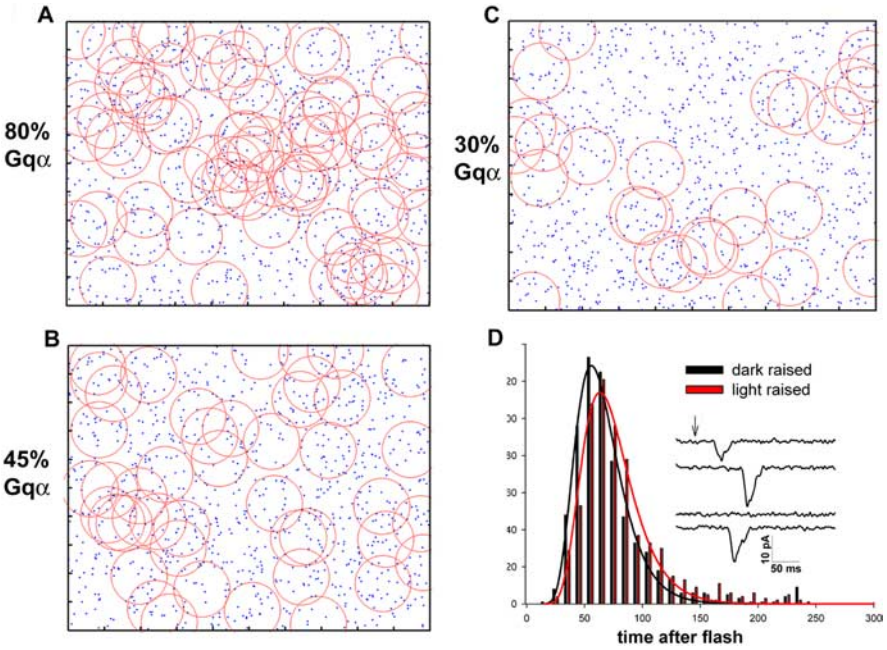

Figure 8. Model simulation portraying the ability of activated rhodopsin to encounter G_q molecules when G_q concentration in a microvillus is reduced. ***A–C***, Three examples of random generated images (Matlab 6.5) representing the area of a single microvillus that contains 1000 rhodopsin molecules (blue) and different amounts of heterotrimeric G_q molecules. The red circles represent the area covered by the Brownian motion of G_q molecules in 0.1 s, assuming *D* of 0.03 $\mu m^2/s$. ***A***, 80% of maximal level of membrane G_q. ***B***, Forty-five percent of G_q molecules. ***C***, Thirty percent of G_q molecules. ***D***, Prolonged illumination that decreases membrane $G_q\alpha$ concentration increases the averaged latency of the single photon responses. The histograms plot bump-latency distribution as a function of time in bins of 10 ms for dark-raised (black) and light-raised (red) WT flies. The smooth curve is the log-normal function that best fits the data. There is a highly significant difference between the two histograms ($p < 0.01$; $n = 6$). ***D*** shows that the peak amplitude of the bump latency distribution is shifted by 7.5 ms after prolonged illumination that reduces $G_q\alpha$ concentration. The inset presents an example of responses to repeated stimulations with constant intensity dim red light ($-\log I = 4.1$) of light-raised WT flies that elicited, on average, 0.4 bumps/stimulus.

Together, the model simulation shows that it is possible to explain the nonlinear relationship between the reduction in sensitivity to light and the reduction in the available G_q molecules during prolonged illumination (Fig. 8*A–C*).

An interesting prediction of the above model is that the average latency of the quantum bumps is expected to increase when the available concentration of G_q molecules is reduced by prolonged illumination or by mutation. In Figure 8*D*, we tested whether a reduction of $G_q\alpha$ to ~30% by illumination changes the bump latency distribution. To measure the bump latency distribution, we repeatedly applied very dim test flashes that elicited ~0.4 bumps per flash on the average (Fig. 8*D*, inset). The plot of the bump latency distribution (Fig. 8*D*) shows that a reduction of membrane $G_q\alpha$ to ~30% by prolonged illumination resulted in a highly significant ($p < 0.01$) increase of 7.5 ms of the peak bump latency distribution that well fits the log-normal distribution. A slightly larger increase in the peak of the bump latency distribution (of 10 ms) was observed in the illuminated heterozygote $Gq\alpha^1$/+ mutant in which membrane $G_q\alpha$ was reduced to ~ 15% (data not shown).

Reduction in $G_q\alpha$ level has only minor effect on the kinetics of response to light (Fig. 8*D*) in contrast to reduction in PLC level that has a strong effect on response kinetics (see Discussion). This demonstrates that the reduction in concentration of $G_q\alpha$ has only minor influence on the dwell time before a newly activated $G_q\alpha$ finds PLC, even when $G_q\alpha$ level is reduced (see Discussion).

Together, theoretical considerations reveal that diffusion of G_q in the microvillar membrane is sufficient to account for rhodopsin-G_q interaction in a microvillus during very dim light as long as the G_q concentration is large in dark adapted cells. However, when membrane $G_q\alpha$ concentration is largely reduced after prolonged illumination, the G_q molecules available for interaction with the active rhodopsin can easily become the limiting factor of excitation if the diffusion coefficient of the microvillar G_q is only threefold slower than that of tissue culture cells. The increase in bump latency distribution after prolonged illumination (Fig. 8*D*) is fully consistent with this notion, thus, strongly supporting the hypothesis that the translocation of $G_q\alpha$ underlies long-term adaptation.

Discussion

Genetic dissection of long-term adaptation

Multiple mechanisms have been shown to reduce the sensitivity to light during or after prolonged illumination in *Drosophila* photoreceptors: translocation of the TRPL channel is involved in adaptation to dim background light (Bähner et al., 2002). Arr2 translocates from the cell body to the rhabdomere after illumination and it is necessary for fast termination of the light response (Fig. 5*B*, inset) (Yamada et al., 1990; Byk et al., 1993; Dolph et al., 1993). In addition, recycling of the phosphorylated photopigment reduces the photopigment level (Alloway and Dolph, 1999;

Alloway et al., 2000; Kiselev et al., 2000) and, consequently, the probability of quantum catch. However, our data show that none of these mechanisms can account for the long-term adaptation described in the present study. Additional studies have found that prolonged intense illumination reduced the photopigment level (Lee and Montell, 2004). However, when we measured the photopigment levels, no significant difference was found under the two illumination conditions (Fig. 5*D*). Because of the linear relationship between the photopigment level and the sensitivity to light that characterizes invertebrate photoreceptors (Hamdorf, 1979), a fivefold reduction in the photopigment level is required to account for the fivefold reduction in sensitivity to light after prolonged illumination, making such a mechanism unsuitable to explain long-term adaptation.

It may be argued that a reduction in the PLC levels underlies long-term adaptation. However, a previous study that measured the PLC level of the fly microvilli before and during prolonged illumination did not find any reduction in the PLC level after illumination (Bähner et al., 2002). Moreover, other studies have shown that a reduction of PLC level to ~20% has negligible effect on the sensitivity to light and only increased the bump latency (Pearn et al., 1996; Scott and Zuker, 1998; Cook et al., 2000). This is explained by the dual function of the light activated PLC as an activator and a negative regulator of the transduction cascade because of its GTPase activating protein (GAP) function (Cook et al., 2000). Accordingly, at low PLC levels, $G_q\alpha$ stays active for a long time until it encounters a PLC. In contrast, active rhodopsin has a short lifetime during which it must encounter the G-protein to induce excitation. This is why a reduction in $G_q\alpha$ level but not in PLC has a strong effect on the sensitivity to light (Scott and Zuker, 1998; Cook et al., 2000).

The experiments using flies with mutations that interfere with the targeting of $G_q\alpha$ to the membrane provide conclusive evidence that light-regulated translocation of $G_q\alpha$ underlies long-term adaptation. Strikingly, the $G\beta_e$ and $ninaC^{P235}$ mutations (Kosloff et al., 2003; Cronin et al., 2004) concomitantly slowed down the recovery of sensitivity to light in the dark after prolonged illumination and inhibited long-term adaptation (Fig. 4). The $G\beta_e$ mutation most likely inhibits the association of $G_q\alpha$ to the membrane, whereas the $ninaC^{P235}$ mutation affects the actin cytoskeleton and hence $G_q\alpha$ movement. The fact that impairments of the retune of $G_q\alpha$ to the signaling membrane is the common denominator between these two completely different mutations strongly supports the hypothesis that the hitherto unexplored physiological role of light-induced translocation of $G_q\alpha$ (Kosloff et al., 2003; Cronin et al., 2004) is to regulate the sensitivity to light during or after prolonged illuminations.

Long-term adaptation and its relationship to the kinetics of $G_q\alpha$ translocation

To further support the notion that translocation of $G_q\alpha$ in and out of the signaling membrane underlies long-term adaptation, the kinetics of light-induced $G_q\alpha$ translocation (Kosloff et al., 2003) was compared with the kinetics of long-term adaptation in WT flies (Fig. 7*B*,*C*). This comparison was complicated by the fact that changes in membrane concentration of $G_q\alpha$ affect the sensitivity to light in a highly nonlinear manner (Fig. 7*A*). When the nonlinear relationship between the reduction in sensitivity to light during prolonged illumination and membrane $G_q\alpha$ concentration was taken into account, the kinetics of $G_q\alpha$ translocation was similar to that of long-term adaptation (Fig. 7*C*). The relatively small discrepancy at long time periods may be explained by the incorporation of some $G_q\alpha$ molecules into membranes other than the signaling membrane (e.g., endoplasmic reticulum) before their incorporation into the signaling membranes. Indeed, the study of Cronin et al. (2004) did show that in the dark the return of $G_q\alpha$ back to the rhabdomere took >2.5 h to be completed. Future studies will have to address this issue.

To explain the nonlinear dependence of the sensitivity to light on $G_q\alpha$ concentration, we examined theoretically the feasibility that heterotrimeric G_q will encounter an activated rhodopsin when the ratio of G_q/rhodopsin of 1:10 is reduced by fourfold and the diffusion coefficient of G_q in the microvilli is slower than that of tissue culture cells because of the condensed packing of signaling proteins in the microvilli. The outcome of these calculations suggests that a reduction in $G_q\alpha$ level in the signaling membrane can lead to the observed reduction in the sensitivity to light caused by light-induced translocation of $G_q\alpha$. The calculations of the diffusion of G_q in a microvillus suggest that the nonlinear dependence of the sensitivity shift on membrane $G_q\alpha$ concentration is caused by the large amount of G_q protein in the microvilli, which is presumably required to achieve the high sensitivity of the photoreceptors to light in dark-raised flies.

A shift of the bump latency distribution after prolonged illumination

The latency distribution of the bumps in WT flies is relatively narrow (~50 ms width at half maximum) (Fig. 8*D*) and reflects the short lifetime of active rhodopsin. If a light-induced reduction in the available microvillar G_q molecules makes G_q the limiting factor of excitation, it is expected that a large reduction in membrane $G_q\alpha$ will increase the average bump latency as found in the present study. This finding is consistent with previous measurements of the bump-latency distribution of WT and the homozygote $Gq\alpha^1$, which showed that the average bump latency of ~60 ms in WT flies increased to ~100 ms in the mutant (Scott and Zuker, 1998).

The bump latency distribution is sensitive to a reduction in PLC level (Scott and Zuker, 1998). However, a relatively modest reduction in PLC level causes a dramatic increase in both the averaged bump latency and the width of the bump latency distribution (to several hundreds milliseconds), in contrast to the relative small effect on these parameters when $G_q\alpha$ is reduced (Fig. 8*D*) (Scott and Zuker, 1998). Therefore, it is unlikely that the modest increase in bump latency after prolonged illumination arises from a reduction in the PLC level.

The fact that light-induced translocation of $G_q\alpha$ also significantly increased the averaged bump latency (Fig. 8*D*) constitutes additional strong support for the hypothesis that translocation of $G_q\alpha$ underlies long-term adaptation.

We found similar average bump amplitudes in dark- and light-raised flies despite large changes in membrane $G_q\alpha$ concentration (Fig. 1). The similar average bump amplitudes at different membrane $G_q\alpha$ concentrations may arise from compensatory mechanisms that keep the average bump size constant even when $G_q\alpha$ is reduced to 15%. Possible compensatory mechanisms are the change in the $G_q\alpha/G_q\beta$ ratio (Elia et al., 2005) and the involvement of regulators of G-protein signaling (RGSs) (De Vries and Gist, 1999) in fly photoreceptors (Elmore et al., 1998). Both $G\beta$ and RGSs have been shown to have strong effects on GAP activity in vertebrate rods (Keresztes et al., 2004) and they may have similar roles in the determination of the lifetime of $G_q\alpha$ and, hence, the lifetime of PLC molecules when $G_q\alpha$ concentration is reduced. This issue requires additional investigation.

Physiological implications of long-term adaptation through $G_q\alpha$ translocation

The transformation of each absorbed photon into a bump requires vast amounts of mobile membrane $G_q\alpha$ protein (Hardie and Raghu, 2001). Although such high concentrations of membrane $G_q\alpha$ have an obvious advantage during dim lights, they pose an unnecessary load on a highly demanding transduction cascade during daylight, when the sensitivity to each absorbed photon is not required. Translocation of $G_q\alpha$ out of the signaling membrane during prolonged illumination typical for day light is an efficient mechanism to reduce the load on the transduction machinery at an early stage of the transduction cascade.

A mechanism similar to that described in the present study was previously found in vertebrate rods. In vertebrates, a massive light-dependent translocation of the photoreceptor-specific G-protein transducin occurs between the functional compartments of rods. Up to 90% of transducin translocates from rod outer segments to other cellular compartments on the time scale of tens of minutes. The reduction in the transducin content of the rod outer segments is accompanied by a corresponding reduction in the amplification of the rod photoresponse, allowing rods to operate in illumination up to 10-fold higher than would otherwise be possible (Sokolov et al., 2002). The similarity in the physiological effect of light-induced G-protein translocation between vertebrate and invertebrate photoreceptors suggests that this mechanism of long-term adaptation is a common theme in the animal kingdom.

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