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Mechanisms of Light Adaptation in *Drosophila* Photoreceptors

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Summary

Phototransduction in *Drosophila* is mediated by a phospholipase C (PLC) cascade culminating in activation of transient receptor potential (TRP) channels [1, 2]. Ca^{2+} influx via these channels is required for light adaptation, but although several molecular targets of Ca^{2+}-dependent feedback have been identified [3], their contribution to adaptation is unclear. By manipulating cytosolic Ca^{2+} via the Na^+/Ca^{2+} exchange equilibrium, we found that Ca^{2+} inhibited the light-induced current (LIC) over a range corresponding to steady-state light-adapted Ca^{2+} levels (0.1–10 μM Ca^{2+}) and accurately mimicked light adaptation. However, PLC activity monitored with genetically targeted PIP_2-sensitive ion channels (Kir2.1) was first inhibited by much higher (≥ ~50 μM) Ca^{2+} levels, which occur only transiently in vivo. Ca^{2+}-dependent inhibition of PLC, but not the LIC, was impaired in mutants (*inaC*) of protein kinase C (PKC). The results indicate that light adaptation is primarily mediated downstream of PLC and independently of PKC by Ca^{2+}-dependent inhibition of TRP channels. This is interpreted as a strategy to prevent inhibition of PLC by global steady-state light-adapted Ca^{2+} levels, whereas rapid inhibition of PLC by local Ca^{2+} transients is required to terminate the response and ensures that PIP_2 reserves are not depleted during stimulation.

Results

Photoreceptors in both vertebrates and invertebrates generate discrete electrical events, known as quantum bumps, in response to absorption of single photons. In *Drosophila*, quantum bumps represent the concerted opening of ~10–20 Ca^{2+} permeable TRP channels, most probably localized within a single microvillus [1, 4, 5]. The channels, which are encoded by *trp* and *trp-like (trpl)* genes [6–8], are activated downstream of PLC without involvement of $InsP_3$ receptors, most likely via diacylglycerol (DAG) or one of its lipid metabolites [2, 9, 10]. Whereas vertebrate rods saturate with photon fluxes of ~10^3 photons per photoreceptor s^{-1} [11], as in most invertebrate microvillar photoreceptors, *Drosophila* continues light adapting up to the brightest daylight intensities, approaching 10^6 photons s^{-1} [12]. We sought to identify the molecular mechanisms responsible for light adaptation by exploring the Ca^{2+} dependence of different components of the transduction cascade.

Ca^{2+} Dependence of the LIC

The high Ca^{2+} permeability of the light-sensitive channels in *Drosophila* results in a massive Ca^{2+} influx into the microvilli during the light response. From resting levels in the dark of ~160 nM [13], Ca^{2+} concentrations in the microvilli are believed to increase transiently to ~1 mM before relaxing to values of maximally ~10 μM during steady-state adaptation [4, 14]. The major homeostatic mechanism involved in controlling this Ca^{2+} influx is an electrogenic Na^+/Ca^{2+} exchanger encoded by the *calX* gene [15–17], strongly expressed in the photoreceptor microvillar membrane [17, 18]. Assuming a stoichiometry of 3 Na^+:1 Ca^{2+}, and in the absence of other fluxes, the Na^+/Ca^{2+}exchanger should generate an equilibrium internal Ca^{2+} concentration (Ca_i) determined by the external Ca^{2+} concentration (Ca_o), the Na^+ gradient (Na_i/Na_o), and E, the membrane voltage [19].

$$[Ca_i] = [Ca_o]\frac{[Na_i]^3}{[Na_o]^3}e^{\frac{EF}{RT}} \quad (1)$$

We exploited this behavior to manipulate cytosolic Ca^{2+} during whole-cell patch clamping of dissociated photoreceptors. For example, with 20 mM internal Na^+ in the patch pipette, Na^+/Ca^{2+} exchange can be driven in reverse mode by partial substitution of external Na^+ for Li^+; this generates an outward exchange current and raises internal Ca^{2+} into the micromolar range. Responses to test flashes were rapidly inhibited by such solution changes but recovered on Na^+ reperfusion, which results in forward Na^+/Ca^{2+} exchange extruding the accumulated Ca^{2+} at the expense of Na^+ influx, now generating a transient inward exchange current (Figure 1; see Figures S2 and S3 in the Supplemental Data available with this article online). In theory, virtually any internal Ca^{2+} concentration can be achieved by varying external and internal Na^+, allowing the Ca^{2+} dependence of the light response to be explored over a wide range.

In wt photoreceptors recorded with 20 mM Na_i and 110 mM Na_o (predicted Ca_i at –70 mV, 564 nM), the amplitude of the peak LIC in comparison to controls with 10 mM internal Na^+ (Ca_i 70 nM) was reduced by ~25%, indicative of inhibition of the LIC by even submicromolar Ca^{2+} concentrations. Sensitivity was further progressively reduced as Ca_i was raised by perfusion with decreasing external Na^+ concentrations. Assuming the predicted Ca_i equilibrium values (Equation 1), the estimated IC_{50} was ~1 μM, with ≥90% inhibition being achieved with $[Ca_i] \geq 10$ μM.

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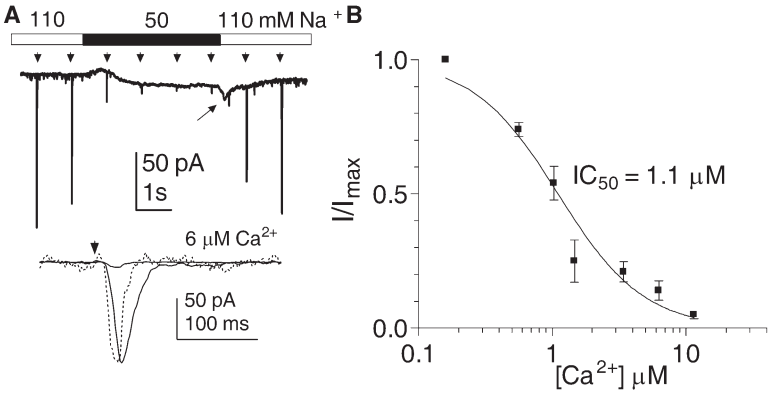

Figure 1. Inhibition of the LIC by Reverse Na^+/Ca^{2+} Exchange

(A) Responses to brief (1 ms) flashes (~100 effective photons, arrowheads) as the control (110 mM Na^+) solution was substituted for 50 mM Na^+/60 mM Li^+ (predicted Ca_i^{2+} 6 μM with 20 mM Na_{in} at –70 mV, Equation 1). This activated reverse Na^+/Ca^{2+} exchange, generating a small outward current, and rapidly suppressed the response. On reperfusion, a transient inward current (arrow) represents activation of forward Na^+/Ca^{2+} exchange extruding the accumulated Ca^{2+} and restoring sensitivity. The inset shows superimposed, averaged responses before and during perfusion. The suppressed response is reproduced normalized to the control (dotted line: scaled 20-fold).

(B) Dose-response function for suppression of the LIC by Ca^{2+} (nominal $[Ca^{2+}]$ calculated from Equation 1); peak responses (I/I_{max}) were normalized to responses immediately before Na^+ substitution and were, in addition, corrected for the 25% suppression by 20 mM Na_{in} in comparison to controls with 10 mM Na_{in} (see Ca2+ Dependence of the LIC). Mean ± SEM on the basis of n = 5–13 cells per data point, with various external Na^+ concentrations and holding potentials of –55, –70, and –85 mV. Data were fitted with apparent IC_{50} 1.1 μM and Hill slope of 1.4.

Ca^{2+} Mimics Light Adaptation

The profound inhibition of the LIC suggested that raising Ca^{2+} in this range also effectively mimicked light adaptation. To confirm this, we compared the effects of background light adaptation with manipulation of Ca^{2+} by the exchanger. As in most photoreceptors, although background illumination suppresses the response to flashes of a given intensity, large responses can still be elicited by further increasing the intensity (Figure 2). Raising Ca^{2+} in the dark closely mimicked this behavior, with both reverse Na^+/Ca^{2+} exchange and background illumination resulting in a shift in response-intensity function, best described by a simple multiplicative reduction in response amplitude across the range of currents that could be accurately voltage clamped. Under physiological conditions, when the currents are transformed into voltages, this should result in approximately parallel shifts of the response-intensity function along the intensity axis. Reverse Na^+/Ca^{2+} exchange also accurately mimicked another major feature of light adaptation, namely the acceleration of response kinetics (Figure 2E). These results show that raising Ca^{2+} in the dark accurately mimics the major features of light adaptation, indicating that Ca^{2+} is not only required, but is also *sufficient* for light adaptation.

Ca^{2+} Dependence of PLC Activity

Recent evidence indicated that Ca^{2+} influx via TRP channels inhibits PLC activity, thereby preventing the near total PIP_2 depletion that occurs when Ca^{2+} influx is compromised [20]. However, the Ca^{2+} concentrations required for this inhibition and whether this inhibition contributes to light adaptation are unknown. In order to monitor PIP_2 hydrolysis by PLC, we expressed a modified version of the inwardly rectifying PIP_2-sensitive ion channel ($Kir2.1^{R228Q}$) in the microvillar membrane as a biosensor for PIP_2 [21]. For avoiding contamination by the LIC, the Kir2.1 channels were expressed in *trpl;trp* double mutants or in *trpl* mutants recorded in the presence of La^{3+} to block the TRP channels, so that the only electrophysiological response to light consisted of a reversible suppression of the constitutive Kir2.1 current reflecting hydrolysis of PIP_2 by PLC.

Strikingly, when we manipulated internal Ca^{2+} via the Na^+/Ca^{2+}exchanger equilibrium, even regimes more extreme than those that had profoundly inhibited the LIC had no significant effect on light-induced PLC activity (Figure 3C; Figure S1). In an alternative approach, we loaded cells via the patch pipette with Ca^{2+} buffered at high concentrations (100–200 μM) with the low-affinity Ca^{2+} buffer NTA, but we again found no significant reduction in PLC activity (n = 8 cells; data not shown). This failure to inhibit PLC might indicate that neither approach is capable of driving microvillar Ca^{2+} to the predicted high levels. Thus, Ca^{2+} delivered via the patch pipette can be expected to be extruded by the Na^+/Ca^{2+} exchanger in the microvilli [17, 18], whereas Ca^{2+} entering the microvilli via reverse Na^+/Ca^{2+} exchange may rapidly diffuse into the cell body, preventing Ca^{2+} from reaching the predicted equilibrium value. To circumvent these problems, we combined high Ca^{2+} in the pipette solution with reverse Na^+/Ca^{2+} exchange regimes designed to yield matched equilibrium Ca^{2+} values over the range of ~50 μM–1 mM. Under these conditions, reverse Na^+/Ca^{2+} exchange reversibly suppressed PLC activity in all cells (n = 15 cells) with an apparent IC_{50} of ~76 μM Ca^{2+} (Figures 3A and 3C).

These results confirm that Ca^{2+} inhibits PLC as previously reported [20] but only at very high Ca^{2+} levels such as those reached transiently by Ca^{2+} influx through the light-sensitive channels [14]. Because of the technical difficulty of achieving the predicted steady-state equilibrium Ca_i (for further discussion, see Figures S2 and S3 and associated text), the curves describing the inhibition of both LIC and PLC should be regarded as approximate, and the IC_{50} estimates as nominal, upper values. However, there seems to be little question that very high levels of Ca^{2+} (>>10 μM) are required for inhibition of PLC, that the IC_{50} for the LIC (and light adaptation) is close to ~1 μM, and that there is essentially a complete separation of the inhibitory ranges for LIC and PLC, respectively. This strongly suggests that light adaptation is mediated primarily downstream of PLC.

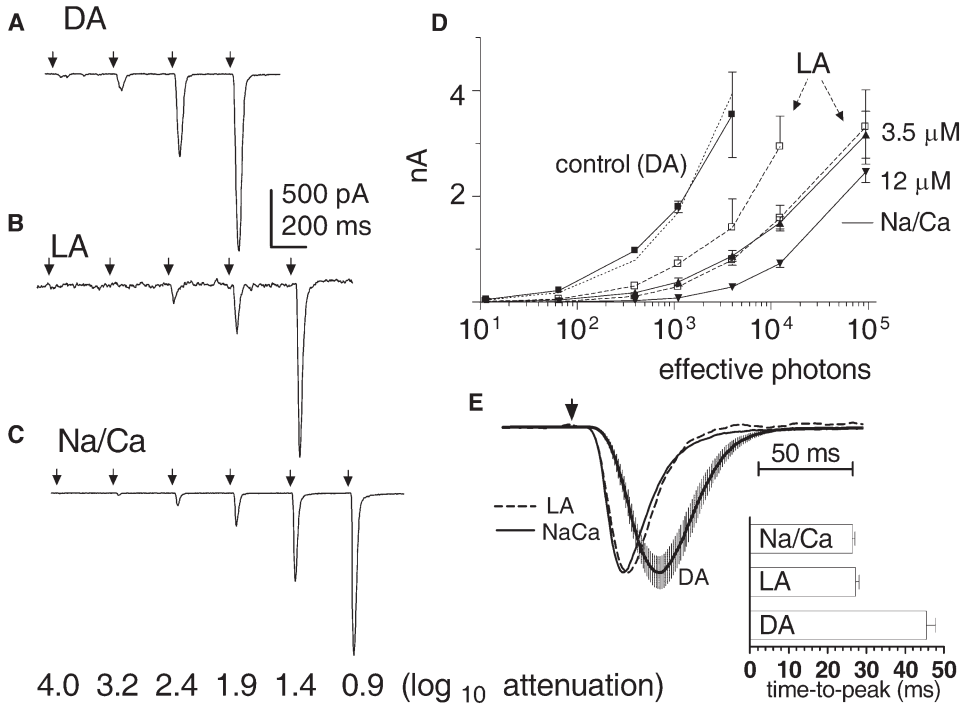

Figure 2. Manipulating Ca^{2+} by Na^+/Ca^{2+} Exchange Mimics Light Adaptation

Responses to brief (5 ms) flashes (arrows) of increasing intensities (log_{10} attenuation indicated, log 0 ≈ 10^5 effectively absorbed photons); (A) dark-adapted (DA), (B) light-adapted (LA; background ~22,000 photon s^{-1}, generating ~175 pA plateau current), and (C) in the dark exposed to 60 mM Na^+ (predicted Ca_i 3.5 μM). Both light adaptation and Na^+/Ca^{2+} exchange suppressed responses at any given intensity, but brighter flashes still elicited large responses.

(D) Averaged (mean ± SEM; n = 5–6 cells) response-intensity functions under dark-adapted conditions, two light-adaptation regimes (LA; dotted lines 4,000 and ~22,000 photons s^{-1}), and during reverse Na^+/Ca^{2+} exchange (60 and 40 mM Na^+; predicted Ca_i^{2+} 3.5 μM and 12 μM). The stippled line through the DA data points are the data from 3.5 μM Na/Ca, scaled 4.5-fold, showing that over this range, responses are simply reduced by a constant factor.

(E) Normalized, averaged responses to brief flashes (arrow) generating peak responses between 150 and 500 pA (in the linear range); the light-adapted (LA; 22,000 photons s^{-1}, dotted trace) responses were similar to those recorded in the dark with 60 mM external Na^+ (Na/Ca, solid trace) and were accelerated in comparison to the dark-adapted response (DA; n = 5–6 cells; for clarity, SEM range is only shown for DA response). The bar graph summarizes the mean time-to-peak values (± SEM) for these cells.

Ca^{2+} Dependence of Channel Activity

The $InsP_3$ receptor is not involved in excitation or adaptation in *Drosophila* [22]; however, both TRP and TRPL channels are sensitive to Ca^{2+}-dependent inactivation [23–25], raising the surprising possibility that the major features of light adaptation might be mediated at the level of the channels. To test this further, we quantified the Ca^{2+} dependence of inhibition of the light-sensitive channels themselves. To measure this, we exploited the so-called rundown current (RDC), which develops after a few minutes of whole-cell recording with pipette solutions containing no ATP, and which represents spontaneous activity of the light-sensitive TRP channels dissociated from the transduction cascade [26, 27]. The RDC was rapidly and reversibly inhibited by reverse Na^+/Ca^{2+} regimes with a Ca^{2+} dependence indistinguishable from that of the LIC itself (Figures 3B and 3C). This is entirely consistent with the suggestion that light adaptation is primarily mediated by Ca^{2+}-dependent inhibition of the light-sensitive channels, although we cannot exclude the contribution of additional mechanisms downstream of PLC with a similar Ca^{2+} dependence.

PKC Is Not Required for Ca^{2+}-Dependent Inhibition of LIC

Both PLC and the light-sensitive TRP channels are coassembled in multimolecular complexes in the microvilli via the scaffolding protein INAD [28]. Another integral component of these transduction complexes is a Ca^{2+}- and DAG-dependent PKC encoded by the *inaC* gene. *inaC* mutants have a severe defect in response termination and are unable to light adapt normally [29, 30]; however, although both TRP and the INAD have been identified as potential PKC substrates [31, 32], PKC's role in response termination and adaptation has remained obscure. We first asked whether PKC was required for the Ca^{2+}-dependent inhibition of the light response by measuring the Ca^{2+} dependence of the LIC in *inaC* mutants. Flash responses in *inaC* are initially indistinguishable from those in the wt but fail to terminate normally, leaving a long tail, which decays over a period of ~1 s [29, 30]. As Ca^{2+} was raised, both components were strongly inhibited with a dose dependence indistinguishable from that measured in wt flies, indicating that the Ca^{2+}-dependent inhibition of the LIC is independent of PKC (Figure 4B).

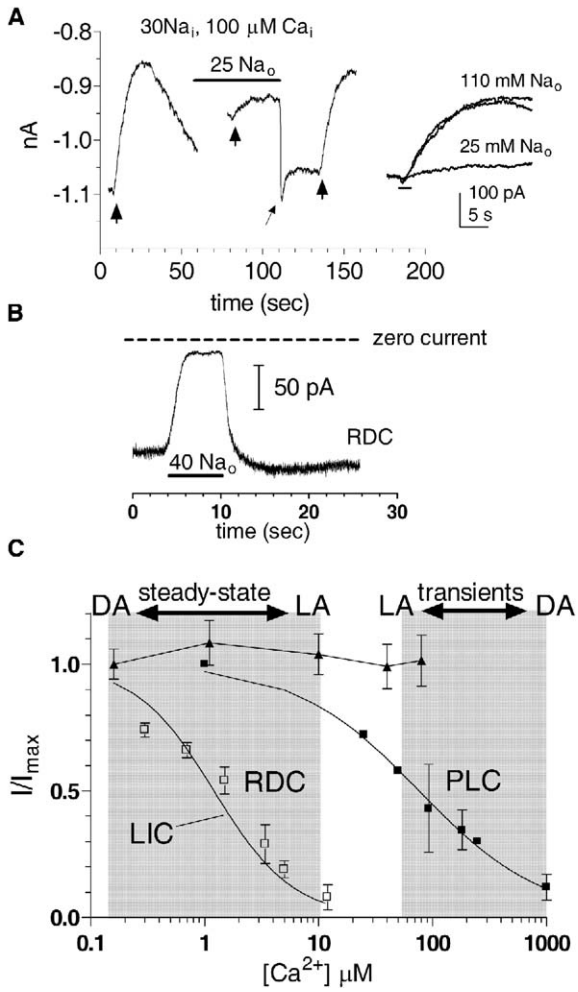

Figure 3. Ca^{2+} Dependence of PLC Activity and RDC

(A) PIP_2-sensitive $Kir2.1^{R228Q}$ current isolated in *trpl;trp* double mutant and recorded with 100 μM internal Ca^{2+} buffered by 10 mM Na_3NTA; a 2 s flash (vertical arrows) containing ~2×10^4 effective photons suppressed the constitutive Kir current (–1.1 nA) by ~20%, indicating corresponding depletion of PIP_2 by PLC. After substitution for 25 mM Na^+ (bar: predicted Ca_i 93 μM at –84 mV), PLC activity was reversibly inhibited. Note the electrogenic Na^+/Ca^{2+} current transient (small arrow); otherwise, the high microvillar Ca^{2+} had little effect on the Kir2.1 current. The inset (right) shows the same traces aligned and superimposed on a faster time scale.

(B) Spontaneous TRP channel activity (rundown current = RDC) was reversibly inhibited by perfusion with 40 mM Na^+ (predicted Ca^{2+}: 12 μM) and recorded from a wt photoreceptor ~8 min after establishing the whole-cell configuration with no nucleotides in the electrode. Parallel experiments with cells expressing Kir2.1 channels indicate that at this time, no detectable PIP_2 remains in the microvilli.

(C) Ca^{2+} dependence of PLC activity (I/I_{max} = Kir2.1 current suppression normalized to suppression in the same cells recorded in 110 mM Na^+ by flashes containing ~2×10^4 effective photons). Closed black triangle: without internal Ca^{2+} added (no effect; n = 5–10 cells per data point, 20 mM internal Na^+; *trpl;Kir2.1*^{R228Q} in presence of 40 μM La^{3+}); closed black square: with equivalent predicted [Ca^{2+}] buffered by 10 mM Na_3NTA in pipette solution (n = 4 cells per data point; points without error bars are data from single cells; data fitted with apparent IC_{50} 76 μM and Hill slope = 0.8; data from *trpl;Kir2.1*^{R228} + La^{3+} and *trpl;trp,Kir2.1*^{R228Q} pooled). In contrast, the Ca^{2+}-dependent inhibition of the RDC (open square; n = 4–8 cells per point) was indistinguishable from inhibition of the LIC (solid line; from Figure 1). Estimates of the Ca^{2+} ranges experienced by the microvilli during steady-state light adaptation (160 nM dark adapted to10 μM light adapted) and during transient incremental responses (~1 mM when dark adapted, 50 μM when light adapted) are indicated for comparison.

Second, we explored the possible role of PKC in inhibition of PLC by expressing $Kir2.1^{R228Q}$ channels in *inaC* mutants. In wt flies, Ca^{2+} influx via the light-sensitive TRP channels effectively inhibits PLC so that only modest reductions in PIP_2 levels are observed even with the brightest intensities [21]. However, if Ca^{2+} influx is reduced by genetic elimination of the TRP channels (i.e., in *trp* mutants), modest light stimuli effectively deplete PIP_2 from the microvilli [20]. Although *inaC* mutants have the normal complement of TRP (and TRPL) channels [30], PIP_2 was depleted by light at least as effectively as in the *trp* mutant (Figures 4C and 4D), suggesting that PLC fails to be effectively inhibited by Ca^{2+} in *inaC*. After depletion, the Kir2.1 current recovered in the dark as PIP_2 was resynthesised, with a time course ($t_{1/2}$ = 62 ± 11 s, n = 6) indistinguishable from that previously reported [21], indicating that PIP_2 resynthesis was unaffected in *inaC* mutants.

These results indicate that PKC is required for the effective Ca^{2+}-dependent inhibition of PLC and termination of the response but is not directly required for the major features of light adaptation, manifest in the Ca^{2+}-dependent inhibition and acceleration of the LIC. Instead, the failure to inhibit PLC in *inaC* results in light-induced PIP_2 depletion and a collapse of excitation so that adaptation is masked indirectly as a consequence. The results also suggest a simple interpretation of the deactivation defect in *inaC*, namely the additional response to the continued PLC activity, which otherwise terminates as soon as Ca^{2+} enters.

Although these results redefine PKC's role in *Drosophila* phototransduction, it is not clear whether the conspicuously high Ca^{2+} levels required to inactivate PLC are needed to activate PKC or whether PKC is required to enable PLC to be inhibited by such Ca^{2+} levels. The relevant PKC phosphorylation target also remains uncertain: PLC is not known as a substrate for PKC in *Drosophila*; however, PLC is organized into a multimolecular signaling complex with PKC and the TRP channel protein by the PDZ domain scaffolding protein INAD [28, 33, 34]. INAD itself has been reported to be a PKC substrate [31], raising the possibility that phosphorylation of INAD could indirectly modulate the activity of the PLC or its susceptibility to Ca^{2+}-dependent inhibition.

Conclusion: Mechanism and Strategy of Light Adaptation

Detailed theoretical considerations [4] as well as in vivo Ca^{2+} indicator measurements [13, 14] in a related dipteran fly (*Calliphora*) indicate that in dark-adapted photoreceptors, Ca^{2+} influx transiently raises microvillar Ca^{2+} from ~160 nM in the dark to ~1 mM after illumination, but Ca^{2+} then rapidly returns to resting levels by diffusion into the cell body and extrusion by Na^+/Ca^{2+} exchange. During light adaptation, continued Ca^{2+} in-

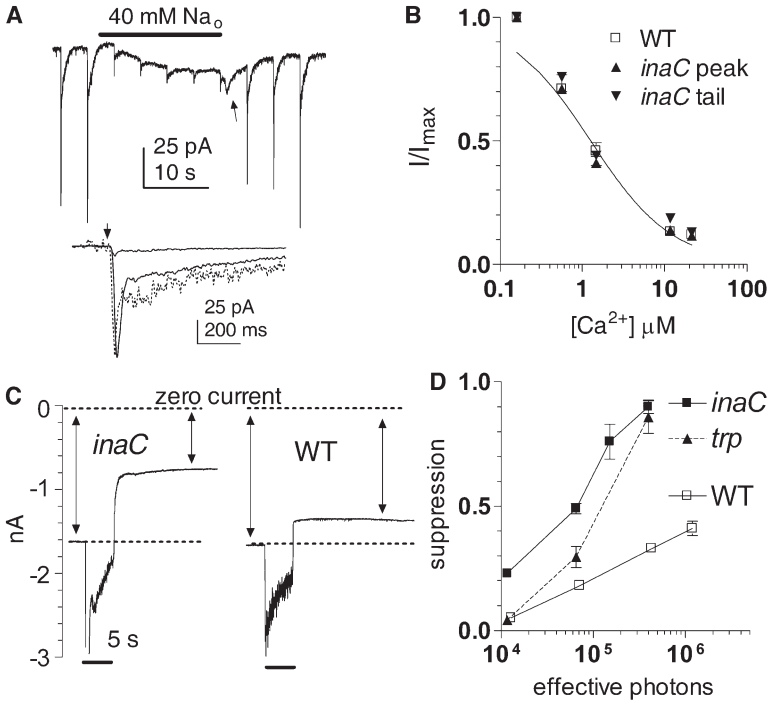

Figure 4. PKC Is Required for Ca^{2+}-Dependent Inhibition of PLC but Not the LIC

(A) Responses to brief flashes (~100 photons) during substitution with 40 mM Na^+ in an *inaC* mutant were rapidly suppressed in a similar fashion to wt (c.f. Figure 1). The arrow indicates the transient inward exchange current. The inset shows responses on a faster time scale (dotted line; inhibited response scaled 10-fold for comparison with control).

(B) Dose dependence of Ca^{2+}-dependent inhibition of both the peak response (closed black upward triangle) and the tail of the response (closed black downward triangle) in *inaC* (mean ± SEM; n = 6–16 cells per data point) was indistinguishable from parallel experiments in wt flies (open white square; n = 3–10 cells; separate data set from Figure 1; fitted with IC_{50} 1.25 μM Hill slope 0.9).

(C) In an *inaC* photoreceptor expressing $Kir2.1^{R228Q}$ channels (left trace), the PIP_2-sensitive current was suppressed by more than 50% with a 5 s stimulus (bar: ~6×10^4 photons). In a wt background (right), the same stimulus suppressed the current by only ~20%.

(D) Normalized response-intensity function for suppression of $Kir2.1^{R228Q}$ current in *inaC* (n = 4–6 cells per data point), with wt and *trp* backgrounds (data from [21]) showing that the *inaC* PIP_2 depletion phenotype is at least as severe as that in *trp*.

flux results in a progressive increase in the steady-state Ca^{2+} concentration in the cell body and microvilli, reaching saturating values of ~10 μM under bright adaptation [14, 35]. Under light-adapted conditions, incremental flashes generate greatly reduced inward currents (Figure 2) so that the Ca^{2+} transients in the microvilli now reach only ~50 μM. Strikingly, these global steady-state (160 nM–10 μM) and localized transient (~1 mM–50 μM) Ca^{2+} ranges map closely onto the estimated Ca^{2+}-dependent operating ranges of the LIC and PLC, respectively (Figure 3). In particular, the steady-state light-adapted Ca^{2+} levels closely match the inhibitory range of the LIC, which also closely mimicked the major features of light adaptation, including gain reduction and acceleration in response kinetics. Our results suggest that these features of adaptation are primarily mediated by the Ca^{2+}-dependent inhibition of the light-sensitive TRP channels, independently of PKC. Both TRP and TRPL are reported to have one or more calmodulin binding sites (CBS) [7, 34], suggesting a Ca-calmodulin-dependent mechanism, and indeed, calmodulin mutants and mutants of the TRPL CBS have both been reported to have defects in Ca^{2+}-dependent inactivation [24]. The surprising conclusion that adaptation is mediated primarily at the level of the channels is not without precedent; a similar situation has been reported in olfactory receptors, where Ca-calmodulin-dependent modulation of cAMP-gated channels has been proposed as the primary mechanism of olfactory adaptation [36].

In contrast, PLC activity was unaffected by the steady-state Ca^{2+} concentrations reached during light adaptation, but it was inhibited over the range of concentrations experienced during the Ca^{2+} transients. As a functional rationale, we propose that it is important to maintain high PLC activity during light adaptation in order to ensure rapid responses. Macroscopic responses represent the linear summation of quantum bumps, and, hence, their kinetics are jointly determined by quantum-bump duration and bump latency (reviewed in [10]). Bump latency represents the time taken for second messenger concentration to exceed threshold for channel activation and is therefore critically dependent on the rate of PLC activity. Why then inhibit PLC at all? First, the deactivation defect in *inaC* mutants suggests that PKC-dependent inhibition of PLC by Ca^{2+} transients is required to terminate the light response. Second, light-activated PLC activity in *Drosophila* is exceptionally high, and without feedback by Ca^{2+}, a single effectively absorbed photon depletes all the PIP_2 of at least one microvillus within less than a second [20, 21]. Under normal conditions, Ca^{2+} influx via the TRP channels appears essential to rapidly inhibit PLC, thereby preventing this precipitous loss of PIP_2 [20].

Experimental Procedures

Flies

Drosophila melanogaster were raised in the dark at 25°C. The wild-type (wt) strain was white-eyed (w^{118}) Oregon. For monitoring PIP_2 hydrolysis, experiments were performed on flies expressing genetically modified Kir2.1 channels ($Kir2.1^{R228Q}$) as an electrophysiological biosensor for PIP_2 [20, 21]. For isolation of the PIP_2-sensitive Kir2.1 current, the channels were expressed either in a double mutant, $trpl^{302};trp^{343}$, lacking both classes of light-sensitive channels [24, 37] or in $trpl^{302}$ and recorded in the presence of La^{3+} to block the TRP channels. Experiments were performed in $inaC^{P209}$, a null mutant of an eye-enriched PKC, to test the role of PKC [29].

Whole-Cell Recordings

Dissociated ommatidia were prepared from recently eclosed adult flies and transferred to a recording chamber on an inverted Nikon Diaphot microscope [20, 38]. The control bath solution contained 110 mM NaCl, 10 mM KCl, 4 mM CsCl, 10 mM *N*-Tris-(hydroxymethyl)-methyl-2-amino-ethanesulphonic acid (TES), 4 mM $MgCl_2$, 1.5 mM $CaCl_2$, 25 mM proline, and 5 mM alanine. Cells were exposed to 50–100 μM ouabain for ~1 min before recording to block Na/K ATPase. Extracellular Na^+ concentration was varied from 110 to 0 mM by equimolar substitution with LiCl. The standard intracellular solution was 20 mM NaCl, 120 mM K gluconate, 10 mM TES, 4 mM Mg ATP, 2 mM $MgCl_2$, 1 mM NAD, and 0.4 mM Na GTP. For some experiments, Ca^{2+} in the pipette was buffered with 10 mM Na_3 nitrilo-triacetic acid (NTA) with the WinMaxC program used to calculate the free Ca^{2+} concentrations. K gluconate was reduced as necessary to maintain osmolarity. pH of all solutions was adjusted to 7.15. Data were recorded at 20 ± 1°C with Axopatch 1-D or 200 amplifiers and analyzed with pCLAMP 8 or 9 software (Axon Instruments). Cells were stimulated by a green-light-emitting diode with intensities calibrated in terms of effectively absorbed photons by counting quantum bumps at low intensities in wt flies (e.g., [5]).

Supplemental Data

Detailed Experimental Procedures and three supplemental figures are available at http://www.current-biology.com/cgi/content/full/15/13/1228/DC1/.

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