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Review

Phototransduction in *Drosophila melanogaster*

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Summary

As in most invertebrate microvillar photoreceptors, phototransduction in *Drosophila melanogaster* uses a G-protein-coupled phosphoinositide pathway, whereby hydrolysis of phosphatidyl inositol 4,5-bisphosphate (PIP_2) by phospholipase C generates inositol 1,4,5-trisphosphate ($InsP_3$) and diacyl glycerol (DAG), leading to activation of two classes of Ca^{2+}-permeable light-sensitive channel, encoded by the *trp* and *trpl* genes. In some invertebrate photoreceptors, excitation is mediated by release of Ca^{2+} from intracellular stores by $InsP_3$; however, in *Drosophila melanogaster*, recent evidence suggests instead that a lipid messenger, such as DAG, its metabolites and/or the reduction in PIP_2 levels, may mediate excitation. Like vertebrate rods, *Drosophila melanogaster* photoreceptors generate quantum bumps in response to single photons, but their kinetics is approximately 10–100 times faster, and this reflects a fundamentally different strategy incorporating a threshold, positive and negative feedback by Ca^{2+} acting downstream of phospholipase C and a refractory period.

Key words: phototransduction, *Drosophila melanogaster*, phosphoinositide, light-sensitive channel, calcium, inositol trisphosphate, vision, TRP, diacyl glycerol, PIP_2, phospholipase C.

Introduction

Two major classes of photoreceptor have emerged during the course of animal evolution: ciliary photoreceptors, typified by vertebrate rods and cones, and the microvillar or rhabdomeric photoreceptors typical of arthropods and most molluscs. Whilst the phototransduction cascade in rods and cones is understood in great detail, there are still major uncertainties surrounding invertebrate phototransduction. This is compounded by the apparent diversity in the underlying mechanisms in different species and the fact that the general class of mechanism (phospholipase-C-activated Ca^{2+} influx) is not fully understood in any cell. This review focuses on the fruit fly *Drosophila melanogaster*, on which most recent studies have concentrated because of its unique potential for genetic manipulation and the ability to obtain high-quality electrophysiological recordings under whole-cell patch-clamp conditions.

In all ocular photoreceptors, incident light is absorbed by a membrane-bound visual pigment, rhodopsin, and transduced into conductance changes in the plasma membrane by a G-protein-coupled signalling cascade. Whilst other G-protein-coupled receptors are activated by chemical messengers, rhodopsin is activated by absorption of light by the covalently bound chromophore 11-*cis* retinal (or, in dipteran flies, 11-*cis* 3-hydroxy retinal; Vogt and Kirschfeld, 1984). The resulting photoisomerization to all-*trans* retinal triggers the conversion of rhodopsin (Rh) to the active metarhodopsin (M) state, which catalyses the activation of a heterotrimeric G-protein. As with all such G-proteins, this involves nucleotide exchange (GTP for GDP) and subsequent dissociation of the Gα subunit, which remains active until the bound GTP is hydrolysed. In vertebrates, the G-protein (transducin) binds to and activates the effector enzyme, phosphodiesterase (PDE), leading to hydrolysis of cGMP. In contrast, in most invertebrates, the effector enzyme is phosphoinositide-specific phospholipase C (PLCβ), which hydrolyses the minor membrane phospholipid phosphatidyl inositol 4,5-bisphosphate (PIP_2) to produce soluble inositol 1,4,5-trisphosphate ($InsP_3$) and diacylglycerol (DAG) (Fig. 1). By an as yet unknown mechanism, which very likely differs in different invertebrates, this results in the activation of cation-permeable channels and membrane depolarisation. More generally, the phosphoinositide cascade is of central importance in controlling cellular Ca^{2+} levels, both by releasing Ca^{2+} from $InsP_3$-sensitive stores and by activating Ca^{2+} influx through specific channels in the plasma membrane, often *via* so-called store-operated Ca^{2+} channels which are activated by the reduction of levels of Ca^{2+} in the store lumen (Berridge et al., 2000). The central role of PLC in invertebrate photoreceptors is not disputed, but how activation of PLC is linked to opening of the light-sensitive channels remains one of the major unresolved questions in sensory transduction.

The light-sensitive channels: TRP and TRPL

The light-sensitive conductance in *Drosophila melanogaster* is highly Ca^{2+}-permeable (Hardie, 1991) and mediated by at

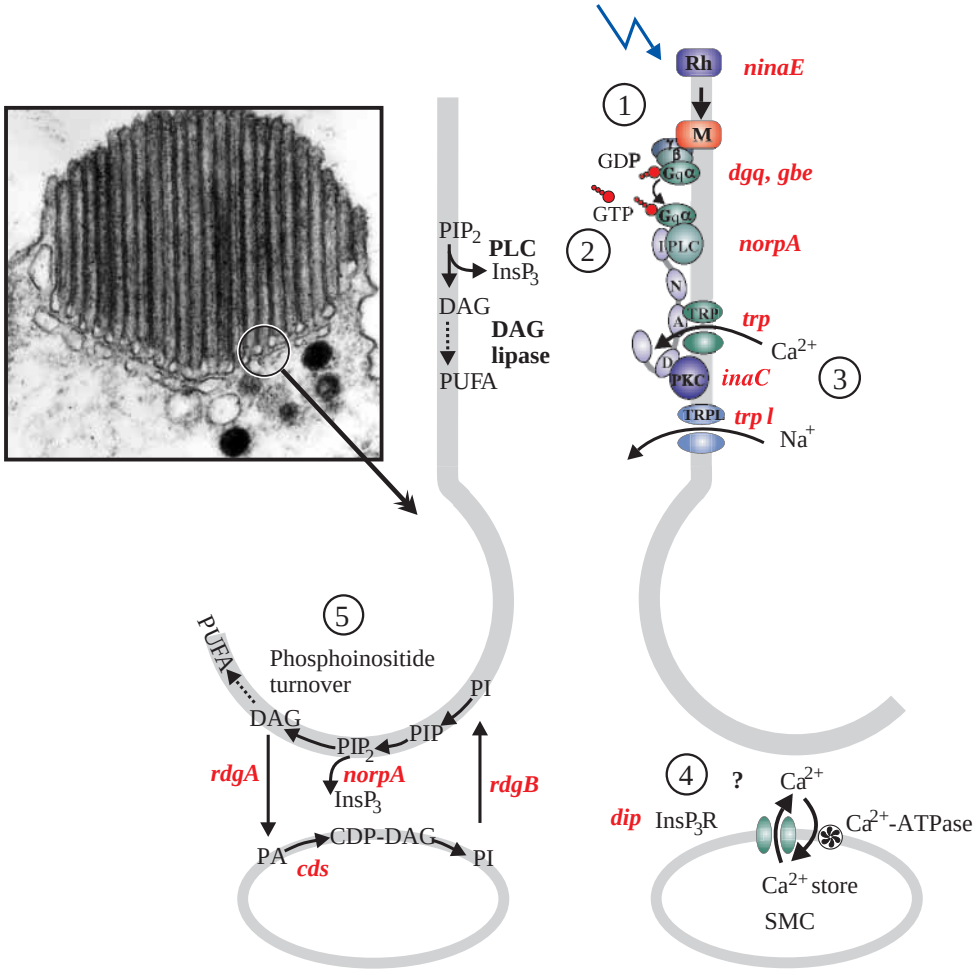

Fig. 1. *Drosophila melanogaster* phototransduction cascade. Inset: cross-section of a *Drosophila melanogaster* rhabdomere (electron micrograph courtesy of Dr A Polyanovsky), which is composed of some 30 000 microvilli, each approximately 1–2 μm in length and 60 nm in diameter. Each microvillus contains approximately 1000 molecules of rhodopsin and most elements of the phototransduction machinery. An enlargement of the circled area in the inset, showing the base of one microvillus with associated phototransduction machinery, is shown schematically in the main figure. Activation: (1) photoisomerization of rhodopsin to metarhodopsin (Rh→M, encoded by the *ninaE* gene) activates heterotrimeric G_q protein *via* GTP–GDP exchange, releasing the $G_q\alpha$ subunit. Genes and mutants for both α (*dgq*) and β (*gbe*) subunits have been identified. (2) $G_q\alpha$ activates phospholipase C (PLC; *norpA* gene), generating inositol 1,4,5-trisphosphate ($InsP_3$) and diacyl glycerol (DAG) from phosphatidyl inositol 4,5-bisphosphate (PIP_2). DAG is also a potential precursor for polyunsaturated fatty acids (PUFAs) *via* DAG lipase (gene yet to be identified in any eukaryote). (3) Two classes of light-sensitive channel (TRP and TRPL; *trp* and *trpl* genes) are activated by an unknown mechanism. Several components of the cascade, including the ion channel TRP, protein kinase C (PKC, *inaC* gene) and PLC are coordinated into a signalling complex by the scaffolding protein, INAD, which contains five PDZ domains. (4) At the base of the microvilli, a system of submicrovillar cisternae (SMC) has traditionally been presumed to represent Ca^{2+} stores endowed with $InsP_3$ receptors ($InsP_3R$; *dip* gene) and smooth endoplasmic reticulum Ca^{2+}-ATPase; however, the SMC may play a more important role in phosphoinositide turnover (5): DAG is converted to phosphatidic acid (PA) *via* DAG kinase (*rdgA* gene) and to CDP-DAG *via* CD synthase (*cds* gene) in the SMC. After conversion to phosphatidyl inositol (PI) by PI synthase, PI is transported back to the microvillar membrane by a PI transfer protein (*rdgB* gene). PI is converted to PIP_2 *via* sequential phosphorylation (PI kinase and PIP kinase).

least two classes of channel encoded, at least in part, by the *trp* (*t*ransient *r*eceptor *p*otential) gene and a homologue with approximately 40 % sequence identity, *trpl* (*trp-like*) (Hardie and Minke, 1992; Montell and Rubin, 1989; Niemeyer et al., 1996; Phillips et al., 1992). *trp* encodes an approximately 8 pS Ca^{2+}-selective (P_{Ca}:P_{Na} approximately 100:1, where *P* is permeability) ion channel (TRP) which can be isolated in *trpl* mutants, whilst *trpl* encodes an approximately 40 pS non-selective cation channel (P_{Ca}:P_{Na} approximately 4:1) responsible for the residual current in *trp* mutants (Reuss et al., 1997). The predicted sequences of both TRP and TRPL, with six transmembrane helices, represent single subunits of putative tetrameric channels, but there is some uncertainty as to whether the channels form homo- or heteromultimers *in vivo* (Reuss et al., 1997; Xu et al., 1997). A third *trp* homologue (*trp-γ*) has recently been identified in photoreceptors, and it is reported to form heteromultimers with TRPL (Xu et al., 2000). Why the photoreceptor requires two or more classes of channel is unclear. Under normal conditions, at least 95 % of the light-induced current is probably carried by the TRP channels (Reuss et al., 1997), and the light-induced current in *trpl* mutants is almost indistinguishable from wild type. Although some subtle phenotypes have been reported in intracellular recordings and electroretinograms from *trpl* mutants (Leung et al., 2000), the relationship between these altered responses and the properties of the TRPL channels is unclear.

TRP is the defining member of a novel family of ion channels, with approximately 20 mammalian isoforms divided into several subfamilies [for a review, see (Harteneck et al., 2000; Clapham et al., 2001)]. Isoforms most closely related to *Drosophila melanogaster trp* and *trpl* form a distinct subfamily (TRPC1–TRPC7), all of which appear to be activated

downstream of PLC and possibly include channels responsible for store-operated Ca^{2+} entry. The more distantly related TRPs include a bewildering variety of channels, such as the vanilloid receptor (VR1 or capsaicin receptor=TRPV1) involved in thermal nociception (Caterina et al., 1997), osmotically activated channels (Strotmann et al., 2000), Ca^{2+} transporters in gut and kidney (Hoenderop et al., 1999; Peng et al., 1999), mechanosensitive ion channels found in *Drosophila melanogaster* sensory hairs (Walker et al., 2000) and novel channels of obscure function, such as TRPM7 (=TRP-PLIK) and TRPM2 (=LTRPC2) notable for harbouring enzymatic domains in their C termini (Perraud et al., 2001; Runnels et al., 2001).

The enigma of excitation

The most familiar action of PLC is to produce $InsP_3$ and, in some arthropods such as *Limulus polyphemus*, light-induced $InsP_3$ production and the release of Ca^{2+} from $InsP_3$-sensitive stores are essential steps in excitation [for a review, see (Nasi et al., 2000)]. However, in *Drosophila melanogaster*, this now seems unlikely. First, the massive rise in intracellular [Ca^{2+}] following stimulation is almost entirely due to influx *via* the light-sensitive channels (Cook and Minke, 1999; Hardie, 1996b; Peretz et al., 1994; Ranganathan et al., 1994). Second, excitation cannot be mimicked by releasing caged $InsP_3$ (Hardie and Raghu, 1998) or caged Ca^{2+} (Hardie, 1995) or by depleting Ca^{2+} stores with the Ca^{2+}-ATPase inhibitor thapsigargin (Hardie, 1996a). Third, severe or null mutations in the only $InsP_3$ receptor ($InsP_3R$) gene in *Drosophila melanogaster* have no detectable effect upon the response to light (Acharya et al., 1997; Raghu et al., 2000a). Nevertheless, a role for $InsP_3$ cannot yet be entirely abandoned: for example, it was recently reported that 2-APB, an albeit non-specific membrane-permeant blocker of $InsP_3$ receptors, could reversibly block the light response in *Drosophila melanogaster* photoreceptors (Chorna-Ornan et al., 2001). It should also be recognised that negative results in response to pharmacological application of $InsP_3$ or other agents should be treated with caution, both because of the relative inaccessibility of the microvillar environment and because very high local concentrations are likely to be generated during physiological responses.

Given the requirement for PLC, alternative excitatory signals might include DAG or the reduction in PIP_2 levels. The most familiar action of DAG is to activate protein kinase C (PKC) in concert with Ca^{2+}, but mutants of eye PKC have defects only in response inactivation and adaptation, leaving excitation unaffected (Hardie et al., 1993; Smith et al., 1991). However, DAG has other roles, such as a precursor for polyunsaturated fatty acids (PUFAs) *via* the action of DAG lipase. A recent study showed that both TRP and TRPL channels could be activated by PUFAs downstream of PLC (Chyb et al., 1999). The significance of this result has been questioned since PUFAs can uncouple mitochondria, and ATP depletion has also been shown to activate the light-sensitive channels (Agam et al., 2000). However, this cannot explain the activation of heterologously expressed TRPL channels by PUFAs in excised inside-out patches (Chyb et al., 1999). More recently, Estacion et al. (Estacion et al., 2001) found that heterologously expressed TRPL channels can also be activated by DAG, but reported that some of the actions of both DAG and PUFAs may be indirect *via* activation of PLC in the excised patches. The activity of TRPL channels in excised patches was suppressed by application of PIP_2, suggesting PIP_2 depletion as a potential contributory factor to channel gating. This seems unlikely to account for activation of TRPL *in vivo* since, under conditions in which PIP_2 is severely depleted, TRPL channels in the *trp* mutant become completely inactivated and can be reactivated by light only after PIP_2 is resynthesised. Nevertheless, under the same conditions of PIP_2 depletion, TRP channels tend to remain constitutively activated, suggesting that PIP_2 depletion might reward further attention as a mechanism of activation of TRP (Hardie et al., 2001).

Independent evidence for an excitatory role for DAG comes from the *rdgA* mutant. The *rdgA* gene encodes DAG kinase, which inactivates DAG by converting it to phosphatidic acid (Masai et al., 1993). *rdgA* mutants undergo severe retinal degeneration, and the photoreceptors show no response to light. When investigated with whole-cell voltage-clamp techniques, the TRP channels in *rdgA* mutants are constitutively active, and it seems that the resultant Ca^{2+} influx may cause the severe degeneration because this was rescued in *rdgA*;*trp* double mutants (Raghu et al., 2000b). The response to light was also rescued in the double mutant, but failed to terminate normally, indicating that DAG kinase is required for response termination. The constitutive activity of the TRP channels and the deactivation defect would be consistent with a role for DAG in excitation. However, DAG kinase is also the first enzymatic step in the resynthesis of PIP_2, so that PIP_2 levels and the kinetics of PIP_2 recycling may also be affected in the *rdgA* mutant.

In summary, there is little evidence to support a role for $InsP_3$ in phototransduction in *Drosophila melanogaster*, whilst several recent studies have suggested that alternative actions of PLC (i.e. the production of DAG, PUFAs and/or a reduction in PIP_2 levels) may be important. Resolution of the mechanism of excitation might require, *inter alia*, identification and characterization of ligand-binding sites on the channel molecules, biochemical analysis of light-induced lipid metabolism and molecular identification and mutant analysis of any further gene products required for activation. In this respect, a mutation of a novel protein (INAF) with no known homologues has recently been shown to mimic aspects of the *trp* phenotype, suggesting that it may be required for TRP activation (Li et al., 1999).

Response termination

For efficient signal transduction, particularly to maximise the temporal resolution of the response, it is clearly important

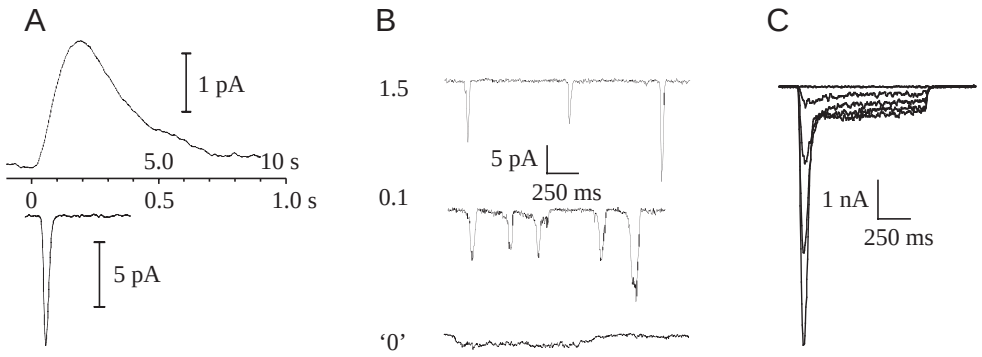

Fig. 2. Voltage-clamped responses from dissociated *Drosophila melanogaster* photoreceptors. (A) Quantum bumps in a vertebrate [top, toad rod, courtesy of Professor T. Lamb; data from (Whitlock and Lamb, 1999)] compared with *Drosophila melanogaster* (below). Note the different scales; the kinetics of the toad bump is approximately 100 times slower than that of *Drosophila melanogaster*. (B) *Drosophila melanogaster* quantum bumps recorded in normal (1.5 mmol l^{-1}), intermediate (0.1 mmol l^{-1}) and '0' (<100 nmol l^{-1}) Ca^{2+}. The amplitude is reduced and the kinetics greatly slowed in low external [Ca^{2+}]. In intermediate concentrations, a slow rising phase appears to trigger a full-sized bump after a brief delay [adapted from (Henderson et al., 2000)]. (C) Responses to 1 s steps of light of increasing intensity (maximum approximately 50 000 effectively absorbed photons per second); the rapid peak-to-plateau transitions are a direct manifestation of Ca^{2+}-dependent light adaptation [adapted from (Raghu et al., 2000a)].

that the response is also rapidly inactivated. As in any transduction cascade, it is essential that the activity of each element is efficiently terminated.

Activated rhodopsin is inactivated by binding to arrestin

Drosophila melanogaster photoreceptors actually express two arrestin isoforms, one of which, *arr2*, appears to play the dominant role in inactivation (Dolph et al., 1993). Rhodopsin is also multiply phosphorylated by rhodopsin kinase, and in vertebrates this is essential for inactivation (Chen et al., 1995). But, in *Drosophila melanogaster*, flies expressing a truncated rhodopsin construct in which the phosphorylation sites are deleted show no defects in electrical responses or in the ability of arrestin to bind to rhodopsin (Vinos et al., 1997), implying that the phosphorylation plays no direct role in phototransduction.

Activity of both the GTP-bound $G_q\alpha$ subunit and PLC is terminated by the GTPase activity of the G-protein

The intrinsic GTPase activity of the G-protein is much too slow to account for the rapid response termination, and it is now clear that, as in rods, the effector enzyme itself (PDE in rods, PLC in flies) acts as a GTPase-activating protein (GAP) to accelerate GTP hydrolysis. This is most clearly seen in *norpA* hypomorphs with reduced levels of PLC, in which responses can be elicited up to a minute after absorption of light. Presumably, this represents the time taken for individual activated G-proteins to diffuse in the membrane before encountering a rare PLC molecule, in turn demonstrating that $G_q\alpha$ remains active until it encounters PLC (Cook et al., 2000; Scott and Zuker, 1998). In vertebrate rods, additional proteins (RGS9=regulator of G-protein signalling and Gβ5) are also required for GAP activity of the effector enzyme (Arshavsky and Pugh, 1998). Whether homologous proteins play similar roles in *Drosophila melanogaster* photoreceptors is not known.

Light-sensitive channels

Both classes of light-sensitive channel are rapidly inactivated by Ca^{2+}, e.g. (Reuss et al., 1997). This may involve calmodulin (CaM), since TRP contains one and TRPL two calmodulin-binding sites (Chevesich et al., 1997; Warr and Kelly, 1996) and light responses in *Cam* hypomorphs or flies expressing a mutant TRPL construct with one or other calmodulin-binding site deleted show delayed response inactivation (Scott et al., 1997). The TRP channel is also subject to a voltage-dependent Mg^{2+} block, which intensifies over the physiological range of membrane potentials (–70 to 0 mV). This would appear to be an elegant and economic mechanism for gain reduction during light adaptation when the membrane depolarises by some 30–40 mV (Hardie and Mojet, 1995).

Protein kinase C

Mutants of the photoreceptor PKC (*inaC*) have severe defects in response deactivation and light adaptation (Hardie et al., 1993; Smith et al., 1991). At least two PKC phosphorylation targets have been identified, namely the TRP channel and the INAD scaffolding protein (Huber et al., 1998; see further below), although whether either of these is responsible for the *inaC* deactivation phenotype remains to be seen.

Quantum bump generation

Many photoreceptors share the remarkable ability of responding to single photons (Fig. 2), but microvillar photoreceptors do so by opening channels, whilst in vertebrate rods, the channels close. Since approximately 10 000 channels are open in the dark in a typical rod, several hundred channels must close to generate a detectable response of approximately 1 pA; however, in invertebrates, in which channels are closed at rest, the opening of even one channel might, in principle, be sufficient. In fact, the average *Drosophila melanogaster* quantum bump of approximately 10 pA represents simultaneous activation of only approximately 15 channels (Henderson et al., 2000), which might reasonably be expected to be contained within a single microvillus. Perhaps as a consequence of their high gain, vertebrate rods have only a

limited ability to adapt to light, saturating with photon fluxes of approximately 500 photons s^{-1}. In contrast, fly photoreceptors not only detect single photons but also light-adapt (Fig. 2C) and continue responding sensitively under the brightest daylight intensities, equivalent to approximately 10^6 photons s^{-1} photoreceptor^{-1} (Howard et al., 1987; Juusola and Hardie, 2001).

Vertebrate quantum bumps have short, rather constant latencies, but relatively slow rise times. In *Drosophila melanogaster*, there is a finite but variable latency (20–100 ms) following which the bump rises and falls approximately 10–100 times faster than in rods (Fig. 2). This difference reflects fundamentally different mechanisms of bump generation. In vertebrates, amplification begins at the earliest stage, each rhodopsin molecule activating numerous G-proteins. In *Drosophila melanogaster*, all effective amplification in terms of bump amplitude appears to take place downstream of PLC, since hypomorphic mutations in either G_q or PLC have little or no effect on bump amplitude or waveform, affecting only the latency or quantum efficiency of bump generation (Pak et al., 1976; Scott and Zuker, 1998). When G_q levels are reduced in G_q hypomorphs, latency is little affected but quantum efficiency is greatly reduced because most metarhodopsin molecules are rapidly inactivated by binding to arrestin before they have a chance to encounter G_q. In contrast, reduced PLC levels in *norpA* hypomorphs lead to dramatic increases in bump latency (in some cases to more than 1 min) but have little effect on quantum efficiency because, once activated, $G_q\alpha$ remains active indefinitely until it binds to a rare PLC molecule (see above) (Cook et al., 2000; Scott and Zuker, 1998). In G_q and *norpA* hypomorphs, each bump is probably generated by one G-protein and one PLC molecule. A single G-protein may also be sufficient to generate a bump in wild-type photoreceptors, but it seems likely that a variable number will be activated, determined stochastically by the probability of metarhodopsin encountering either arrestin or a second, third, etc. G-protein. Such stochasticity might contribute to the relatively broad latency distribution of fly and other invertebrate photoreceptors.

The amplification downstream of PLC and the rapid inactivation of the quantum bump are both dependent upon Ca^{2+} influx through the light-sensitive channels. Bump amplitude is reduced approximately 10-fold at low external Ca^{2+} concentrations, whilst bump duration increases dramatically (Henderson et al., 2000). At intermediate external Ca^{2+} concentrations, bumps develop with a small slow rising phase that abruptly accelerates to yield a full-sized bump (Fig. 2B), suggesting that Ca^{2+} influx must reach a threshold before triggering a cycle of positive and negative feedback responsible for amplification and rapid inactivation. A plausible mechanism for facilitation would be that Ca^{2+} increases the affinity of the channel for the putative second messenger, so that the near-threshold concentration of transmitter that builds up during the latent period suddenly becomes saturating.

In mutants of arrestin or calmodulin (which affects arrestin binding indirectly), activated rhodopsin (metarhodopsin) lifetime is greatly prolonged. Bump waveforms are unaffected in such mutants, but each absorbed photon gives rise to multiple trains of bumps separated by 100–200 ms (Scott et al., 1997). This indicates that the same rhodopsin molecule can generate a second bump after a brief refractory period. The first evidence for a refractory period came from paired flash experiments in *Calliphora vicina* showing that the response to a second flash was completely abolished by an adapting flash of intensity just sufficient to ensure that every microvillus had absorbed at least one photon. Complete inactivation was only transient, substantial sensitivity recovering within 50 ms (Hochstrate and Hamdorf, 1990). The refractory period is probably caused by extremely high Ca^{2+} concentrations that are believed to exceed 200 μmol l^{-1} during the lifetime of the bump (Oberwinkler and Stavenga, 2000; Postma et al., 1999) and might end when the Ca^{2+} inside the microvilli has been removed by diffusion and/or by the Na^+/Ca^{2+} exchanger [within approximately 100 ms and faster when light-adapted, according to measurements in *Calliphora* (Oberwinkler and Stavenga, 2000)]. Accordingly, if Na^+/Ca^{2+} exchange is temporarily blocked by removing external Na^+, a single flash containing sufficient photons to activate the majority of microvilli renders the photoreceptor refractory to further stimulation until the Na^+ is returned to the bath (S. Moore and R. C. Hardie, unpublished data).

Under light-adapted conditions, each quantum bump becomes progressively reduced in amplitude and duration as a result of the negative feedback from the raised steady-state $[Ca^{2+}]$; nevertheless, noise analysis suggests that the photoreceptors continue acting as linear photon counters up to daylight intensities (Wong, 1982; Howard et al., 1987; Juusola and Hardie, 2001). This can be readily understood if it is assumed that each microvillus is a semi-autonomous unit of excitation and can be recycled for action within 100 ms, or less when light-adapted, thereby allowing the 30 000 microvilli in each *Drosophila melanogaster* rhabdomere to process in excess of 30 000 photons s^{-1}, as observed experimentally (Juusola and Hardie, 2001). After taking into account the approximately 1–2 log unit attenuation of the incident light flux by the pupil pigment granules, which migrate towards the rhabdomere when light-adapted, this is sufficient to avoid saturation under the brightest daylight intensities (Howard et al., 1987; Juusola and Hardie, 2001).

The INAD signalling complex

Phototransduction in flies represents the fastest G-protein-coupled signalling system known. Without doubt, a major factor in the speed of this cascade is the extreme localization of excitation, probably to a single microvillus. Coupled with the lack of substantial amplification (in terms of numbers of G-protein and PLC molecules activated), this minimizes delays due to diffusion either of proteins in membranes or of transmitter molecules, whilst the tiny microvillar lumen results in rapid and massive rises in $[Ca^{2+}]$ essentially coincident with the light-sensitive current (Postma et al.,

1999; Oberwinkler and Stavenga, 2000). An additional factor may be the fact that many of the cascade elements appear to be assembled into a supramolecular signalling complex organised by a 'scaffolding protein' INAD. First identified by a subtle mutant phenotype on response inactivation (Shieh and Niemeyer, 1995), the *InaD* gene (*i*nactivation *n*o *a*fterpotential) encodes a scaffolding protein with five so-called PDZ domains, each of which binds to a specific partner protein (Chevesich et al., 1997; Huber et al., 1996; Tsunoda et al., 1997). It is generally accepted that these include TRP, PKC and PLC. INAD has also been reported to bind TRPL, rhodopsin, calmodulin and a non-conventional myosin NINAC (no inactivation no afterpotential) (Montell, 1999), although this has not been confirmed by other authors. INAD also has the potential to multimerize *in vitro*, possibly forming extended 'rafts'. It has been proposed that the resulting supramolecular complex is the unit of signalling underlying the quantum bump and, in support of this, severe mutations of the *InaD* gene result in defects in the bump waveform (Scott and Zuker, 1998). However, the INAD protein is also required for the localization of PKC, TRP and PLC to the microvillar membrane (Tsunoda et al., 1997) so, at present, it is not possible to distinguish whether it is the integration of the elements into a supramolecular complex or simply their presence in the microvillus in appropriate concentrations which is required for normal rapid response kinetics.

Concluding remarks and outlook

All photoreceptors are faced with the conflicting goals of extreme sensitivity, rapid response speed and large dynamic range. As exemplified by *Drosophila melanogaster*, invertebrate photoreceptors have exploited the phosphoinositide pathway to achieve these aims, easily outstripping vertebrate photoreceptors in terms of the combination of sensitivity, speed and adaptational performance. Physiological and molecular genetic analysis of *Drosophila melanogaster* phototransduction has led to a deeper understanding of how this exceptional performance is achieved, but major outstanding issues, such as how the light-sensitive channels are activated and precisely how Ca^{2+} regulates the sensitivity and speed of the cascade, remain to be resolved. The *Drosophila melanogaster* eye is also an important genetic model for phosphoinositide signalling more generally and has led to the discovery and characterization of novel classes of signalling molecules of widespread importance. Notable amongst these are the TRP-related ion channels, responsible for Ca^{2+} influx in many systems, and the scaffolding protein INAD. With the recent publication of the *Drosophila melanogaster* genome and the concurrent development of functional genomic tools, there is now an excellent prospect of resolving the outstanding problems in phototransduction within the foreseeable future.

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