Phototransduction in *Drosophila*

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Highlights

- *Drosophila* phototransduction uses canonical Gq/PLC/TRP channel cascade
- Photoreceptors respond to single photons 10-100x faster than vertebrate rods
- Photoreceptors contract in response to light
- TRP channels activated by physical effects of PIP_2 depletion and protons?
- Ultracompartmentalization and Ca^{2+}- dependent feedback key to performance

Abstract

Phototransduction in *Drosophila's* microvillar photoreceptors is mediated by phospholipase C (PLC) resulting in activation of two classes of Ca^{2+}-permeable channels, TRP and TRPL. Here we review recent evidence on the unresolved mechanism of their activation, including the hypothesis that the channels are mechanically activated by physical effects of PIP_2 depletion on the membrane, in combination with protons released by PLC. We also review molecularly explicit models indicating how Ca^{2+}-dependent positive and negative feedback along with the ultracompartmentalization provided by the microvillar design can account for the ability of fly photoreceptors to respond to single photons 10-100x more rapidly than vertebrate rods, yet still signal under full sunlight.

Introduction

Vision throughout the animal kingdom is based on rhodopsins, at least three subfamilies of which arose before the deuterostome (chordate and vertebrate) and protostome invertebrate lineages diverged over 550 Mya [1,2]. Ciliary C-opsins and G_o-opsins couple to cyclic nucleotide based machinery, exemplified by ciliary rods and cones [3]. Rhabdomeric R-opsins, found in microvillar photoreceptors typical of many invertebrate eyes, use the phosphoinositide (PI) cascade, involving phospholipase C (PLC) and TRP channels [4,5]. In many phyla both R-opsin and C-opsin/G_o-opsin based photoreceptors still co-exist in the same animals, typically with one used for vision, and the other for non-visual tasks such as circadian entrainment. Even mammalian retinae harbour a third class of photoreceptor, so-called intrinsically photo-sensitive retinal ganglion cells, which express an R-opsin (melanopsin) and a PI cascade similar to that in *Drosophila* [**6,7]. This review covers recent advances in microvillar phototransduction in *Drosophila*, which is also an influential genetic model for the PI cascade more generally. The major focus is on the unresolved mechanism of activation, whilst briefly reviewing Ca^{2+}-dependent feedback and computational models that account for photoreceptor performance.

The Phototransduction cascade

Fly photoreceptors are exquisitely sensitive, responding to single photons with kinetics ~10-100x faster than in vertebrate rods (Figure 1), yet like cones can rapidly adapt over the full diurnal range. Eight photoreceptors form a repeating unit, the ommatidium, beneath each of the ~750 facets of the *Drosophila* compound eye (Figure 1). The phototransduction compartment, the light-guiding rhabdomere is formed by a stack of ~30000 microvilli, each containing all the essential elements of the transduction cascade [8-11]. Many of these are generic elements found in any PI cascade, including the G-protein coupled receptor (rhodopsin, encoded by *ninaE*), heterotrimeric G-protein (Gq), phospholipase C (PLCβ4, encoded by *norpA*), and two related Ca^{2+}-permeable cation channels encoded by the *transient receptor potential* (*trp*) and *trp*-like (*trpl*) genes (Figure 1C). TRP and TRPL are thought to assemble as distinct homo-tetrameric channels [*12], although TRP also requires an accessory, single pass transmembrane protein, INAF for full functionality [13,14]. Several components, including PLC, TRP, protein kinase C (PKC) and myosin III (NINAC) are assembled into multimolecular signalling complexes by the scaffolding protein, INAD with its 5 PDZ domains [8,11].

Mechanism of activation

The usual suspects

Drosophila TRP was known to be activated via PLC from the time of its discovery as the first TRP channel [15-17]. PLC hydrolyses phosphatidyl-inositol 4,5 bisphosphate (PIP_2), yielding diacylglycerol (DAG), inositol 1,4,5 trisphosphate ($InsP_3$) and a proton (Figure 2), but which product(s) ultimately activate the channels remains controversial [8,9,11]. $InsP_3$ and Ca^{2+} stores contribute to excitation in some microvillar photoreceptors (e.g. *Limulus*), but not in *Drosophila* [18,19]. This would seem to leave DAG, well known to activate some related vertebrate TRPCs [20], as the obvious alternative candidate. Indeed, evidence for an excitatory role for DAG has come from mutants of *rdgA*, encoding DAG kinase (DGK), which controls DAG levels by phosphorylating it to phosphatidic acid (PA, Figure 2B). In *rdgA* mutants TRP and TRPL channels become constitutively active resulting in severe retinal degeneration. Hypomorphic mutations in PLC (*norpA*), have severely attenuated light responses; but in *norpA,rdgA* double mutants, not only is the degeneration in *rdgA* rescued but the residual *norpA* light response is greatly facilitated, representing a striking reciprocal genetic rescue [21,22]. These phenotypes would seem most simply explained if DAG is the excitatory messenger, or at least required for channel activation. Thus, mutations in DGK might elevate DAG levels generated by basal PLC activity – potentially accounting for constitutive channel activation – as well as amplifying the effect of residual light-induced DAG generation in PLC hypomorphs [21].

There are, however, problems with this model [23]. Firstly, exogenous DAG usually fails to activate native or heterologously expressed TRP or TRPL channels. A possibly significant exception is a report that native TRP channels can be activated by DAG in excised patches from isolated rhabdomeres [*24]. However, responses were very sluggish (tens of seconds delay), and from a preparation in a physiologically severely compromised state. Secondly, available biochemical measurements failed to show raised DAG levels in *rdgA* mutants despite a reduction in PA [25]. Thirdly, DGK immunolocalises, not to the microvilli where the rest of the transduction machinery resides, but to smooth endoplasmic reticulum abutting their base [26]. However, it should be noted: i) that *rdgA* generates multiple transcripts (10 annotated in http://www.flybase.org) so that there could be a rhabdomeric isoform unrecognised by available antibodies; ii) *rdgA* has a PDZ-binding motif and may interact with the INAD signalling complex [27]; iii) ATP was reported to suppress DAG-stimulated TRP activity in excised patches from isolated rhabdomeres, but not in *rdgA* mutants, suggesting DGK activity in the patches [24].

Whilst most authors find DAG an ineffective agonist, all agree that TRP and TRPL are potently activated by polyunsaturated fatty acids (PUFAs), which could in principle be released from DAG by an appropriate lipase (Figure 2) [28-30]. In apparent support, DAG lipase mutants (*inaE*) have severely attenuated light responses [31]. However, *inaE* encodes *sn-1* DAG lipase, which rather than PUFAs, releases mono-acyl glycerols (Figure 2), which are also poorly effective agonists (Hardie R.C. unpubl.). For PUFA generation, either an *sn-2* DAG lipase or an additional enzyme (MAG lipase) would be required; but there is no evidence for either in photoreceptors and no evidence that PUFAs are generated in response to light [24]. *Rolling blackout* (*rbo*), mutants of which also have an impaired light response, was also suggested as a lipase involved in phototransduction [32], but was recently identified as a homologue of Efr3, a scaffolding protein which recruits PI 4-kinase to the membrane, suggesting the *rbo* phenotype might reflect a defect in PIP_2 synthesis [33].

Recently Lev *et al.* [30] supported a role for PUFAs, after confirming that TRPL channels expressed in HEK cells could be activated by PUFAs but not by DAG. They reported that activation of the channels via PLC was suppressed by a DAG lipase inhibitor, consistent with PUFAs generated from DAG as the endogenous agonist. However, given that PUFAs are effective agonists, it is only to be expected that in cell types capable of generating PUFAs, activation could be achieved by this mechanism. As the authors later conceded [34], this therefore contributes little to the question of whether the channels are activated by endogenous PUFAs in the photoreceptors.

Protons and bilayer mechanics

PLC activity has at least two further consequences: PIP_2 depletion and proton release (Figure 2). The latter is usually ignored; however, a proton is released for each PIP_2 hydrolysed and Huang *et al.* [*35] measured a rapid light-induced and PLC-dependent acidification in the rhabdomeres. They also found that the strict *combination* of PIP_2 depletion and acidification achieved by protonophores, rapidly and reversibly activated both TRP and TRPL channels in photoreceptors (Figure 3). The findings have been questioned [11] since the protonophore used (dinitrophenol = DNP) is a mitochondrial uncoupler and native TRP channels become spontaneously activated following ATP depletion [36]. However, activation of channels by DNP in PIP_2-depleted photoreceptors was equally effective and reversible with or without ATP in the patch electrode and was unaffected by the ATP-synthetase inhibitor oligomycin, which did, though, prevent activation of the channels by mitochondrial uncoupling [*35].

PIP_2 modulates the activity of numerous ion channels, including many mammalian TRP isoforms [37]. It is usually believed to do so via PIP_2 binding domains on the channels, but

recent evidence raises the possibility that the light-sensitive TRP/TRPL channels may be regulated by physical effects of PIP_2 depletion on the membrane [**38]. PIP_2 is an integral membrane phospholipid and cleavage of its bulky and highly charged inositol headgroup by PLC (Figures 2 & 3) effectively reduces membrane area, volume and phospholipid crowding. Might this generate sufficient forces (e.g. membrane tension, changes in lateral pressure profile, thickness and/or curvature) to mechanically gate the channels, in combination with protons? Evidence for this included the remarkable finding that light induced rapid contractions of the photoreceptors (Figure 3C). These photomechanical responses, measured by atomic force microscopy, had latencies shorter than the electrical response, were abolished in PLC mutants and interpreted as the synchronized contractions of microvilli as PIP_2 was hydrolysed in their membrane [**38]. It was also shown that: i) known mechano-sensitive channels (gramicidin) responded to light when incorporated into the membrane in place of the native light-sensitive channels; ii) light responses mediated by the native channels were facilitated by hypo-osmotic solutions (Figure 3D); and iii) cationic amphiphiles, which should insert into, and crowd the inner leaflet of the bilayer, potently inhibited the light response [**38]. A role of physical membrane properties in channel activation was also proposed by Parnas *et al.* [39] who reported that osmotic swelling, PIP_2 sequestration by poly-lysine, and PUFAs all had similar effects in enhancing activity of TRPL channels expressed in *Drosophila* S2 cells, whilst PUFA-induced channel activity was suppressed by the mechano-sensitive channel inhibitor GsMTx-4.

Ca^{2+}-dependent feedback

Ca^{2+} influx mediates ~30% of the light-induced current [40], has profound effects upon gain and kinetics and mediates light adaptation via multiple targets including the channels. In Ca^{2+}-free solutions, both onset and termination of the light induced current are slowed ~10-fold indicating sequential positive and negative feedback by Ca^{2+} influx. The effects of removing extracellular Ca^{2+} are mimicked by mutation of a single negatively charged residue (Asp^{621}) in the pore of the TRP channel, which converts the normally Ca^{2+}-selective channel (P_{Ca}:P_{Na} >50:1) to a monovalent ion channel with negligible permeability for Ca^{2+} [41]. Positive feedback by Ca^{2+} is essential for rapid kinetics and high gain, and is mediated by facilitation of TRP (but not TRPL) channels, and possibly PLC [8,42]. The Ca^{2+} dependence of both positive (EC_{50} ~300 nm) and negative feedback (IC_{50} ~1 μM) on the channels have been estimated by manipulating cytosolic Ca^{2+} via the Na^+/Ca^{2+} exchanger equilibrium [43,44]. Negative feedback acts on both TRP and TRPL channels, is responsible for rapid termination of the quantum bump, and is sufficient to account for the major features of light adaptation [43]. The molecular basis of Ca^{2+}-dependent channel regulation is unclear. Both TRP and

TRPL contain calmodulin (CaM) binding sites, but their role is uncertain. TRP has 28 identified phosphorylation sites, redundantly controlled by multiple kinases and phosphatases [*45,46,47]. But again, their function is enigmatic and neither positive nor negative feedback of the channels is obviously affected in mutants of the eye-specific, Ca^{2+} dependent PKC, *inaC* [43].

Eye-specific PKC is however, required for inhibition of PLC, which occurs at the much higher (>50 μM) concentrations reached transiently in the microvillus during a quantum bump [43]. Since PLC is not known to be a PKC target, it has been suggested that this inhibition maybe mediated indirectly by phosphorylation of the scaffolding protein INAD. In particular, the PDZ4/5 domains of INAD form a supramodule that switches between two conformational redox states via a cys-cys bridge which forms in response to illumination in a PKC – and also pH dependent – manner. In the oxidized state it dissociates from its target (both PLC and TRP have been proposed as partners) potentially modulating its activity [48,49].

A third target, active metarhodopsin (M*), is rapidly (time constant ~20ms) inactivated by Ca^{2+}-dependent binding to arrestin (Arr2) [50]. The Ca^{2+}-dependence is abolished in mutants of both calmodulin (*cam*) and *ninaC*, which encodes a CaM binding myosin III, abundantly expressed in the microvilli. This suggests that MyoIII sequesters Arr2 in the dark preventing it from binding to M*, but that as soon as the quantum bump is initiated, Ca^{2+} influx promotes release of Arr2, which then rapidly binds and inactivates M* (Figure 1C) [50].

Ca^{2+} has yet further targets, particularly in the visual pigment cycle, but these do not seem to directly influence the electrophysiological response [8,50].

Quantum bumps and Computational models

Fly photoreceptors respond to single photons yet continue signalling in full sunlight with the fastest kinetics of any photoreceptors. Their microvillar organization, along with Ca^{2+}-dependent feedback is critical for this performance. Each quantum bump is generated within the confines of one microvillus, with macroscopic currents representing the summation of quantum bumps across the microvillar ensemble. A single activated metarhodopsin (M*) is believed to activate ~5-10 Gq proteins by random diffusional encounters. Each released Gqα subunit binds and activates a PLC molecule, several of which must be activated to generate sufficient excitatory "message" (putatively local membrane perturbation and protons) to overcome a Ca^{2+}-dependent threshold required to activate the first TRP channel [42,44]. In

fully dark-adapted cells this happens with a stochastically variable latency of ~15-100 ms (mean ~40 ms). Within the tiny volume of a microvillus a single Ca^{2+} ion already represents a concentration of ~1 μM, and Ca^{2+} influx through even one TRP channel rapidly raises Ca^{2+} throughout the microvillus, facilitating activation of most of the remaining ~20 channels in the microvillus, resulting in an "all-or-none" quantum bump. This transiently raises Ca^{2+} within the affected microvillus to mM levels, terminating the bump by Ca^{2+}-dependent inactivation of the channels and preceding steps of the cascade. During light-adaptation, accumulation of Ca^{2+} entering via many microvilli raises steady-state Ca^{2+} throughout the cell to maximally ~10 μM; this inhibits both TRP and TRPL channels, progressively reducing quantum bump currents, whilst depolarization of the cell and activation of voltage-activated K channels results in further global reduction in voltage gain. This conceptual model of quantum bump generation [21] has been combined with experimentally determined parameters to generate molecularly explicit computational models that accurately predict quantum bump waveforms and their latency distribution (Figure 4) [8,51,52,**53].

Once the bump has terminated, the affected microvillus is temporarily refractory to further photoisomerizations for a stochastically variable period of ~100 ms. This may simply reflect inhibition by the transiently high Ca^{2+} levels, but may also reflect more subtle molecular events, such as the reversible conformational changes in INAD [48]. Far from compromising sensitivity, the refractory period contributes seamlessly to light adaptation. With increasing photon flux, the proportion of microvilli in a refractory state at any one instant increases, progressively reducing effective quantum efficiency (Q.E.); however, with ~30000 microvilli, even during bright sunlight (~10^6 photoisomerizations per photoreceptor per second) a significant fraction will always be recovering from the refractory state. Modelling and experiment confirm that the reduction in Q.E. is balanced by the increase in photon arrival, so that the overall rate of effectively absorbed photons simply plateaus. This means that a high rate of information transfer is maintained in responses to naturalistic stimuli over a broad range of intensities (Figure 4E) [**53]. Despite sacrificing photons, this strategy enables perceptually consistent estimates of real-world light contrast patterns over a large illumination range, in an energy efficient manner [54].

Conclusions

Ultracompartmentalization inherent in the microvillar design, combined with Ca^{2+}-dependent feedback, can account for many aspects of the performance of fly photoreceptors. Nevertheless, the final "messenger" of excitation downstream of PLC remains unresolved. Although there is evidence implicating DAG and/or PUFAs, it is not compelling. Recent

evidence suggests that the channels may be activated by the combination of two neglected consequences of PLC activity: the physical effects of PIP_2 depletion on the membrane, and acidification. A more specific hypothesis is that the physical state of the membrane following PIP_2 depletion favours a conformational state of the channels with an accessible protonatable site (perhaps previously buried within the bilayer), which promotes channel gating when protonated. It is of course premature to accept this as the final solution. Not only must the hypothesis be further tested and refined, it must also be reconciled with existing evidence, such as the *rdgA* mutant phenotypes. Here it may be pertinent to recall that DGK not only metabolises DAG, but is also the first step in the resynthesis of PIP_2 (Figure 2B). Therefore, *rdgA* mutants may have reduced PIP_2 and raised DAG levels in their microvilli, potentially approximating the physical state of the membrane following PIP_2 hydrolysis. In addition, PA is a facilitator of PI(4)P 5-kinase [55] so that the final step of PIP_2 synthesis might also be compromised in *rdgA* (Figure 2B). Finally, TRP family members are notorious for being polymodally regulated and it may not be surprising to find that multiple signals contribute to channel activation or that the channels behave differently in different expression systems [34].

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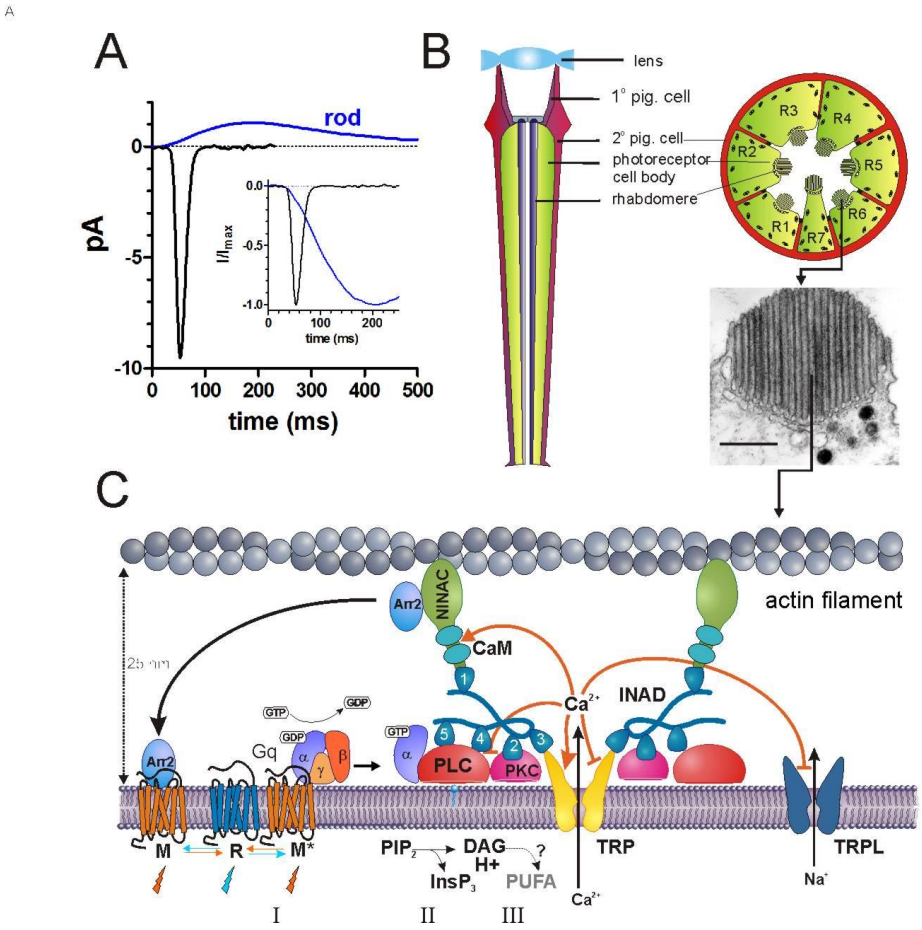

Figure 1 Photoreceptors and transduction cascade in *Drosophila*.

A Single photon responses (quantum bumps) in *Drosophila* and mouse rod (blue): inset, normalised to compare kinetics **B** Left: section of an ommatidium showing two photoreceptors with their rhabdomeres (~80 μm long). Right: in cross-section, rhabdomeres R1-R6 (λ_{max} 480 nm) surround the central R7 (UV-sensitive). R8 (blue/green sensitive) lies proximally in the ommatidium. The electron-micrograph shows one rhabdomere, with one row of its stack of ~30000 microvilli (scale bar 0.5 μm). **C** Elements of the cascade in a 'half' microvillus. Photoisomerization of rhodopsin (R) to metarhodopsin (M) activates Gq via GDP-GTP exchange (I), releasing the Gq*α* subunit; Gq*α* activates phospholipase C (PLC), generating $InsP_3$, diacylglycerol (DAG) and a proton from PIP_2 (II). Two classes of light-sensitive channels (TRP and TRPL) are activated downstream of PLC (III**)**. Ca^{2+} influx feeds regulates multiple targets, including both channels, PLC (via PKC)- and arrestin (Arr2, via CaM and NINAC = myosin III). Several components, including TRP, PKC, and PLC are assembled into signalling complexes by one or other of five PDZ domains (1–5) in the scaffolding protein, INAD, which may be linked to the central actin filament via NINAC. Modified from [8].

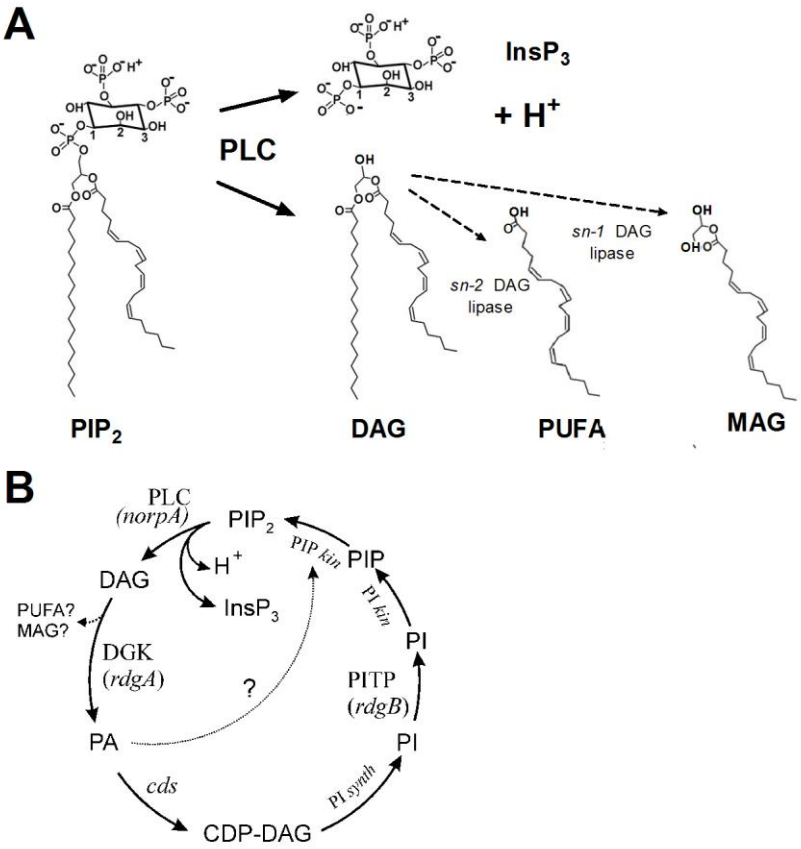

Figure 2 Phosphoinositide pathways

A Hydrolysis of PIP_2 by PLC releases $InsP_3$ and a proton, leaving DAG in the membrane. In principle DAG could be further metabolised by *sn-2* DAG lipase to PUFAs (*e.g.* linolenic acid), or by *sn-1* DAG lipase (*inaE* gene) to generate monoacyl glycerol (MAG). **B** PI turnover cycle: phosphorylation of DAG to PA by DGK (encoded by *rdgA*) is the first step in the PIP_2 resynthesis pathway, whilst PA is also a potential activator of PI(4)P-5 kinase (*PIP kin*).

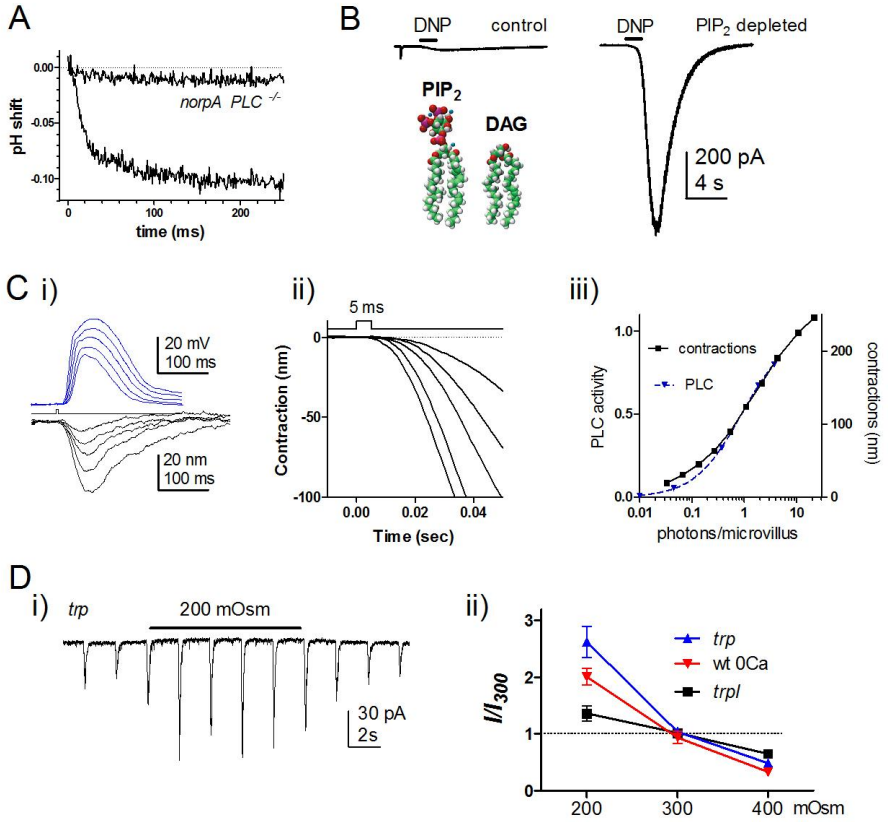

Figure 3 Activation by protons, PIP_2 depletion and bilayer mechanics

A Rapid light-induced acidification measured with pH indicator dye (loaded via patch pipette), is abolished in a PLC mutant (*norpA*). **B** The protonophore DNP fails to activate channels under control conditions, but following PIP_2 depletion, rapidly and reversibly activates the light-sensitive channels: from [35]. Inset shows molecular models of PIP_2 and DAG illustrating the physical effect of PIP_2 hydrolysis by PLC. **C** i) Contractions, measured by atomic force microscopy, elicited by flashes of increasing intensity (200-8000 effective photons). Voltage responses to same flashes shown above (blue); ii) contractions elicited by brighter flashes (up to ~10^6 photons) on faster time base; iii) intensity dependence of contractions (black squares) overlaps the intensity dependence of PLC activity (blue triangles: measured using fluorescent pH assay: Hardie R.C. unpubl.). **D** i) voltage-clamped responses to flashes of light in a *trp* mutant were reversibly facilitated by perfusion with hypo-osmotic solution (200 mOsm); ii) current amplitudes in hyper- and hypo-osmotic solutions normalised to control values in 300 mOsm bath in *trp* and *trpl* mutants and wild-type flies in absence of Ca^{2+}: from [**38].

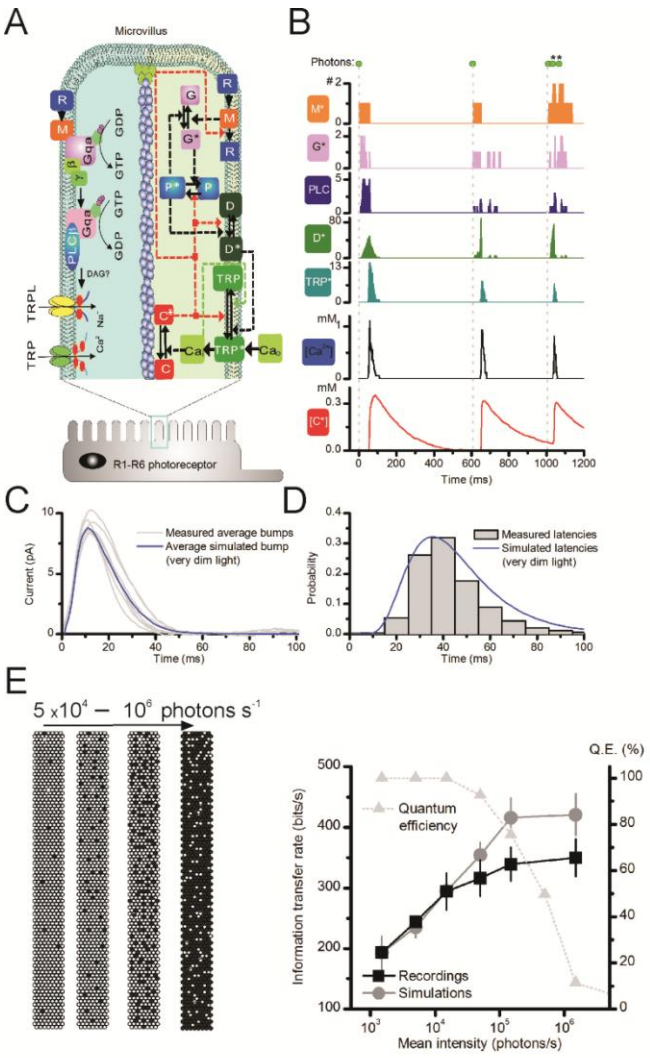

Figure 4 Modelling bumps and light adaptation

A Microvillar phototransduction reactions. M*, metarhodopsin; C*, Ca^{2+}-dependent feedback; D*, DAG (proxy for messenger of excitation); P*, G protein-PLC complex. **B** Reactions modelled in a stochastic framework: simulations show how elementary responses (bumps) to captured photons (green circles) are generated: after a variable latency, 5-15 TRP-channels open, mediating Ca^{2+} and Na^{+} influx. Ca^{2+}-dependent feedback (red) results in a refractory period. ** two photons arriving during refractory period fail to activate channels. **C,D** Average recorded and simulated bumps and their latency distributions are similar.

E As photon flux increases ($5x10^4 – 10^6$ photons absorbed/second), an increasing proportion of microvilli are refractory (black) at any one instant, reducing quantum efficiency (Q.E.); but the overall effective sampling rate (absorbed photons x Q.E.) and hence information transfer rate, plateaus in both simulations and intracellular voltage recordings from the intact fly: from [**53].