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Calcium Influx via TRP Channels Is Required to Maintain PIP_2 Levels in *Drosophila* Photoreceptors

Roger C. Hardie,*‖ Padinjat Raghu,* Suzanna Moore,* Mikko Juusola,† Richard A. Baines,‡ and Sean T. Sweeney§#

*Department of Anatomy
†Department of Physiology
‡Department of Zoology
§Department of Genetics
Cambridge University
Downing Street
Cambridge
United Kingdom

Summary

The *trp* (*transient receptor potential*) gene encodes a Ca^{2+} channel responsible for the major component of the phospholipase C (PLC) mediated light response in *Drosophila*. In *trp* mutants, maintained light leads to response decay and temporary total loss of sensitivity (inactivation). Using genetically targeted PIP_2-sensitive inward rectifier channels (Kir2.1) as biosensors, we provide evidence that *trp* decay reflects depletion of PIP_2. Two independent mutations in the PIP_2 recycling pathway (*rdgB* and *cds*) prevented recovery from inactivation. Abolishing Ca^{2+} influx in wild-type photoreceptors mimicked inactivation, while raising Ca^{2+} by blocking Na^+/Ca^{2+} exchange prevented inactivation in *trp*. The results suggest that Ca^{2+} influx prevents PIP_2 depletion by inhibiting PLC activity and facilitating PIP_2 recycling. Without this feedback one photon appears sufficient to deplete the phosphoinositide pool of ~4 microvilli.

Introduction

The light sensitive current in *Drosophila* is mediated by two classes of Ca^{2+} permeable channels, TRP and TRPL. Both channels are activated downstream of phospholipase C and are the prototypical members of an emerging family of ion channels responsible for a variety of receptor mediated Ca^{2+} influx phenomena (Montell and Rubin, 1989; Hardie and Minke, 1992; Phillips et al., 1992; Niemeyer et al., 1996; reviewed in Harteneck et al., 2000). TRP, the major charge carrier, is highly Ca^{2+} permeable ($P_{Ca}:P_{Na} > 100$); in *trp* (*transient receptor potential*) mutants lacking this channel, the remaining light induced current (LIC) is carried by TRPL channels (Niemeyer et al., 1996), which have a reduced Ca^{2+} permeability ($P_{Ca}:P_{Na} \approx 4$) (Reuss et al., 1997). Ca^{2+} influx via these channels is responsible for virtually all the massive (high micromolar) light-induced Ca^{2+} rise in *Drosophila* photoreceptors (Peretz et al., 1994; Ranganathan et al., 1994; Hardie, 1996; Cook and Minke, 1999; Minke and Hardie, 2000).

The *trp* mutation is so named because the response to light decays to baseline during prolonged illumination, following which the photoreceptor becomes temporarily unresponsive (Cosens and Manning, 1969; Minke et al., 1975). Despite over 30 years of investigation, the underlying cause of this phenotype is still controversial. Minke (1982) concluded that the phenotype represented exhaustion of some factor required for excitation. It was later proposed that this factor was Ca^{2+}, that Ca^{2+} influx via TRP channels was required for refilling Ca^{2+} stores and that the decay represented store depletion (Minke and Selinger, 1991). This influential hypothesis led to the proposal that TRP represented a store-operated Ca^{2+} influx pathway. While some vertebrate TRP homologues are strong candidates for store-operated channels (review Harteneck et al., 2000), the role of Ca^{2+} stores in *Drosophila* phototransduction has become controversial (Acharya et al., 1997; Raghu et al., 2000; Sullivan et al., 2000). Recently, Scott et al. (1997) proposed an alternative explanation for the *trp* phenotype—namely Ca^{2+} dependent inactivation of the TRPL channels. In support of this, they reported that the decay was abolished in the absence of external Ca^{2+}. These results were challenged by Cook and Minke (1999), who found that responses in *trp* did decay in Ca^{2+}-free solutions and that the subsequent response inactivation was more profound.

We have also demonstrated that TRPL channels are subject to Ca^{2+}-dependent inactivation (Reuss et al., 1997), but doubted whether this was related to the inactivation following the *trp* decay, since Ca^{2+}-dependent inactivation is a normal feature of wild-type photoresponses (Hardie and Minke, 1994; Hardie and Minke, 1995). However, in view of the questionable role of Ca^{2+} stores, we wondered whether depletion of another factor might underlie the *trp* decay. One candidate is phosphatidylinositol 4,5 bisphosphate (PIP_2), the substrate for PLC, which is the key effector enzyme in *Drosophila* phototransduction (Bloomquist et al., 1988). To test this, we used a genetically targeted PIP_2-sensitive ion channel as a biosensor to provide evidence that PIP_2 is depleted during the *trp* decay, and showed that mutations in the PIP_2 recycling pathway prevent recovery of sensitivity following inactivation. Our results suggest that Ca^{2+} influx normally protects the photoreceptor from PIP_2 depletion by inhibiting PLC and facilitating PIP_2 recycling. The data allow quantitative estimates of the amplification of the transduction cascade and have interesting implications for the strategy of phototransduction.

Results

Ca^{2+} Dependence of *trp* Phenotype

To gain insight into the *trp* phenotype, we quantified its intensity dependence using whole-cell voltage clamped light responses. In normal Ca^{2+}, we found that the light

‖Correspondence should be addressed (e-mail: rch14@hermes.cam.ac.uk).
#Present address: Department of Biochemistry and Biophysics, University of California, San Francisco, Parnassus Avenue, Box 0448, San Francisco, California 94143.

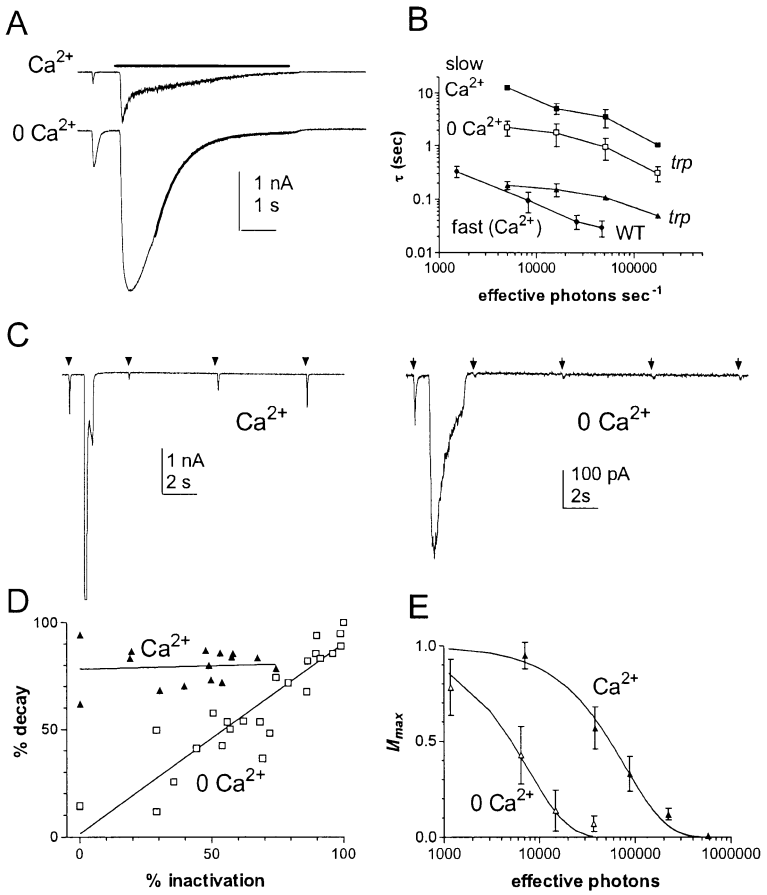

Figure 1. *trp* Decay and Response Inactivation

(A) Responses of *trp* photoreceptors to 5 s light pulses (50,000 effective photons/s) in normal (1.5 mM Ca^{2+}) and Ca^{2+}-free bath. In 1.5 mM Ca^{2+} (top trace), responses decayed with two kinetic components (see also Figure 3); in Ca^{2+} free, responses were facilitated and decayed with a single component. Traces fitted with single (Ca^{2+} free) or double exponentials (1.5 mM Ca^{2+}).

(B) Summary of decay time constants (mean ± SD, n = 5–9 cells). Closed square, slow component in 1.5 mM Ca^{2+}; closed triangle, fast component, 1.5 mM Ca^{2+}; open square, Ca^{2+}-free slow component; closed circle, fast component in wild type (WT).

(C) In zero Ca^{2+}, the decay induced by a 2 s stimulus (35,000 effective photons) led to long term response inactivation to dim test flashes (arrows), but in 1.5 mM Ca^{2+} (left), despite extensive rapid Ca^{2+} dependent decay, a brighter, 500 ms stimulus (~10^5 effective photons, peak response clipped) resulted in little long-term inactivation.

(D) Inactivation (estimated from response to test flash 10 s after the inactivating stimulus) was strongly correlated with decay in Ca^{2+}-free solution (open square, linear regression, $r^2 = 0.84$), but not in normal Ca^{2+} (closed triangle, $r^2 = 0.17$).

(E) Residual sensitivity (*I*/*Imax*) as a function of effective photon content of inactivating stimulus (closed triangle, 3 s stimuli in 1.5 mM Ca^{2+}; open square, 0.5 s stimuli in Ca^{2+}-free bath; n = 5–7 cells). Data fitted assuming each photon inactivates 0.38 microvilli in Ca^{2+} and 4.1 microvilli in Ca^{2+}-free bath (Equation 2).

induced current (LIC) in *trp* decayed toward baseline with two distinct kinetic components: a rapid (subsecond) decay which appeared similar to, though slightly slower than, the Ca^{2+}-dependent inactivation seen in wild-type photoreceptors, and a much slower decay which returned the current to baseline over a period of 1–5 s (Figure 1; see also Figure 3). In most cases the entire decay time course could be well fitted by the sum of two exponentials, although at higher intensities a damped oscillation was often observed in the response waveform (e.g., Figure 1C).

We suspected that the faster component represented the Ca^{2+} dependent inactivation of TRPL channels previously described (Reuss et al., 1997; Scott et al., 1997). To test this, we perfused *trp* cells with Ca^{2+}-free Ringer's. As previously reported, this immediately resulted in pronounced facilitation due to alleviation of Ca^{2+} dependent inactivation (Reuss et al., 1997). In confirmation of Cook and Minke (1999), we found that stimuli that induced decay under normal conditions still induced decay in Ca^{2+}-free conditions. However, the fast component was absent and responses now decayed with a single exponential time course, ~3× faster than the slower component recorded in the presence of Ca^{2+} (Figures 1A and 1B).

To test if either component of decay was correlated with response inactivation, we delivered dim test flashes before and after stimuli of various intensities and duration. Only in the absence of Ca^{2+} was the response inactivation (% reduction in response) correlated with the extent of the decay (Figures 1C and 1D). By contrast, in normal Ca^{2+}, brief stimuli (<1 s), which induced as much as 80% fast decay, resulted in relatively little loss of sensitivity, which recovered quickly (<20 s), and only longer stimuli that induced slow decay resulted in significant long term inactivation.

We quantified the intensity dependence of response inactivation from similar data by plotting the residual sensitivity ($S = I/I_{max}$) as a function of the intensity of the inactivating stimulus that can be accurately calibrated in terms of effectively absorbed photons (i.e., effectively activated rhodopsin molecules; see Experimental Procedures). Since the number of microvilli (*m*) in the rhabdomere can also be estimated (~30,000, using morphological data from Suzuki et al., 1993), the fraction (*f*) of microvilli that fail to absorb a photon in a flash of effective photon content *p* can be simply calculated from Poisson statistics as

$$f = e^{(-p/m)} \quad (1)$$

Assuming that the residual sensitivity is determined by the fraction of microvilli remaining unaffected by a given stimulus, it is then possible to express inactivation in terms of the number of microvilli inactivated by each effectively absorbed photon. Namely, if one photon inactivates n microvilli, the residual sensitivity is predicted by

Table 1. Response Inactivation

	1.5 mM Ca^{2+}	0 Ca^{2+}
Wild type	NA	1.7 ± 1.1 (9)
trp^{343}	0.42 ± 0.13 (6)	4.2 ± 1.5 (6)
$rdgB^{KS222}$	0.39 ± 0.16 (4)	3.1 ± 0.7 (9)
cds^{1}	1.0 ± 0.3 (2)	4.7 ± 1.4 (3)

Number of microvilli inactivated by each effectively absorbed photon under different conditions leading to long-term response inactivation attributable to PIP_2 depletion. Data estimated from the residual sensitivity 10 s after inactivating stimuli of various intensities from Equations 2 and 3, expressed as mean ± SD (number of cells).

$$S = e^{(-p/(m/n))} \quad (2)$$

from which

$$n = -\ln S \times (m/p) \quad (3)$$

In the presence of Ca^{2+}, brief stimuli (0.5 s) failed to induce any significant inactivation in *trp* beyond wild-type controls (not shown); with stimuli of long duration (3 s), the loss of sensitivity was fitted assuming each photon effectively inactivated ~0.4 microvilli. In zero Ca^{2+}, inactivation was independent of stimulus duration (between 0.5 and 5s), but enhanced 10-fold, each photon effectively inactivating ~4 microvilli (Figure 1E; Table 1).

Kir2.1 Channels as Biosensors of PIP_2

Because we doubted whether the *trp* phenotype reflected Ca^{2+} store depletion, we sought evidence for an alternative explanation, namely, depletion of PIP_2—the substrate for the key effector enzyme in phototransduction. Support for this might be provided by biochemical measurements of microvillar PIP_2, but such measurements would be technically challenging and difficult to relate to the in vivo *trp* decay. We therefore developed a strategy for monitoring PIP_2 dynamics in vivo. Specifically, we exploited the properties of the Kir2.1 inward-rectifier channel, the activity of which is believed to be primarily determined by binding to PIP_2 (Huang et al., 1998; Rohacs et al., 1999). We expressed the human Kir2.1 channel in *Drosophila* photoreceptors under control of the rhodopsin (Rh1) promoter (see Experimental Procedures). The Kir2.1 transgene included an inframe EGFP tag, and predominant or exclusive expression in the microvillar membrane was confirmed by observation of fluorescence in both the intact eye (Figure 2) and dissociated ommatidia. All cells ($n > 100$) expressing Kir2.1 channels displayed a large constitutive potassium conductance immediately on establishing the whole-cell configuration, but light responses were unaffected. The conductance (IRK) showed the well-known inwardly rectifying properties of Kir2.1 as well as its characteristic voltage dependent block by external Cs^+ (Figure 2). Under control conditions, the IRK current was maintained for the lifetime of the recording (up to 20 min) as long as ATP was included in the pipette. In the absence of ATP, the current gradually decayed, as is typical for the behavior of Kir channels and normally attributed to degradation of PIP_2 by lipid phosphatases (Huang et al., 1998).

Following stimulation by light under control conditions, the IRK current showed at most a slight suppres-

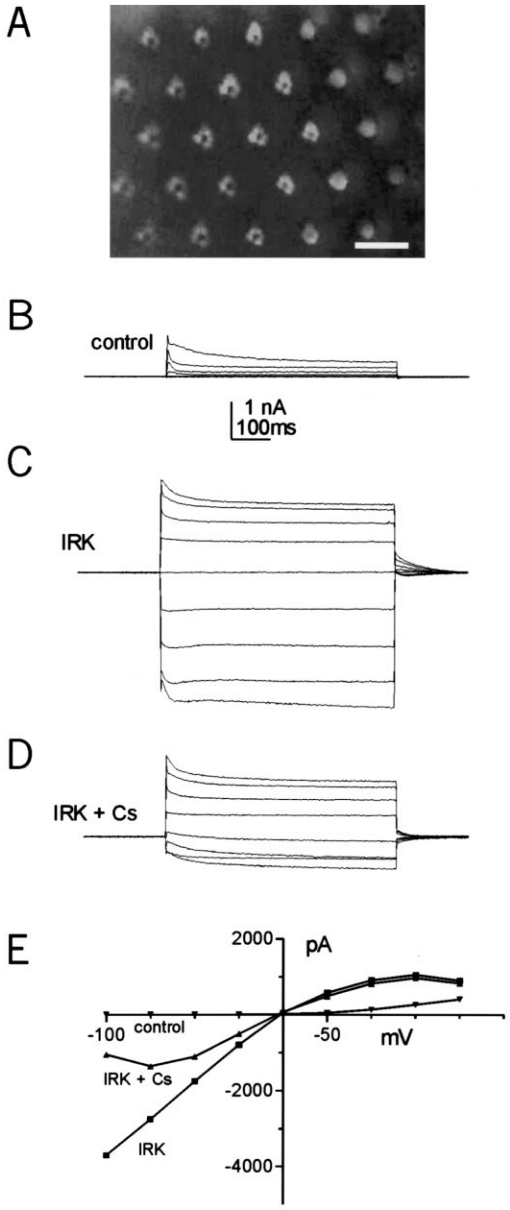

Figure 2. Functional Expression of Kir2.1 in *Drosophila* Photoreceptors

(A) Fluorescence of *Drosophila* rhabdomeres in Kir2.1-EGFP expressing flies observed in the intact eye using optical neutralization of the cornea (see Methods). The central rhabdomeres (R7 and R8) appeared dark, since Rh1GAL4 drives expression only in R1–6 cells. Scale bar 20 μm.

(B–D) Voltage clamped currents evoked by voltage steps from 0 mV to −100 mV (10 mV steps) from a holding potential of −70 mV in a control (B: Rh1GAL4/+ heterozygote), and Kir2.1 expressing photoreceptor (C and D). Bath contained 10 mM K^+. In control cells, outward currents representing voltage gated outward currents (I_A and delayed rectifier) were elicited at voltages above ~−50 mV; in Kir2.1-expressing cells these are superimposed on a large IRK, which was partially blocked in a voltage dependent manner by 4 mM Cs (D).

(E) I-V traces of currents in (B–D) determined at end of the voltage steps (open triangle, control; closed square, IRK). Note the voltage-dependent block in the presence of Cs^+ (closed triangle).

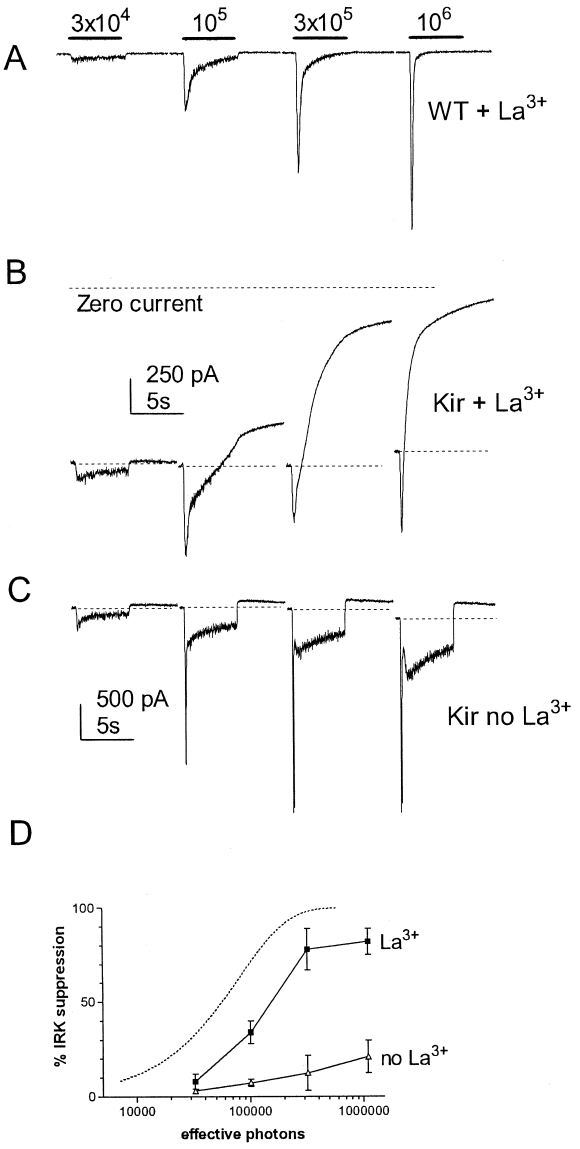

Figure 3. *trp* Decay Correlates with IRK Suppression

(A and B) Responses to 5 s stimuli (bars) of increasing intensity (number of effectively absorbed photons indicated) in bath containing 40 μM La^{3+} to mimic the *trp* phenotype.

(A) In a control cell, the lowest intensity evoked a maintained response, but a partial decay of the LIC was apparent with the second flash (10^5 photons) and decay was essentially complete with the two highest intensities.

(B) Similarly, significant IRK suppression was first seen with 10^5 photons and was nearly complete at the two higher intensities. The constitutive IRK current in the dark (dotted lines) was −1.3 nA; zero current level also shown by a dotted line.

(C) Responses in a Kir2.1 expressing cell in normal bath (no La^{3+}) show the typical peak/plateau relation of the LIC but only slight suppression of IRK (constitutive current ∼−1 nA).

(D) Intensity dependence of IRK suppression for 5 s flashes in the presence (closed square) and absence (open triangle) of La^{3+}. Mean ± SD, n = 4–7 cells for each data point. Dotted line: intensity dependence of *trp* response inactivation ($100 \times [1 - I/I_{max}]$, from Figure 1E).

sion (maximally ∼20%, Figure 3C). This may represent loss of PIP_2, but we cannot exclude some modulation of the current by the large ion fluxes associated with the LIC. In order to mimic the *trp* phenotype, the photoreceptors were exposed to La^{3+} (40 μM), which completely blocks the TRP channels while leaving TRPL channels unaffected, and quantitatively mimics diverse aspects of the physiological *trp* phenotype (e.g., Hochstrate, 1989; Hardie and Minke, 1992). Under these conditions, the IRK current was now profoundly suppressed with an intensity dependence that closely matched that of *trp* decay and response inactivation (Figures 3B and 3D). Light-induced ion fluxes are substantially reduced under these conditions, due to the block of TRP channels, leaving PIP_2 depletion as the most obvious explanation for the suppression of IRK. Following suppression, the IRK current recovered to preillumination levels over a period of ∼1 min ($t_{1/2}$ = 32 ± 5 s, n = 11; see, e.g., Figure 4), presumably reflecting PIP_2 resynthesis.

Mutations in the PIP2 Recycling Pathway Prevent Recovery from Inactivation

PIP_2 is resynthesised by conversion of DAG to phosphatidic acid, synthesis of phosphatidylinositol (PI) via CDP-diacylglycerol, transport of PI to plasma membrane, and serial phosphorylation to PIP_2 (Batty et al., 1998). If *trp* decay and inactivation are due to PIP_2 depletion, then recovery from inactivation should be impaired in mutants of this pathway. We examined two such mutants, namely $rdgB^{KS222}$, a severe hypomorph of PI transfer protein (PITP) (Vihtelic et al., 1993), and cds^1, which lacks CDP-diacylglycerol synthase (Wu et al., 1995). As long as flies were strictly dark reared, we found that responses to moderate flashes in *rdgB* were indistinguishable from wild type under control conditions. In *cds,* peak amplitudes were slightly reduced (∼50% of wild-type controls) and time-to-peaks slightly delayed (∼70 ms cf. 50 ms in wild type), probably indicative of reduced PIP_2 levels. Quantum bumps were indistinguishable from wild type in both mutants (data not shown).

To mimic the *trp* phenotype, *rdgB* or *cds* photoreceptors were exposed to La^{3+}. A dim flash was delivered to test sensitivity, following which cells were challenged with a single 5 s stimulus of intensity just sufficient to induce full decay. While *trp* photoreceptors (or wild type in the presence of La^{3+}) fully recovered sensitivity from such flashes within ∼90 s, in *rdgB* (n = 7) and *cds* (n = 5), no recovery was seen for the duration of the experiment (Figure 4). Interestingly, in most cases (12/15), similar stimuli delivered to *rdgB* in the absence of La^{3+} resulted in responses which failed to terminate, leaving channels constitutively activated (Figure 4C). Similar behavior has also been reported in electroretinogram recordings (Milligan et al., 1997). The lack of any recovery in *rdgB* or *cds* may seem surprising because PI and PIP kinases are presumably still present and capable of synthesizing PIP_2 from PI in the microvilli. However, these kinases probably operate too quickly for their effect to be noticed, i.e., PI is converted to PIP_2 "on demand" during the response. This implies not only all the PIP_2, but also all microvillar PI was depleted by the inactivating flashes.

From in vitro studies and by sequence homology, the *rdgB* protein is assumed to function as a PITP and is thus expected to be an essential component of the PI recycling pathway (Vihtelic et al., 1993; Milligan et al.,

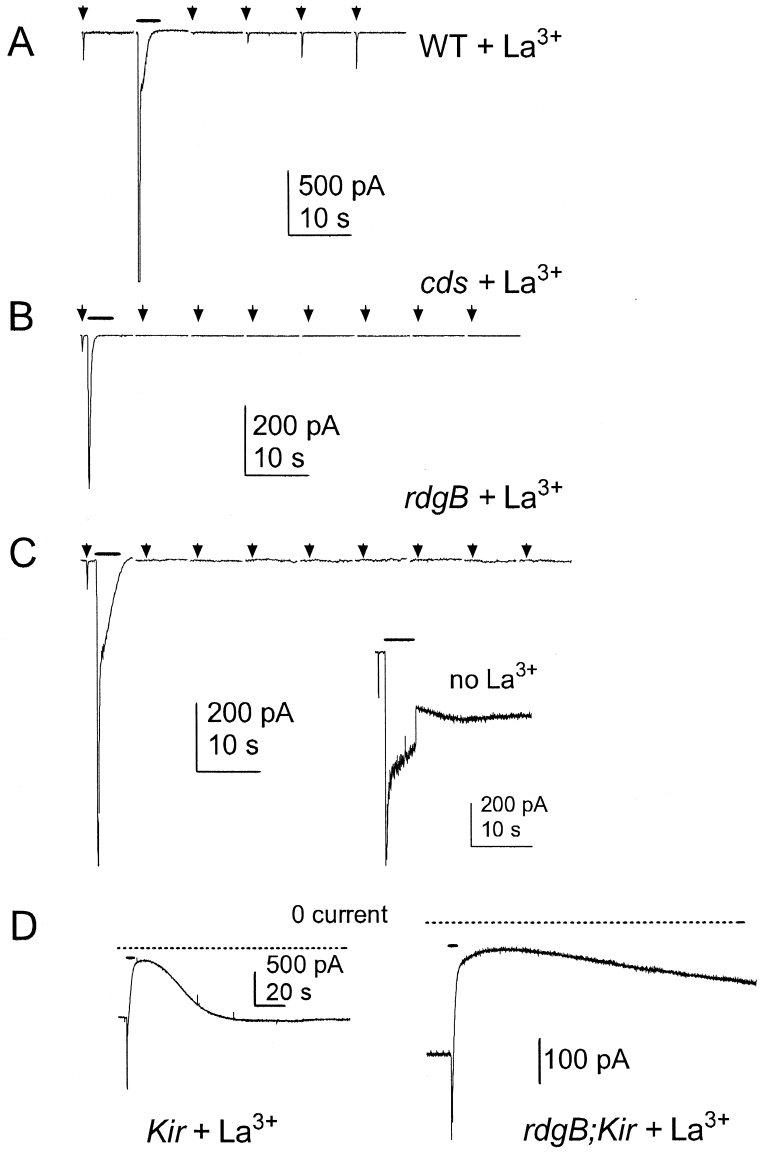

Figure 4. Mutations in PIP$_2$ Recycling Pathway Prevent Recovery from Response Inactivation

(A) In a wild-type (WT) cell bathed in 40 μM La^3, a stimulus of intensity and duration just sufficient to induce complete decay (5 s, ~200,000 photons) inactivated the response to a dim test flash (500 photons, arrows), which subsequently recovered over ~1 min (20 s between start of each trace).

(B and C) In photoreceptors from *rdgB* or *cds* mutants exposed to La^{3+}, similar stimuli also led to decay; but there was no recovery for the duration of the experiment (30 s between traces).

(C) Inset: response to same stimulus in *rdgB* without La^{3+} failed to terminate.

(D) Suppression of IRK current in photoreceptors expressing Kir2.1 channels, exposed to La^{3+} and stimulated with a 5 s inactivating flash. Left: in an otherwise wild-type fly, IRK was suppressed by ~70%, recovering within 60 s. Right: on an *rdgB* background, recovery from suppression was nearly abolished.

1997). However, there is no in vivo evidence for this assumption. To test whether PI recycling was in fact impaired, we generated *rdgB* mutant lines expressing Kir2.1 channels. After the light-induced suppression in the presence of La^{3+}, the IRK current in otherwise wild-type flies fully recovered with a half-time of ~30 s (see above). In dark-reared *rdgB;Kir* flies after one flash of intensity and duration just sufficient to induce decay, the IRK current was similarly suppressed but then recovered by at most ~50% over a period of several minutes ($n = 6$, Figure 4D), probably reflecting some limited, slow transfer of PI by residual RDGB protein in this hypomorph.

Response Inactivation in Ca^{2+}-Free Solutions

Because the primary defect in *trp* mutants is the absence of a Ca^{2+} permeable channel, the simplest explanation for the various manifestations of the *trp* phenotype would be the reduced Ca^{2+} influx, in which case removing external Ca^{2+} might be expected to mimic the *trp* phenotype. Although prolonged stimuli delivered to wild-type cells in Ca^{2+}-free solution do not normally lead to response decay unless cells have been exposed to Ca^{2+} for long periods (Hardie and Minke, 1992), this might reflect differences between TRP and TRPL channels, such as their relative sensitivity to putative second-messenger molecules or mechanism of inactivation. Therefore, we asked, instead, whether the *trp* response inactivation phenotype was mimicked in Ca^{2+}-free solutions. Sensitivity in wild type normally recovers to ~80% of control levels within seconds of even the brightest "inactivating" stimuli; however, in the absence of Ca^{2+}, sensitivity was progressively reduced with increasing intensity, sensitivity recovering slowly with a time course ($t_{1/2} = 47 \pm 10$ s, $n = 6$; Figure 5B) very similar to that following response inactivation in *trp* or wild type in the presence of La^{3+} ($t_{1/2} = 52 \pm 6$ s, $n = 7$; e.g., Figure 4A). With inactivating flashes containing more than ~20,000

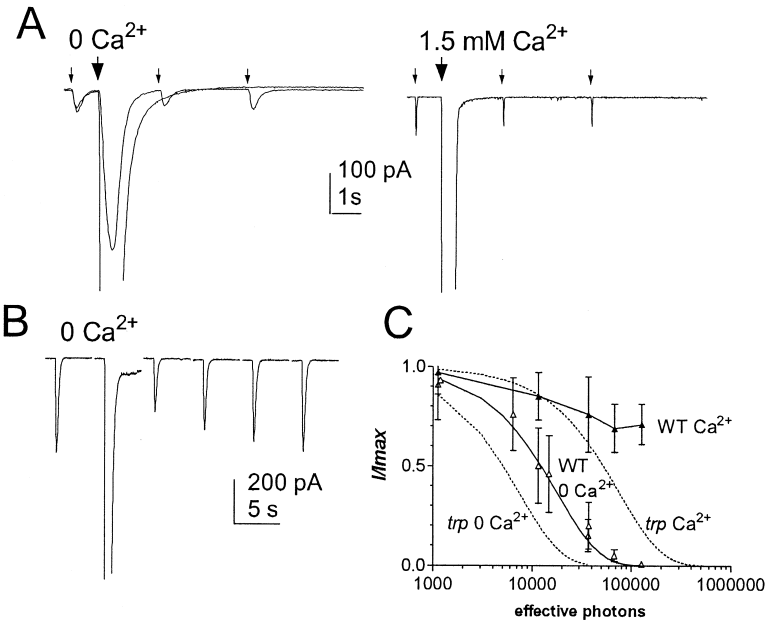

Figure 5. Response Inactivation in Ca^{2+}-Free Ringer's

Sensitivity in a wild-type photoreceptor was probed with dim test flashes (small arrows) delivered before and after an inactivating stimulus of either 1000 or 35,000 photons.
(A) The photoreceptor was completely inactivated following the brighter stimulus in 0 Ca^{2+} (left) but not in 1.5 mM Ca^{2+} (right).
(B) Time course of recovery from inactivation in Ca^{2+}-free solution following an inactivating flash containing ~26,000 photons: 28 s between traces.
(C) The residual sensitivity (I/I_{max}) in 0 Ca^{2+} (open triangle) was fitted assuming each effectively absorbed photon inactivated 1.7 microvilli (Equation 2); closed triangle data obtained in an identical fashion in normal Ca^{2+} (mean ± SD, n = 4–5 cells). Dotted lines: equivalent data from *trp* photoreceptors (from Figure 1E).

effective photons, responses often failed to terminate, leaving channels constitutively activated, sometimes indefinitely, reminiscent of the behavior of *rdgB* photoreceptors in the presence of Ca^{2+}. Quantitatively, the number of microvilli (1.7) effectively inactivated per photon in Ca^{2+}-free solutions was ~4× greater than in *trp* in normal Ca^{2+} and within a factor of ~2 of that measured in *trp* in Ca^{2+}-free solutions (Figure 5; Table 1).

To test whether PIP_2 depletion was also responsible for inactivation in zero Ca^{2+}, we again recorded from *rdgB* and *cds* photoreceptors. Because responses in *rdgB*, in particular, often failed to terminate with brighter flashes, we used relatively dim flashes repeated at 30 s intervals in Ca^{2+}-free solutions. In wild-type controls, this was sufficient time for the response to recover fully between flashes; however, in *rdgB* and *cds*, each flash induced an exponential and irreversible reduction in sensitivity (Figure 6; see also Wu et al., 1995). The sensitivity loss was well fitted, assuming each photon inactivated ~3 microvilli. Similar experiments performed in the presence of Ca^{2+} also led to a progressive irreversible loss of sensitivity, but ~8× as many photons were required to achieve the same degree of inactivation (Table 1), implying that 8× less PIP_2 was hydrolyzed per absorbed photon. These results show that removing Ca^{2+} closely mimics the *trp* inactivation phenotype, and that this is also likely to be due to depletion of PIP_2.The close quantitative correspondence between response inactivation measured in *trp* and *rdgB* (Table 1) raises the possibility that the *trp* phenotype may reflect a defect in PIP_2 recycling (see Discussion).

Ca^{2+} Rescues the *trp* Inactivation Phenotype

It has been suggested that reduced Ca^{2+} influx is not sufficient to explain the *trp* phenotype because it could not be rescued by raising extracellular or intracellular

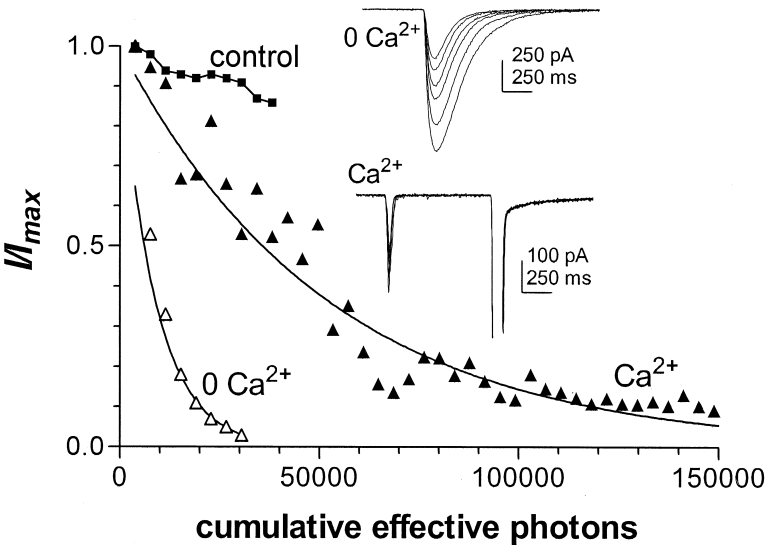

Figure 6. Response Inactivation in *rdgB*

Insets: responses to 10 ms flashes repeated at 30 s intervals in an *rdgB* photoreceptor exposed to Ca^{2+}-free solution: each flash (~2000 photons) induced a progressive reduction in sensitivity. In 1.5 mM Ca^{2+} repeated flashes of twice the intensity induced significantly less inactivation (sensitivity probed with a dim test flash before each flash as the large responses to the inactivating stimulus were inadequately clamped). The plot of normalized peak responses (I/I_{max}) confirms that loss of sensitivity was more severe in Ca^{2+}-free bath (open triangle) than in 1.5 mM Ca^{2+} (closed triangle); wild-type controls in Ca^{2+}-free bath (closed square) showed little inactivation under these conditions. Curves were fitted assuming that each absorbed photon inactivates 3.4 microvilli in Ca^{2+} free but only 0.58 microvilli in the presence of Ca^{2+} (Equation 2). See Table 1 for statistics on all cells.

Ca^{2+} (Scott et al., 1997; Cook and Minke, 1999). However, it may not be possible to reproduce wild-type microvillar Ca^{2+} levels in *trp* mutants by raising extracellular Ca^{2+} because their light-sensitive channels (i.e., TRPL channels) are ~25× less permeable to Ca^{2+} (Reuss et al., 1997), and quantum bumps in *trp* are ~5× smaller (Niemeyer et al., 1996; Henderson et al., 2000). A potentially more powerful method to raise Ca^{2+} is to block Na^+/Ca^{2+} exchange during the response. This is because, following stimulation, Ca^{2+} initially rises to very high (>200 μM) concentrations in the microvilli, but then relaxes quickly (~100 ms) to a much lower steady-state value, due to Na^+/Ca^{2+} exchange (Oberwinkler and Stavenga, 2000). A similar behavior can be expected in *trp*, except the absolute levels reached will be lower due to the reduced Ca^{2+} influx. We reasoned that in *trp,* the transient Ca^{2+} rise might be sufficient to prevent PIP_2 depletion, before falling below some critical value as it is extruded by the exchanger. If this is the case, blocking the exchanger should maintain Ca^{2+} at sufficient levels to prevent depletion. We therefore rapidly perfused photoreceptors with a solution in which Na^+ was substituted for Cs^+ or Li^+ to prevent Na^+/Ca^{2+} exchange, starting shortly (<1 s) before a flash, which would normally inactivate the response in *trp*. The degree of inactivation was probed with a test flash 10 s after the inactivating stimulus, by which time reperfusion of the bath solution had restored exchanger activity and presumably returned microvillar Ca^{2+} near to preillumination levels.

In controls, the response to the test stimulus in *trp* was virtually abolished by the inactivating stimulus as usual, but if Na^+/Ca^{2+} exchange was blocked during the 5 s, inactivating stimulus sensitivity recovered to ~50% (Figure 7). Responses recorded in the absence of Na^+ decayed to baseline more quickly than under control conditions. We presume this represents enhanced Ca^{2+}-dependent inactivation, but it was clearly no longer related to the response inactivation and cannot be considered part of the *trp* phenotype because a similar decay was seen in wild-type flies under these conditions (Figure 7C).

To test whether PIP_2 depletion was prevented under these conditions, we performed similar experiments in flies expressing Kir2.1 channels in the presence of La^{3+}. As shown above, a stimulus of sufficient intensity and duration to induce decay resulted in ~70% suppression of the IRK current indicating substantial loss of PIP_2. However, when Na^+/Ca^{2+} exchange was blocked during the inactivating stimulus, IRK was suppressed by at most 10%–20% (Figure 7D).

Discussion

We have shown that responses in *trp* decay with two kinetic components, but only the slower component, which was accelerated in Ca^{2+}-free solution, was correlated with response inactivation. By targeting a PIP_2-sensitive ion channel to the microvilli, we provided evidence that decay was associated with depletion of PIP_2, suggesting this may underlie the *trp* phenotype. This was supported by two independent mutations in the PIP_2 recycling pathway, both of which prevented recovery from inactivation. Finally we demonstrated that removing extracellular Ca^{2+} mimicked response inactivation, while raising Ca^{2+} could rescue this aspect of the *trp* phenotype. In the following, we discuss the use of Kir channels as PIP_2 biosensors, compare our findings with previous data and models of *trp* decay, and discuss novel aspects of the Ca^{2+}-dependent regulation of PIP_2 metabolism revealed by this study. Finally, we consider the implications of our results for the cellular and molecular strategies underlying the performance of microvillar photoreceptors.

Kir Channels as PIP_2 Biosensors

Only recently have attempts been made to monitor PIP_2 in vivo, in particular using GFP-tagged pleckstrin homology (PH) domains (Stauffer et al., 1998; Varnai and Balla, 1998; Holz et al., 2000). Although this approach has proved valuable for visualizing PIP_2 localization, the PH domain used in most of these studies (from $PLC\delta_1$) has higher affinity for $InsP_3$ than PIP_2, complicating interpretation of dynamic changes (Lemmon et al., 1995; Hirose et al., 1999). We therefore developed an alternative approach for tracking PIP_2, by exploiting the properties of the Kir family of inward rectifier K channels, whose activity has been shown to depend upon binding to PIP_2 (Baukrowitz et al., 1998; Huang et al., 1998; Zhang et al., 1999). We chose Kir2.1 (= IRK1; Kubo et al., 1993), as this is constitutively active, is activated by PIP_2 alone without cofactors, and is reported to have the highest affinity and greatest specificity for PIP_2 of all the Kir family (Huang et al., 1998; Rohacs et al., 1999; Zhang et al., 1999).

Although there are no quantitative dose response data for the PIP_2 dependence of Kir channels, it seems likely that the near total suppression seen in the presence of La^{3+} also represents near total depletion of microvillar PIP_2 and PI. Thus, firstly, the suppression of IRK currents was invariably associated with profound loss of sensitivity to light, entirely consistent with loss of substrate (PIP_2) for phototransduction. Secondly, Kir2.1's affinity for PIP_2 is so high that it has not previously proven possible to suppress its activity by PLC activation (Kobrinsky et al., 2000). The high affinity for PIP_2 may also explain why IRK required slightly higher intensities for suppression than *trp* inactivation (Figure 3D), recovered slightly more quickly ($t_{1/2}$ = 32 s cf. ~50 s for recovery of light response), and, unlike the response to light, showed some limited slow recovery in *rdgB* hypomorphs.

Ca^{2+}-Dependent Regulation of PIP_2

Our results indicate that the stimulus-dependent loss of sensitivity when Ca^{2+} influx is reduced—either by removing extracellular Ca^{2+} or by the *trp* mutation—is due to PIP_2 depletion. Assuming PIP_2 levels are determined by the balance between synthesis and hydrolysis, this could be explained by Ca^{2+}-dependent inhibition of PLC activity, or by Ca^{2+}-dependent facilitation of PIP_2 recycling. Our evidence suggests both may contribute. Firstly, facilitation of PIP_2 recycling cannot readily account for the results found in PIP_2-recycling mutants. Inactivation in *rdgB* and *cds* was irreversible on the time scale of the experiments, indicating there was no effective PIP_2 recycling, and we therefore attribute the

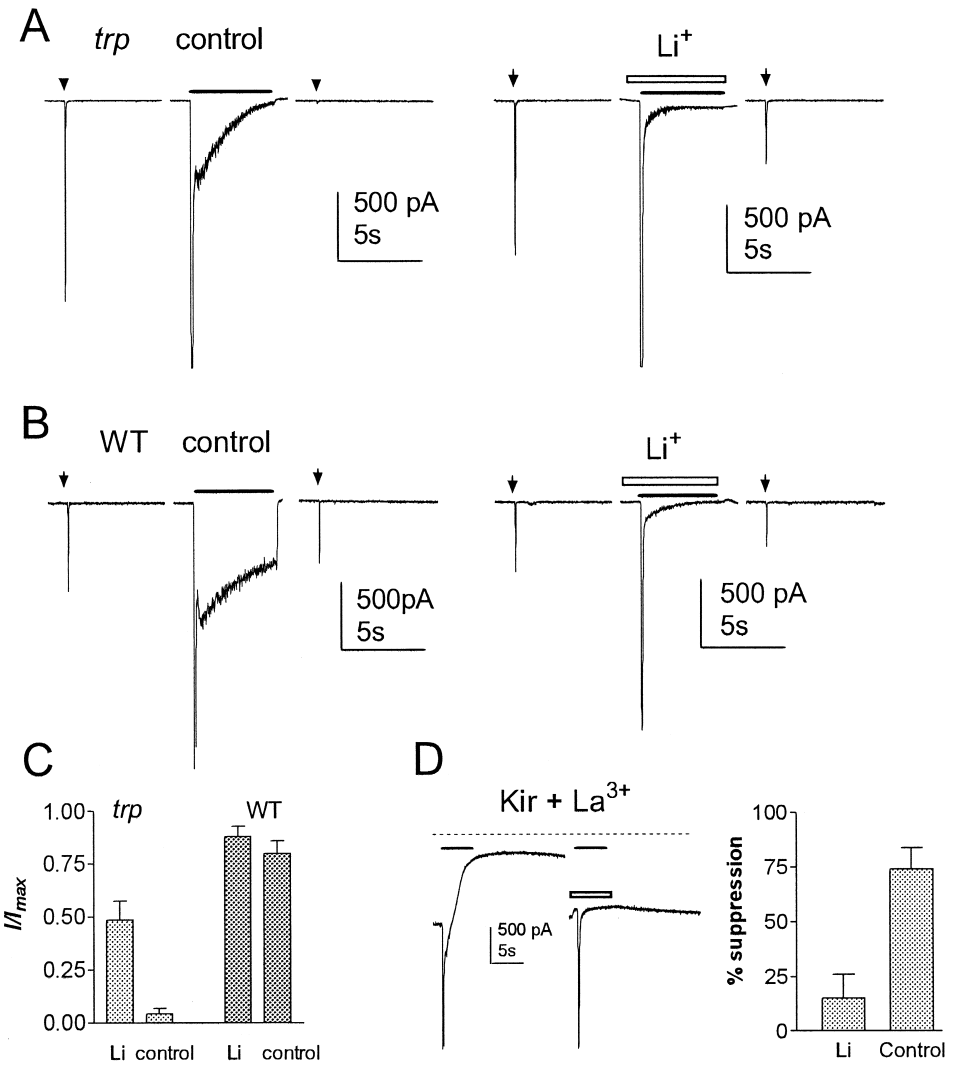

Figure 7. Blocking Na^{2+}/Ca^{2+} Exchange Rescues Response Inactivation in *trp*

In each set of three traces, a test flash preceded a 5 s inactivating stimulus (solid line: ~50,000 photons s^{-1}); sensitivity was probed with an identical test flash delivered 10 s later.

(A) Under control conditions (left), the response to a test flash was reduced to <5%. However, when the same cell was perfused with Na^+-free Ringer (box) during the inactivating stimulus, the response had recovered to ~50%.

(B) Identical experiment performed in wild type (WT), the response recovered to >50% irrespective of perfusion. In both wild-type and *trp* blocking the exchanger resulted in complete decay of the response due to enhanced Ca^{2+}-dependent inactivation.

(C) Histogram showing recovery in *trp* (mean ± SD, n = 6) and WT under control conditions with and without (control) Li^+ perfusion during the stimulus.

(D) Similar experiment performed in flies expressing Kir2.1 channels exposed to La^{3+}. Under control conditions the IRK current was suppressed by the inactivating stimulus; this was prevented by Na^+-free perfusion (right). The histogram compares percent suppression of IRK with and without Li^+ perfusion (mean ± SD, n = 11).

marked Ca^{2+} dependence of inactivation in these mutants to Ca^{2+}-dependent inhibition of PLC activity. Because response inactivation in *trp* and *rdgB* showed similar sensitivity to Ca^{2+} (Figures 1E and 6; Table 1), the same mechanism—i.e., inhibition of PLC activity—seems likely to have been responsible. This suggests that Ca^{2+} influx may also be required to facilitate rapid PIP_2 recycling and that failure of this may be the primary cause of the *trp* phenotype in the presence of Ca^{2+}. This is supported by the close quantitative correspondence between the *rdgB* and *trp* response inactivation phenotypes in both Ca^{2+} and Ca^{2+}-free solutions (Table 1). Although the response in *rdgB* does not normally decay to baseline as in *trp*, as in the case of zero Ca^{2+}, we assume this reflects the different properties of TRP and TRPL channels. Interestingly, we found that stimuli of sufficient intensity and duration to induce decay in *trp* often resulted in failure of response termination in *rdgB* or in wild type in Ca^{2+}-free solutions. This suggests that, although both TRP and TRPL channels require PIP_2 hydrolysis for activation, under some conditions, TRP channels may become constitutively activated following PIP_2 depletion, while TRPL channels inactivate.

Both Ca^{2+}-dependent inhibition of PLC activity and

Ca^{2+}-dependent facilitation of PIP_2 recycling appear required to explain the Ca^{2+}-dependent regulation of PIP_2 metabolism indicated by this study. In principle, PLC activity could be regulated by Ca^{2+} at the level of rhodopsin, G-protein, or PLC. G-proteins are not normally regulated by Ca^{2+}, and recent evidence suggests Ca^{2+} is probably not involved in regulating rhodopsin lifetime in *Drosophila* (Alloway and Dolph, 1999). Therefore, PLC itself should be considered as a likely site of action, and indeed *Drosophila* PLC has been reported to be inhibited in vitro by high micromolar concentrations of Ca^{2+} (Running Deer et al., 1995). Possible mechanisms for Ca^{2+} dependent inhibition of PLC include Ca-calmodulin binding or phosphorylation by a Ca^{2+}-dependent enzyme such as PKC, both of which have been proposed in *Limulus* photoreceptors (Richard and Lisman, 1997; Dabdoub and Payne, 1999). However, in view of the exceptionally high Ca^{2+} concentrations believed to occur in the microvilli following even a single photon absorption, a simple and direct mechanism might be binding of Ca^{2+} to PIP_2, thereby screening its negative charges.

To our knowledge, there is no precedent for Ca^{2+} dependent facilitation of PIP_2 recycling. However, an acidic amino-acid domain adjacent to the PI transfer domain of the RDGB protein has been reported to bind Ca^{2+} in vitro (Vihtelic et al., 1993) suggesting a possible molecular target. Because several steps of the PIP_2 recycling pathway are presumed to occur in the submicrovillar cisternae (specialized endoplasmic reticulum at the base of the microvilli), which are also believed to represent Ca^{2+} stores, we would not exclude the possibility that PIP_2 recycling might be regulated by store Ca^{2+} rather than cytosolic Ca^{2+}.

Resolution of the *trp* Phenotype

Our results support the proposal that exhaustion of some factor required for excitation underlies the *trp* phenotype (Minke, 1982). We have now identified this factor as PIP_2, while we found that the role of Ca^{2+} is indirect, namely to reduce net PIP_2 hydrolysis. Earlier, apparently contradictory studies of *trp* decay can readily be reconciled with these findings. We confirm that response decay and inactivation in *trp* are more profound in zero Ca^{2+} (Cook and Minke, 1999), but explain this by proposing that Ca^{2+} influx during the light response in *trp* is still sufficient to inhibit PLC, but insufficient to facilitate rapid PIP_2 recycling. Scott et al.'s report (1997) of Ca^{2+}-dependent inactivation of TRPL channels is revealed as the first and faster component of *trp* decay in the presence of Ca^{2+}. However, this is unrelated to the response inactivation and cannot be considered part of the mutant phenotype since Ca^{2+} dependent inactivation is a characteristic of wild-type photoreceptors as well.

The proposal that *trp* decay represented depletion of Ca^{2+} stores (Minke and Selinger, 1991; Cook and Minke, 1999) has been a key argument for the hypothesis that release of Ca^{2+} is essential for excitation in *Drosophila* photoreceptors. Together with recent results showing that phototransduction is unaffected by mutations in either the $InsP_3$ or ryanodine receptors (Acharya et al., 1997; Raghu et al., 2000; Sullivan et al., 2000), our explanation for the *trp* phenotype casts doubt on any direct role for Ca^{2+} stores in excitation in *Drosophila* photoreceptors.

Quantitative Considerations

To our knowledge, the only available data on the biochemical amplification of the phototransduction cascade in invertebrate photoreceptors come from studies in the squid, suggesting ~500 PIP_2 molecules are hydrolyzed per activated rhodopsin (Szuts, 1993). From the relationship between the intensity of an inactivating flash and sensitivity loss, it is now possible to make an estimate of the biochemical amplification in *Drosophila*. Without Ca^{2+} influx or PIP_2 recycling, activation of one rhodopsin molecule appears to result in the hydrolysis, not only of the immediately available PIP_2, but also most of the PI in up to ~4 microvilli (Table 1). Data from *Calliphora* (Zinkler et al., 1985) suggest that PI represents ~3% of microvillar lipids which, given the dimensions of the microvilli (1 μm long, 60 nm in diameter), would amount to ~7500 molecules per microvillus. The comparison of response inactivation in the presence and absence of Ca^{2+} suggests that ~10-fold less (i.e., up to ~3000 PIP_2 molecules) may be hydrolyzed per photon under physiological conditions in the presence of Ca^{2+} influx. This would probably represent all the immediately available PIP_2, which is not generally considered to account for more than 10% of total PI.

Strategy of Phototransduction in Microvillar Photoreceptors

Dipteran photoreceptors have the fastest G-protein coupled signaling pathway known: when dark adapted they respond to single photons with large bumps (~10 pA in *Drosophila*,), representing simultaneous activation of about 15 TRP channels (Henderson et al., 2000) with a duration of ~30 ms at 20°C. They also light adapt to cover the entire environmental range of intensities (up to ~10^5–10^6 photons per s) (Howard et al., 1987; Juusola and Hardie, 2001). This performance is remarkable when compared, for example, to amphibian rods, which generate quantum bumps of ~1 pA amplitude with a duration in excess of 1 s and which saturate with photon fluxes of ~500 s^{-1} (Baylor et al., 1979). Previous studies have indicated that quantum bumps in *Drosophila* are triggered downstream of PLC, the final amplification being mediated by Ca^{2+} influx (Henderson et al., 2000). Bump latency however, is limited by the rate of activation of PLC, since hypomorphic mutations in PLC result in normal size quantum bumps but with prolonged latencies (Scott and Zuker, 1998; Cook et al., 2000). Bump latency is the main constraint on response speed in *Drosophila* (Juusola and Hardie, 2001); our results suggest that short latencies are achieved by exceptionally high PIP_2-hydrolysis rates consuming much of the immediately available PIP_2 within one microvillus. The rate of hydrolysis required is so great however, that without Ca^{2+}-dependent feedback, not only the immediately available PIP_2 but also the entire PI reservoir of up to 4 microvilli is consumed within 1 s. The microvillar design of the rhabdomeric photoreceptor ensures that Ca^{2+} influx elicited by a single photon raises Ca^{2+} to extremely high levels during the time course of the quantum bump

(Postma et al., 1999). We recently showed that this Ca^{2+} influx is required for the facilitation responsible for large quantum bumps (Henderson et al., 2000); the present study suggests that it is also essential to rapidly quench PLC activity and accelerate PIP_2 recycling, so as to avoid an otherwise debilitating depletion of substrate. For a short period (50–100 ms), the microvillus then becomes refractory (Hochstrate and Hamdorf, 1990) until Ca^{2+} is extruded by the Na^+/Ca^{2+} exchanger. We suggest that this allows time for PIP_2 to be rapidly replenished and also for molecular inactivation of rhodopsin and the G-protein-PLC complex (Cook et al., 2000). When Ca^{2+} feedback fails, as in the *trp* mutant, this strategy leaves the PI and PIP_2 pool in the microvilli vulnerable to total depletion resulting in response decay and inactivation.

Experimental Procedures

Flies

Flies (*Drosophila melanogaster*) were raised in the dark at 25°C. The wild-type strain was *w* Oregon; mutants included: trp^{P343} (null; Niemeyer et al., 1996); $rdgB^{KS222}$ (severe hypomorph; Milligan et al., 1997); and cds^1 (null, Wu et al., 1995). Flies expressing a UAS-Kir2.1 transgene tagged with an inframe enhanced green fluorescent protein (*p[UAS-EGFP-Kir2.1]*) were generated as described elsewhere (Baines et al., 2001). Two independent transformants (Kir1, on the second, and Kir7, on the third chromosome) were used with no obvious differences. To induce expression in the photoreceptors (*p[UAS-EGFP-Kir2.1]*) flies were crossed to flies expressing a 3kB fragment containing the promoter for R1–6 rhodopsin (Rh1) fused to GAL4 (*p[Rh1GAL4]* (again, 2 independent transformants on the 2nd and 3rd chromosomes were used; provided by J. Treismann and F. Pichaud) and experiments performed on F1 adults. Expression of *EGFP-Kir2.1* was confirmed by GFP fluorescence on dissociated ommatidia, or in the intact animal by using optical neutralization of the cornea (Franceschini and Kirschfeld, 1971) whereby an immersion objective is lowered onto the cornea of an immobilized fly and focused through the facet lenses at the levels of the rhabdomere tips (Figure 2A).

Whole-Cell Recordings

Dissociated ommatidia were prepared as previously described from recently enclosed adult flies (Hardie, 1991; Hardie, 1996) and transferred to the bottom of a recording chamber on an inverted Nikon Diaphot microscope. Unless otherwise stated, the bath was composed of (in mM): 120 NaCl, 5 KCl, 10 TES, 4 $MgCl_2$, 1.5 $CaCl_2$, 25 proline, and 5 alanine. Ca^{2+}-free bath was identical except $CaCl_2$ was omitted and 250 μM EGTA added (free $[Ca^{2+}] < 100$ nM measured fluorimetrically with 5 μM INDO-1). In some experiments, the monovalent cation composition was 110 NaCl, 10 KCl, and 4 CsCl to reduce the amplitude and voltage dependence of the IRK currents. For experiments in Ca^{2+}-free bath or Na^+-free Ringer, cells were plated in normal bath and perfused shortly before the experiment from a broad tipped (~5–10 μm) picospritzer. Intracellular solution was (in mM): 140 K gluconate, 10 TES, 4 Mg ATP, 2 $MgCl_2$, 1 NAD, and 0.4 Na GTP. pH of all solutions was 7.15. Whole-cell voltage clamp recordings were made using electrodes of resistance ~10 MΩ; series resistance values were generally below 25 MΩ and were routinely compensated to 80%. Data were collected and analyzed using an Axopatch 1-D amplifier and pCLAMP 6 or 8 software (Axon Instruments, Foster City, California). Cells were stimulated via a green LED, with maximum effective intensity of $\sim 2 \times 10^5$ photons s^{-1} per photoreceptor. Relative intensities were calibrated using a photomultiplier and converted to absolute intensities in terms of effectively absorbed photons by counting quantum bumps at low intensities (e.g., Henderson et al., 2000).

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References

Acharya, J.K., Jalink, K., Hardy, R.W., Hartenstein, V., and Zuker, C.S. (1997). InsP(3) receptor is essential for growth and differentiation but not for vision in Drosophila. Neuron *18*, 881–887.

Alloway, P.G., and Dolph, P.J. (1999). A role for the light-dependent phosphorylation of visual arrestin. Proc. Natl. Acad. Sci. USA *96*, 6072–6077.

Baines, R.A., Uhler, J.P., Thompson, A., Sweeney, S.T., and Bate, M. (2001). Altered electrical properties in Drosophila neurons developing without synaptic transmission. J. Neurosci. *21*, 1523–1531.

Batty, I.H., Currie, R.A., and Downes, C.P. (1998). Evidence for a model of integrated inositol phospholipid pools implies an essential role for lipid transport in the maintenance of receptor-mediated phospholipase C activity in 1321N1 cells. Biochem. J. *330*, 1069–1077.

Baukrowitz, T., Schulte, U., Oliver, D., Herlitze, S., Krauter, T., Tucker, S.J., Ruppersberg, J.P., and Fakler, B. (1998). PIP_2 and PIP as determinants for ATP inhibition of K-ATP channels. Science *282*, 1141–1144.

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

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

Cook, B. BarYaacov, M., BenAmi, H.C., Goldstein, R.E., Paroush, Z., Selinger, Z., and Minke, B. (2000). Phospholipase C and termination of G-protein-mediated signalling in vivo. Nature Cell Biol. *2*, 296–301.

Cook, B., and Minke, B. (1999). TRP and calcium stores in Drosophila phototransduction. Cell Calcium *25*, 161–171.

Cosens, D.J., and Manning, A. (1969). Abnormal electroretinogram from a *Drosophila* mutant. Nature *224*, 285–287.

Dabdoub, A., and Payne, R. (1999). Protein kinase c activators inhibit the visual cascade in Limulus ventral photoreceptors at an early stage. J. Neurosci. *19*, 10262–10269.

Franceschini, N., and Kirschfeld, K. (1971). Etude optique in vivo des éléments photorécepteurs dans l'oeil composé de *Drosophila*. Kybernetik *9*, 159–182.

Hardie, R.C. (1996). INDO-1 measurements of absolute resting and light-induced Ca2+ concentration in Drosophila photoreceptors. J. Neurosci. *16*, 2924–2933.

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

Hardie, R.C., and Minke, B. (1994). Calcium-dependent inactivation of light-sensitive channels in Drosophila photoreceptors. J. Gen. Physiol. *103*, 409–427.

Hardie, R.C., and Minke, B. (1995). Phosphoinositide-mediated phototransduction in Drosophila photoreceptors: The role of Ca2+ and trp. Cell Calcium *18*, 256–274.

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

Harteneck, C., Plant, T.D., and Schultz, G. (2000). From worm to man: three subfamilies of TRP channels. Trends Neurosci. *23*, 159–166.

Henderson, S.R., Reuss, H., and Hardie, R.C. (2000). Single photon responses in Drosophila photoreceptors and their regulation by Ca^{2+}. J. Physiol. Lond. *524*, 179–194.

Hirose, K., Kadowaki, S., Tanabe, M., Takeshima, H., and Iino, M. (1999). Spatiotemporal dynamics of inositol 1,4,5-trisphosphate that underlies complex Ca2+ mobilization patterns. Science *284*, 1527–1530.

Hochstrate, P. (1989). Lanthanum mimicks the trp photoreceptor mutant of Drosophila in the blowfly Calliphora. J. Comp. Physiol. [A] *166*, 179–187.

Hochstrate, P., and Hamdorf, K. (1990). Microvillar components of light adaptation in blowflies. J. Gen. Physiol. *95*, 891–910.

Holz, R.W., Hlubek, M.D., Sorensen, S.D., Fisher, S.K., Balla, T., Ozaki, S., Prestwich, G.D., Stuenkel, E.L., and Bittner, M.A. (2000). A pleckstrin homology domain specific for phosphatidylinositol 4,5-bisphosphate (PIP_2) and fused to green fluorescent protein identifies plasma membrane PIP_2 as being important in exocytosis. J. Biol. Chem. *275*, 17878–17885.

Howard, J., Blakeslee, B., and Laughlin, S.B. (1987). The intracellular pupil mechanism and the maintenance of photoreceptor signal:noise ratios in the blowfly *Lucilia cuprina*. Proc. R. Soc. Lond. B *231*, 415–435.

Huang, C.L., Feng, S.Y., and Hilgemann, D.W. (1998). Direct activation of inward rectifier potassium channels by PIP_2 and its stabilization by G beta gamma. Nature *391*, 803–806.

Juusola, M., and Hardie, R.C. (2001). Light Adaptation in Drosophila Photoreceptors. I. response dynamics and signaling efficiency at 25 degrees c. J. Gen. Physiol. *117*, 3–25.

Kobrinsky, E., Mirshahi, T., Zhang, H., Jin, T., and Logothetis, D.E. (2000). Receptor-mediated hydrolysis of plasma membrane messenger PIP_2 leads to K^+-current desensitization. Nat. Cell Biol. *2*, 507–514.

Kubo, Y., Baldwin, T.J., Jan, Y.N., and Jan, L.Y. (1993). Primary structure and functional expression of a mouse inward rectifier potassium channel. Nature *362*, 127–133.

Lemmon, M.A., Ferguson, K.M., Obrien, R., Sigler, P.B., and Schlessinger, J. (1995). Specific and high-affinity binding of inositol phosphates to an isolated pleckstrin homology domain. Proc. Natl. Acad. Sci. USA *92*, 10472–10476.

Milligan, S.C., Alb, J.G., Elagina, R.B., Bankaitis, V.A., and Hyde, D.R. (1997). The phosphatidylinositol transfer protein domain of Drosophila retinal degeneration B protein is essential for photoreceptor cell survival and recovery from light stimulation. J. Cell Biol. *139*, 351–363.

Minke, B. (1982). Light-induced reduction in excitation efficiency in the trp mutant of Drosophila. J. Gen. Physiol. *79*, 361–385.

Minke, B., and Hardie, R.C. (2000). Genetic dissection of Drosophila phototransduction. In Handbook of Biological Physics, Vol. 3, D.G. Stavenga, W.J. de Grip and E. N. Pugh, eds. (Elsevier), pp. 449–525.

Minke, B., and Selinger, Z. (1991). Inositol lipid pathway in fly photoreceptors: excitation, calcium mobilization and retinal degeneration. Prog. Retinal Res. *11*, 99–124.

Minke, B., Wu, C.-F., and Pak, W.L. (1975). Induction of photoreceptor noise in the dark in a Drosophila mutant. Nature *258*, 84–87.

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

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

Oberwinkler, J., and Stavenga, D.G. (2000). Calcium transients in the rhabdomeres of dark- and light-adapted fly photoreceptor cells. J. Neurosci. *20*, 1701–1709.

Peretz, A. SussToby, E., RomGlas, A., Arnon, A., Payne, R., and Minke, B. (1994). The light response of Drosophila photoreceptors is accompanied by an increase in cellular calcium: Effects of specific mutations. Neuron *12*, 1257–1267.

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

Postma, M., Oberwinkler, J., and Stavenga, D.G. (1999). Does Ca2+ reach millimolar concentrations after single photon absorption in Drosophila photoreceptor microvilli? Biophys. J. *77*, 1811–1823.

Raghu, P., Colley, N.J., Webel, R., James, T., Hasan, G., Danin, M., Selinger, Z., and Hardie, R.C. (2000). Normal phototransduction in Drosophila photoreceptors lacking an InsP(3) receptor gene. Mol. Cell Neurosci. *15*, 429–445.

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.

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

Richard, E.A., and Lisman, J.E. (1997). Calmodulin (CaM) peptide antagonists block G(q) phospholipase C (PLC) activity in Limulus ventral photoreceptors. Invest. Ophthalmol. Vis. Sci. *38*, 2775–2775.

Rohacs, T., Chen, J., Prestwich, G.D., and Logothetis, D.E. (1999). Distinct specificities of inwardly rectifying K+ channels for phosphoinositides. J. Biol. Chem. *274*, 36065–36072.

Running Deer, J.L., Hurley, J.B., and Yarfitz, S.L. (1995). G protein control of Drosophila photoreceptor specific phospholipase C. J. Biol. Chem. *270*, 12623–12628.

Scott, K., Sun, Y.M., Beckingham, K., and Zuker, C.S. (1997). Calmodulin regulation of Drosophila light-activated channels and receptor function mediates termination of the light response in vivo. Cell *91*, 375–383.

Scott, K., and Zuker, C.S. (1998). Assembly of the Drosophila phototransduction cascade into a signalling complex shapes elementary responses. Nature *395*, 805–808.

Stauffer, T.P., Ahn, S., and Meyer, T. (1998). Receptor-induced transient reduction in plasma membrane PtdIns(4,5)P-2 concentration monitored in living cells. Curr. Biol. *8*, 343–346.

Sullivan, K.M.C., Scott, K., Zuker, C.S., and Rubin, G.M. (2000). The ryanodine receptor is essential for larval development in Drosophila melanogaster. Proc. Natl. Acad. Sci. USA *97*, 5942–5947.

Suzuki, E., Katayama, E., and Hirosawa, K. (1993). Structure of photoreceptive membranes of Drosophila compound eyes as studied by quick-freezing electron microscopy. J. Electron Micr. *42*, 178–184.

Szuts, E.Z. (1993). Concentrations of Phosphatidylinositol 4,5-Bisphosphate and Inositol 1,4,5-Trisphosphate Within the Distal Segment of Squid Photoreceptors. Vis. Neurosci. *10*, 921–929.

Varnai, P., and Balla, T. (1998). Visualization of phosphoinositides that bind pleckstrin homology domains: Calcium- and agonist-induced dynamic changes and relationship to myo-[H-3]inositol-labeled phosphoinositide pools. J. Cell Biol. *143*, 501–510.

Vihtelic, T.S., Goebl, M., Milligan, S., Otousa, J.E., and Hyde, D.R. (1993). Localization of Drosophila retinal degeneration B, a membrane- associated phosphatidylinositol transfer protein. J. Cell Biol. *122*, 1013–1022.

Wu, L., Niemeyer, B., Colley, N., Socolich, M., and Zuker, C.S. (1995). Regulation of PLC-mediated signalling in vivo by CDP-diacylglycerol synthase. Nature *373*, 216–222.

Zhang, H.L., He, C., Yan, X.X., Mirshahi, T., and Logothetis, D.E. (1999). Activation of inwardly rectifying K^+ channels by distinct PtdIns(4,5)P-2 interactions. Nat. Cell Biol. *1*, 183–188.

Zinkler, D., Bentrop, J., and Paulsen, R. (1985). Phospholipids of fly photoreceptor membrane: fatty acid and phosphoinositide metabolism. Verh. Dtsch. Zool. Ges *78*, 303.