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Current Issues in Invertebrate Phototransduction

Second Messengers and Ion Conductances

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Abstract

Investigation of phototransduction in invertebrate photoreceptors has revealed many physiological and biochemical features of fundamental biological importance. Nonetheless, no complete picture of phototransduction has yet emerged. In most known cases, invertebrate phototransduction involves polyphosphoinositide and cyclic GMP (cGMP) intracellular biochemical signaling pathways leading to opening of plasma membrane ion channels. Excitation is Ca^{2+}-dependent, as are adaptive feedback processes that regulate sensitivity to light. Transduction takes place in specialized subcellular regions, rich in microvilli and closely apposed to submicrovillar membrane systems. Thus, excitation is a highly localized process.

This article focuses on the intracellular biochemical signaling pathways and the ion channels involved in invertebrate phototransduction. The coupling of signaling cascades with channel activation is not understood for any invertebrate species. Although photoreceptors have features that are common to most or all known invertebrate species, each species exhibits unique characteristics. Comparative electrophysiological, biochemical, morphological, and molecular biological approaches to studying phototransduction in these species lead to fundamental insights into cellular signaling. Several current controversies and proposed phototransduction models are evaluated.

Index Entries: Transduction; ion channel; phosphoinositide; IP_3; cyclic GMP; Ca^{2+}; calcium; *Limulus*; *Drosophila*; photoreceptor; rhabdom.

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Introduction

The study of phototransduction in invertebrate eyes has been very rewarding, because it offers fundamental insights into the variety of processes underlying cellular signaling and the interactions between these processes. The problem of phototransduction can be stated simply. How does a cell accurately transduce photon information to neural information? However, it seems that its solution will be quite complex. Phototransduction begins with the absorption of light by the photopigment protein, rhodopsin, initiating intracellular biochemical signaling cascades. Neural excitation is initiated by the opening of plasma membrane ion channels. How light-induced cascades are linked with the channel opening has not been solved for any invertebrate species. An outline of intracellular signaling cascades

(Stimulus → Receptor → G-protein →
Effector → Messenger → Target → Effect)

offers only a narrow view of a very broad picture. There may be several cascades operating simultaneously and interactively, offering feedback or feedforward capabilities. Thus, for each enzymatic component activated in excitation, it is important to understand the conditions under which its activation takes place, how its inactivation occurs, and how it is reset. For each step in the cascades, the factors that govern the kinetics of activation and deactivation and the modulation of these factors during normal physiological function must be characterized. All these biochemical features take place in restricted subcellular regions, and the specific suborganellar environmental conditions that support individual reactions are essential features for analysis. Thus, identification and complete characterization of each cascade reaction will be required to understand fully the functioning system. Comparison of electrophysiological measurements with quantitative simulations developed from the characteristics of the intracellular biochemistry will help in evaluation of specific models of phototransduction.

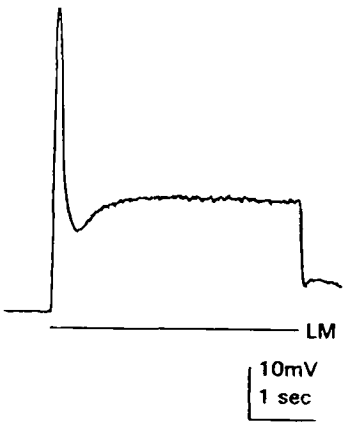

Fig. 1. Light response (receptor potential) recorded from *Limulus* ventral photoreceptor. The membrane depolarized from resting potential –68 mV reached a transient peak and declined to plateau after a brief "dip." The measurement was made with a single microelectrode, using standard methodologies (Johnson and O'Day, 1995). LM indicates light monitor.

A typical response to bright, prolonged illumination in most invertebrate species includes a variable latency period followed by an abruptly rising (depolarizing) membrane potential, V_m, that reaches a transient peak and declines to a plateau potential (see Figure 1). After the end of stimulation, V_m gradually returns to baseline. These physiological features are referred to as response latency, initiation, and termination, respectively. The decline of V_m to plateau reflects a reduction in the photoreceptor sensitivity to light, termed light adaptation, enabling the retina to function effectively over a wide range of illumination intensities. Adaptation can also be quantified as the ratio of response amplitude after an adapting light to that before adaptation (Lisman and Brown, 1975a).

Although no blueprint for invertebrate transduction is known, great progress has been made toward understanding some of the underlying processes. The premier preparations for studying invertebrate transduction have been the rhabdomeral photoreceptors of the horseshoe crab, *Limulus*, and of the fruitfly, *Drosophila*. The ventral photoreceptors of *Limu-*

lus provide an extremely tractable physiological preparation, whereas *Drosophila* retinal photoreceptors offer excellent genetic and molecular approaches. Vital biochemical, physiological, and imaging information and insights come from several other species, principally among dipterans and marine invertebrates.

In *Limulus* and several other marine invertebrates, there is ample evidence that phototransduction is mediated in part by the polyphosphoinositide (PI) pathway (Szuts et al., 1986; Payne et al., 1988; Brown et al., 1991; Nagy, 1991; Mitchell et al., 1995); however the link between PI pathway components and channel opening is unknown. In addition, the soluble intracellular messenger, cyclic GMP (cGMP), can act as a ligand to open ion channels activated in *Limulus* phototransduction (reviews: Bacigalupo et al., 1990; O'Day et al., 1997); however, the link between photon absorption and cGMP chemistry is not known. In *Drosophila* and other flies, a large body of evidence indicates a central role for PI metabolism in phototransduction (reviews: (Hardie and Minke, 1995; Pak, 1995; Ranganathan et al., 1995; Zuker, 1996)). Furthermore, cGMP influences *Drosophila* transduction, but its physiological role remains unclear.

In all species, calcium ion, Ca^{2+}, is an integral component of transduction. Ca^{2+} is essential for light-induced excitation as well as adaptation, whereby the gain (channels opened per incident photon) of transduction is modulated.

In this article, we briefly outline the early events in transduction and then focus on two topics: the second messenger pathways that may underlie excitation and the channels activated in the transduction processes that initiate the light response.

Second Messenger Pathways in Invertebrate Phototransduction

Receptor and G-Protein in Excitation

On absorbing a photon, the rhodopsin chromophore isomerizes, inducing conformational changes in the protein that enable it to bind heterotrimeric G-proteins, continuing the phototransduction cascade. Once bound to rhodopsin, G-proteins are activated after exchange of bound GDP for GTP and separation of the α-subunit (G_α) from the β, γ-subunit ($G_{\beta\gamma}$). Activated G-proteins relay information from the receptor to effector molecules, and both G_α and $G_{\beta\gamma}$ may participate in effector activation. In *Limulus* photoreceptors, an estimated 10 G-proteins are activated per activated rhodopsin (Kirkwood et al., 1989); for *Drosophila* it has been suggested that this ratio is one (Scott et al., 1995). Although the involvement of G-proteins in the activation of effector molecules has been under active investigation, our understanding of the contributions of specific steps is largely inferred by analogy with other signaling systems.

Inactivation of this process is also under active investigation (Bentrop and Paulsen, 1986; Byk et al., 1993; Smith et al., 1995). However, working hypotheses of the inactivation mechanisms can be made by comparing observations from invertebrate preparations with those from vertebrates, where inactivation models have been put forth. It seems clear that the binding of rhodopsin to another regulatory protein, arrestin, is responsible in part for inactivation. Rhodopsin-arrestin binding presumably prevents further G-protein binding and activation by rhodopsin. Phosphorylations of both rhodopsin and arrestin have been proposed to be important steps in inactivation (Lisman et al., 1992; Dolph et al., 1993), although their influence on rhodopsin-arrestin or rhodopsin–G-protein interactions remains unclear (Bentrop and Paulsen, 1986; Byk et al., 1993).

G-protein inactivation occurs through reassociation of G_α with $G_{\beta\gamma}$ following the hydrolysis of the bound GTP to GDP by intrinsic GTPase activity. In some signaling systems, including vertebrate photoreceptors, inactivation of heterotrimeric G-proteins may be accelerated by GTPase accelerating proteins (or RGS proteins (Angleson and Wensel, 1993, Arshavsky et al., 1994; Watson et al., 1996)).

A G-protein of the class G_q activates the PI cascades in *Drosophila* (Lee et al., 1994). Both G_α and $G_{\beta\gamma}$ appear to play physiological roles

(Yarfitz et al., 1991; Dolph et al., 1994; Lee et al., 1994; Running-Deer et al., 1995; Scott et al., 1995), although the modes of action have not yet been determined. Presumably other species with PI-pathway-dependent phototransduction have similar G-protein function. For example, a $G_{q\alpha}$ similar in sequence to that in *Drosophila* has recently been described in *Limulus* photoreceptors (Munger et al., 1996). In the ciliary photoreceptors of scallop, where excitation involves cGMP-gated channels and no PI metabolism (Gomez and Nasi, 1995), G-protein involvement remains to be examined.

Phosphoinositide Signaling Pathway in Excitation

By analogy with other signaling systems, the subsequent steps are thought to include the following. G_q activates phospholipase C (PLC), which cleaves membrane phospholipids, 4,5-phosphatidylinositol bisphosphate (PIP_2) into the two cellular messengers, inositol-(1,4,5)-trisphosphate (IP_3) and diacylglycerol (DAG). These messengers localize to different intracellular environments, where they initiate separate, parallel signaling events. IP_3 is a soluble intracellular messenger that diffuses through the cytosol to activate the IP_3-receptor (IP_3-R), a Ca^{2+} channel on the membranes of the intracellular Ca^{2+} stores. These stores are variously referred to as submicrovillar cisternae (SMC), endoplasmic reticulum, or subrhabdomeral cisternae (Walz, 1982; Calman and Chamberlain, 1982; Suzuki and Hirosawa, 1992). DAG is a lipid soluble messenger that diffuses through the membrane bilayer where it activates protein kinase C (PKC; discussed below).

Cytosolic Ca^{2+} concentration, $[Ca^{2+}]$, is elevated locally by Ca^{2+}-release from SMC owing to IP_3-R activation. This $[Ca^{2+}]$ elevation has multiple physiological consequences in phototransduction, because many components are Ca^{2+}-dependent. In spite of the wealth of evidence for the involvement of PI metabolism and Ca^{2+} release in invertebrate phototransduction, the coupling of the PI pathway to channel opening remains unresolved.

Requirement for PLC

The following observations are among the evidence that the PI pathway is central to *Drosophila* phototransduction: excitation does not occur in mutants lacking functional PLC (Bloomquist et al., 1988); excitation can be recovered at permissive conditions in temperature-sensitive mutants of PLC (Minke, 1979); PLC activity is triggered by light (Szuts et al., 1986; Devary et al., 1987; Suzuki and Hirosawa, 1992). One might expect, however, that exogenous applications of PI pathway component messengers downstream of PLC would induce excitation, and this has not been observed. Excitation in *Drosophila* has not been mimicked by introduction of either IP_3, Ca^{2+}, or DAG in whole-cell recording experiments, calling into question the precise role of PLC (Hardie and Minke, 1995; and Bacigalupo and O'Day, unpublished). Moreover, it has not been determined whether specifically light-induced PLC activity triggers the subsequent steps in transduction. It is possible that the PLC activity triggered by a light stimulus is not required for the generation of the response to that specific stimulus. PLC dependent PI turnover may be required for cellular maintenance and integrity (e.g., reviews: (O'Tousa, 1990; Ranganathan et al., 1995)); and PLC, although light-dependent, may play no direct role in the immediate generation of the electrical response.

IP_3 injection in *Limulus*, in contrast to *Drosophila*, is sufficient to induce channel opening, unless intracellular Ca^{2+} is buffered (Payne et al., 1986), suggesting that Ca^{2+} triggers phototransduction events downstream of the IP_3-R. Thus, the PI pathway appears to be sufficient to cause excitation in *Limulus*, although there are suggestions of parallel, independent excitation pathways (Payne et al., 1988; Frank and Fein, 1991; Nagy, 1993).

Membrane Channels in PI-Dependent Excitation

Recent work on IP_3-dependent excitation in some systems suggests that depletion of intracellular Ca^{2+} stores by IP_3-induced release activates plasma membrane channels indepen-

dent of the elevation of $[Ca^{2+}]$. The gating of these "store-operated" channels (SOC) may be either by direct interaction of the IP_3-R with the SOC or by SOC activation by a diffusing messenger (Clapham, 1995; Petersen et al., 1995). Alternatively, it is proposed that Ca^{2+} store depletion induces translocation of SOC channels in vesicular membranes derived from Ca^{2+} stores and that these SOC-containing vesicles fuse with plasma membranes, generating the electrical response (Somasundaram et al., 1995). The demonstrations that *Drosophila* channel polypeptides, TRP and TRPL, when expressed heterologously, yield currents activated by Ca^{2+} store depletion (Hu and Schilling, 1995; Gillo et al., 1996) provide support for models incorporating SOC channels in PI-dependent excitation (Hardie and Minke, 1995). A basic scheme of PI-pathway dependent excitation involving SOC is diagrammed in Fig. 2.

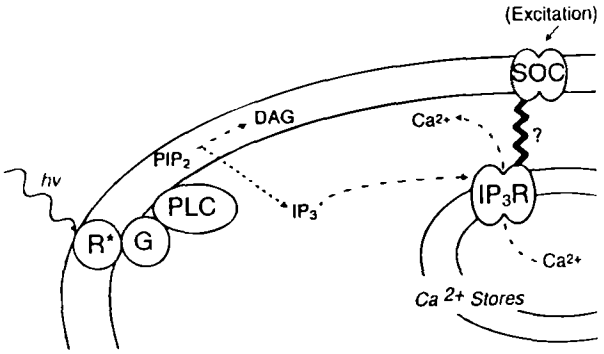

Fig. 2. Basic scheme of PI-pathway-mediated excitation involving SOC channels. R*, activated rhodopsin; G, trimeric G-protein; PLC, activated phospholipase C; PKC, protein kinase C; SOC, Ca^{2+}-store-operated plasma membrane channel; IP_3-R, ER membrane IP_3-receptor Ca^{2+}-channel; PIP_2, membrane phospholipid, 4,5-phosphatidylinositol; IP_3, soluble intracellular messenger, inositol-(1,4,5)trisphosphate; DAG, lipid soluble plasma membrane messenger, diacylglycerol.

Alternatives to SOC-dependent excitation include the possibility that the plasma membrane channels are gated directly by IP_3 generated by PI hydrolysis and/or that some other undetermined ligand gates channels.

Protein Kinase C

An important Ca^{2+}-dependent PI pathway component is PKC, which is activated by Ca^{2+} in concert with DAG and phosphatidylserine (Huang, 1989). In flies, an eye-specific PKC (eye-PKC) and its apparent phosphorylated substrate, INAD (Shieh and Niemeyer, 1995; Shieh and Zhu, 1996; Huber et al., 1996), appear to influence cellular physiology by retarding the termination of light responses and inhibiting light adaptation (Smith et al., 1991; Hardie et al., 1993). PKC and INAD mutants exhibit normal excitation, but dramatically slowed response deactivation. The role of Ca^{2+} as intracellular messenger of light adaptation (Lisman and Brown, 1972; Brown and Lisman, 1975) may stem from its activation of PKC. It has been proposed that INAD and PKC mediate the light-dependent negative feedback underlying light adaptation by modulating the function of PLC (Shieh and Niemeyer, 1995). If this is the case, light adaptation would involve the following sequence of events:

1. PLC-catalyzed synthesis of IP_3 and DAG (*see* Fig. 2),
2. Cytosolic diffusion of IP_3 to the IP_3-R on the SMC membranes, causing
3. Ca^{2+}-mobilization and diffusion to PKC, triggering
4. PKC translocation from cytosol to membrane,
5. Intramembranous diffusion of DAG to PKC,
6. PKC-catalyzed phosphorylation of INAD, causing
7. Downregulation of PLC, and
8. Reducing the gain of subsequent PLC-activating events.

There are other possible roles for PKC. It has been noted that INAD may affect the function of ion channel polypeptide, TRP, with which it coimmunoprecipitates (Huber et al., 1996; Shieh and Zhu, 1996) and which appears to contribute the major component of the light-activated conductance change (*see* Light Responses). In this light, it is interesting that released Ca^{2+} may influence another ion channel polypeptide important in transduction, TRPL (Niemeyer et al., 1996), which has multiple calmodulin binding sites (Phillips et al., 1992) and a site for Ca^{2+}-dependent phosphorylation

by PKC (Warr and Kelly, 1996). Thus, PLC-initiated events may modulate ion channel function in phototransduction.

Alternatively, or additionally, PKC might modify other steps. In vertebrate photoreceptors, PKC phosphorylates rhodopsin (Newton and Williams, 1993) and guanylate cyclase (Wolbring and Schnetkamp, 1996). Although in *Drosophila* rhodopsin does not seem to be a PKC substrate, the issue remains unclear in other invertebrate species. In addition, PKC substrates in other systems include Ca^{2+}-regulatory proteins, Na/Ca exchange, and Ca^{2+}-ATPase, whose analogs in photoreceptors are key to transduction.

cGMP Pathway in Excitation

Although much evidence supports the idea that PI-pathway-dependent signaling is central to transduction, considerable evidence from several species supports the notion that second messenger, cGMP, also plays key roles. The nature of the evidence is different for different preparations.

Limulus

In *Limulus* phototransduction, cGMP appears to serve as a ligand that gates light-sensitive channels. The first single-channel recordings of any light-sensitive conductance were obtained from *Limulus* rhabdomeral membrane using cell-attached patch electrodes (Bacigalupo and Lisman, 1983). When such patches are excised, the channels can be gated by cGMP, but not by Ca^{2+} (Bacigalupo et al., 1991). Furthermore, intracellular injection of cGMP and its nonhydrolyzable analog, 8-Br-cGMP, can induce excitation (Johnson et al., 1986; Bacigalupo et al., 1990; Feng et al., 1991).

Pharmacological and biochemical evidence also points to cGMP metabolism in *Limulus* phototransduction. Inhibitors of guanylate cyclase (GC, the enzyme that synthesizes cGMP) or cGMP-specific phosphodiesterase (cGB-PDE, the enzyme that degrades cGMP) alter phototransduction (Brown et al., 1984; O'Day et al., 1991; Inoue et al., 1992; Faddis and Brown, 1994; Johnson and O'Day, 1995). The effects of these inhibitory agents on excitation exhibit strong dependence on Ca^{2+}, perhaps reflecting the Ca^{2+} dependence of the enzymatic steps (Johnson and O'Day, 1995). Biochemical evidence from *Limulus* indicates that cGMP phosphodiesterase (cGB-PDE) activity is reduced by light (Inoue et al., 1992). Although retinal guanylate cyclase is found in marine invertebrates (Robinson and Cote, 1988), measurements of light-dependent cGMP turnover and changes in cGMP metabolism remain equivocal (Calhoon and Tsuda, 1980; Vandenberg and Montal, 1984; Saibil, 1984; Johnson et al., 1986; Robinson and Cote, 1988; Tsuda et al., 1989; Inoue et al., 1992; Brown et al., 1982).

Drosophila

Several lines of evidence suggest that cGMP is important for transduction in *Drosophila* and other flies; however, precise roles and underlying mechanisms remain unknown. Both soluble (Yoshikawa et al., 1993; Shah and Hyde, 1995) and receptor-type (Liu et al., 1995) GC are expressed in the *Drosophila* retina although their localization in the functioning eye is not firmly established. Cyclic nucleotide phosphodiesterase in *Calliphora* retinas has been reported (Schraermeyer et al., 1993). Additionally, *Drosophila* retinas express a cyclic nucleotide-gated channel (CNGC) gene, whose product codes for a Ca^{2+} channel with properties similar to channels underlying light responses (Baumann et al., 1994 and *see* Channel Peptides section). However, direct measurements from such channels in the native membranes, either in cell-attached or excised membrane patches, have not been made. Indeed, it remains to be established whether such channels are present in the rhabdomeral membranes.

Physiological evidence suggests that cGMP increases sensitivity to light, defined as the conductance change per incident photon. Exogenous nonhydrolyzable analog, 8-Br-cGMP, enhances light responses in voltage-clamped photoreceptors (Fig. 3A). Furthermore, 8-Br-cGMP application alone induces membrane currents that are dependent on the intensity of

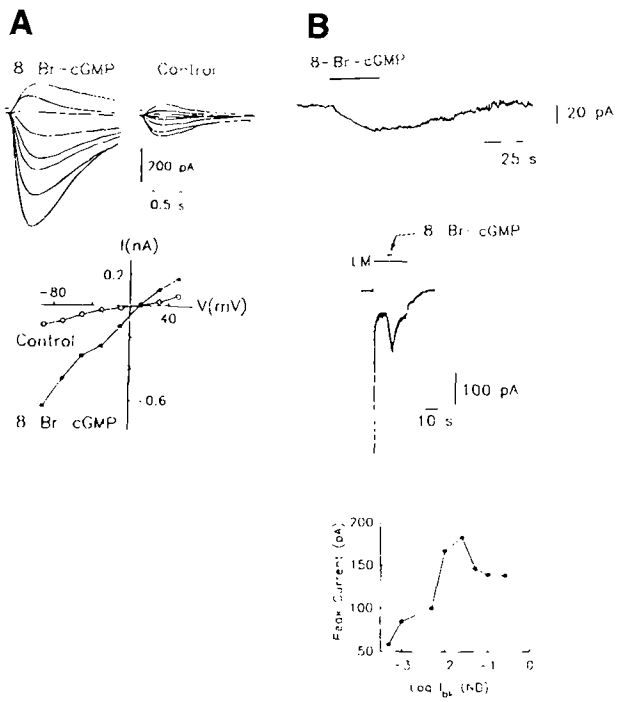

Fig. 3. Effects of exogenous cGMP in *Drosophila* photoreceptors. Membrane-permeant, nonhydrolyzable cGMP analog, 8-Br-cGMP, was introduced ("spritzed") extracellularly to *Drosophila* photoreceptors under tight-seal whole-cell voltage-clamp recording conditions (Bacigalupo et al., 1995). **(A)** Light-induced currents were enhanced by 8-Br-cGMP spritz, compared with control (normal saline) spritz, at all recording voltages. Superimposed are currents recorded at voltages between –90 and +50 mV, in steps of 20 mV. The light stimulus is shown by a bar above each family of traces. A plot of the peak currents measured at each voltage is shown in the lower panel. **(B)** 8-Br-cGMP-spritz induced currents in the dark (top trace) and in the presence of background illumination (middle trace, showing I.M, light monitor, and 8-Br-cGMP application). The magnitude of the current induced by cGMP varied with background light intensity (I_{bk}), as shown in the lower panel. Adapted from the original (Bacigalupo et al., 1995).

background illumination (Fig. 3B). In addition, inhibitors of cGMP metabolism can have profound effects on light-induced excitation in *Drosophila* (Haab et al., 1996), suggesting that endogenous cGMP influences excitation. These results are consistent with direct or indirect roles for cGMP in phototransduction, but specific mechanisms have not been elucidated. As we have noted, response enhancement and direct induction of membrane currents by cGMP are consistent with cGMP as channel ligand (Bacigalupo et al., 1995; O'Day et al., 1997).

cGMP in PI-Dependent Transduction: Models

The notion of light-dependent and Ca^{2+}-dependent cGMP metabolism supports transduction models (e.g., Shin et al., 1993; O'Day et al., 1991b, 1997) in which cytosolic Ca^{2+}, elevated by IP_3-R-mediated Ca^{2+} release from intracellular stores, modulates the activity of GC and of cGB-PDE, thereby inducing transient elevation of cGMP and channel opening. An example of such a model of cGMP-gated channel functioning in PI-dependent excitation is presented in Fig. 4.

In this model, it is assumed that both GC and cGB-PDE activity are depressed by Ca^{2+}, that the Ca^{2-}-affinity for cGB-PDE is higher than that for GC, and that the Ca^{2-} dependence of GC inhibition is steeper than that of cGB-PDE. Biochemical evidence indicating a light-dependent depression of cGB-PDE activity in *Limulus* photoreceptors (Inoue et al., 1992) supports this specific model. A simple kinetic treatment of this model illustrates that light-induced $[Ca^{2+}]$ elevation could yield a transient inward current (Fig. 4B). Here, two enzymes that have directly opposing effects are each depressed by the effects of light. The electrical response to light is governed by the precise balance of the rates of synthesis and degradation of ligand at each point in time. This balance could be tightly regulated by Ca^{2+} owing to the different Ca^{2+} sensitivities of synthesis and degradation. There would be significant cGMP synthesis and degradation in the dark, but degradation must dominate, since dark current (and therefore dark cGMP concentration [cGMP]) is zero. Light would cause a depression of cGMP turnover. The higher Ca^{2+} affinity of the cGB-PDE would yield an early inhibition of cGB-PDE, elevating [cGMP], followed by inhibition of GC, returning [cGMP] to zero as degradation again dominates. The feature of continuous

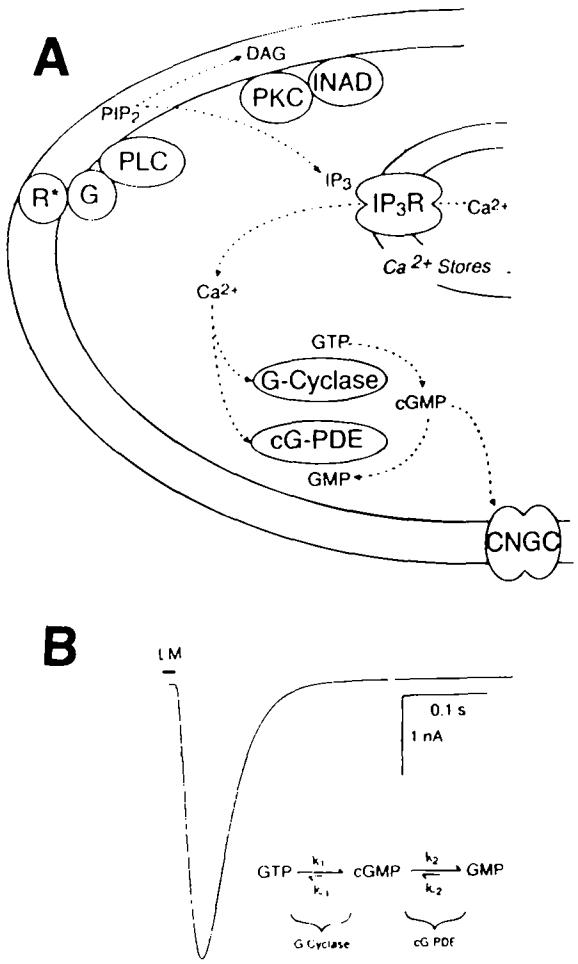

Fig. 4. **(A)** Hypothetical model depicting PI-pathway and cGMP-pathway-dependent excitation. This is designed to reconcile the following features of *Limulus* phototransduction: cGMP-gated channels; PI-pathway-dependent excitation; Ca^{2+}-dependent effects on excitation induced by cGMP-pathway inhibitors. **(B)** Calculated membrane currents based on a kinetic model, similar to that in (A), in which $[Ca^{2+}]$ elevation is sufficient to stimulate cGMP metabolism, elevating [cGMP] near the channels, inducing channel gating. In the reactions GTP $-k1\rightarrow$ cGMP $-k2\rightarrow$ GMP, we assume that k2 $\gg$ k1 and that k1 $\gg$ k-1 and k1 $\gg$ k-2. The Ca dependence of the cGB-PDE and GC-catalyzed reactions is modeled as a sigmoidal relationship between k1 and k2 and $[Ca^{2+}]$. The light-induced $[Ca^{2+}]$ rise is modeled analytically using digitized data from *Limulus* photoreceptors (O'Day and Gray-Keller, 1989); the channel gating by cGMP is modeled in a manner similar to models of the CNGC gating in vertebrate rods (Koutalos and Yau, 1996), with a Hill coefficient of 3.0.

cGMP turnover would enable the cell to respond quickly and efficiently to varying conditions of illumination. Since the light sensitivity is governed by $[Ca^{2+}]$ levels (Lisman and Brown, 1972), and since dark currents in steady state are zero regardless of sensitivity, the rate of cGMP flux should vary with sensitivity: Flux should be higher in sensitive, dark-adapted conditions than in less-sensitive light-adapted conditions.

Quantification of the effects of illumination predicted by this model would entail, among other things, a description of the Ca^{2+} sensitivity of each enzyme and the time-dependent changes in substrate concentration. Quantification is made more complicated by the fact that light-induced current flow increases $[Ca^{2+}]$ (*Drosophila* [Ranganathan et al., 1994; Hardie 1991a,b]; barnacle [Brown and Blinks, 1974; Brown et al., 1988]; scallop [Nasi, 1991; Nasi and Gomez, 1992]; *Limulus*, by shifting Na/Ca exchange equilibrium [O'Day and Gray-Keller, 1989]). Thus, by this or any other model involving multiple Ca^{2+}-dependent steps in activation, light provides a Ca^{2+}-dependent feedback loop, rendering phototransduction nonlinear.

cGMP Pathway as Modulator in Excitation

The observations that implicate the cGMP pathway in *Drosophila* transduction are consistent with a modulatory role for cGMP, rather than (or in addition to) the role as CNGC ligand, discussed above. The possibility that cGMP effects are mediated by cGMP-dependent protein phosphorylation seems reasonable, considering that cGMP-dependent protein kinase (G-kinase)-mediated phosphorylation is the principal known mechanism by which cGMP exerts physiological effects. Two G-kinase genes are reported in *Drosophila* (Kalderon and Rubin, 1989); however, the details of their expression and the identification of substrates are not yet available. In other signaling systems, G-kinase phosphorylates intracellular Ca^{2+} regulatory components, Ca^{2+} pumps, Na/Ca-exchanger, the IP_3-R, and Ca^{2+} channels (Light et al., 1990; Cornwell et al., 1991; Yoshida et al., 1991; Mene et al., 1993; Ehrlich et al., 1994;

Komalavilas and Lincoln, 1994; Sperelakis, 1994; Alioua et al., 1995; Chik et al., 1995). Thus, cGMP may influence excitation by modulating Ca^{2+} regulation, a function vital to normal excitation and adaptation (Fig. 5).

The postulated cGMP-dependent modulation might have extracellular origins. Soluble GC in other systems is activated by membrane-soluble intercellular messengers, notably nitric oxide, NO (Garthwaite, 1991), and carbon monoxide, CO (Schmidt, 1992).

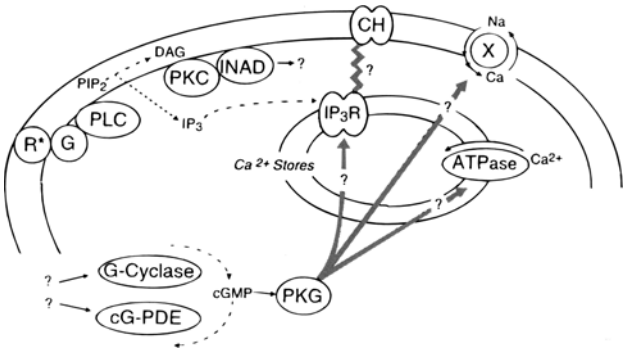

Fig. 5. Hypothetical model in which cGMP modulates light-activated PI pathway via phosphorylation of Ca^{2+} regulatory components. GC, guanylate cyclase; PDE, cGMP-phosphodiesterase; PKG, cGMP-dependent protein kinase; ATPase, Ca^{2+}-pump across SMC membrane into Ca^{2+} stores; X, Na/Ca-exchanger across plasma membrane.

Calcium Ion in Excitation

Investigating the role of Ca^{2+} in transduction has been a difficult task, in part because of the multiple effects of Ca^{2+} in excitation and adaptation, in part because some physiological features appear to respond very quickly to changes in Ca^{2+}, whereas others respond only very slowly, and in part because measuring the temporal and spatial distributions of $[Ca^{2+}]$ is difficult. In all known species, Ca^{2+} is a factor of central importance in excitation and in light adaptation, and it has been the topic of several recent reviews (Hardie and Minke, 1995; Richard et al., 1995; Waltz and Baumann, 1995; Ukhanov and Payne, 1995).

Limulus

In *Limulus* photoreceptors, Ca^{2+} is required for excitation (Bolsover and Brown, 1985), and it serves as a messenger of light adaptation (Lisman and Brown, 1972). A fundamental question is whether the release of Ca^{2+} from intracellular stores is a necessary step in excitation, i.e., whether Ca^{2+} is an obligatory intermediate in excitation. Early transduction models had suggested that Ca^{2+} release is an essential step in transduction. The evidence supporting this view includes:

1. The loss of light-induced excitation accompanying depletion of intracellular Ca^{2+} (Maaz and Stieve, 1980; Bolsover and Brown, 1985);
2. The ability of exogenously introduced IP_3 or Ca^{2+} itself to open the light-dependent channels (Payne et al., 1985; 1986);
3. The fact that Ca^{2+} release and $[Ca^{2+}]$ elevation are induced by light stimuli (Brown and Blinks, 1974) or artificially by injected IP_3 (Corson and Fein, 1987);
4. The failure of IP_3-injection to induce excitation in cells preinjected with Ca^{2+} buffer (Payne et al., 1987), and
5. Inhibition of responses to dim illumination in Ca^{2+}-buffered cells (Lisman and Brown, 1975b).

However, since excitation by bright light is robust in Ca^{2+}-buffered cells (Lisman and Brown, 1975b) and in cells preinjected with Ca^{2+}-release blocker, heparin (Frank and Fein, 1991), it would seem that light-induced excitation can proceed quite well under conditions designed to block release. This argument is equivocal, however, because it is difficult to determine whether either method to prevent Ca^{2+} elevation does so on the time-course and to the extent required to test the model. Heparin has multiple intracellular effects (Palczewski et al., 1991) that can be difficult to separate from one another, and the effects of Ca^{2+} buffers can be difficult to evaluate. If buffering kinetics are too slow, the buffer would fail to prevent a fast light-induced $[Ca^{2+}]$ elevation. If buffering capacity is too small, small IP_3- or dim light-induced $[Ca^{2+}]$ elevation may be successfully blocked, but a sizable light-induced Ca^{2+}-release may overwhelm the buffer. Moreover,

one cannot determine whether the severe inhibition of excitation by excessive buffer (Maaz and Stieve, 1980; Bolsover and Brown, 1985) is owing to blockage of $[Ca^{2+}]$ elevation, as intended, or inhibition of other Ca^{2+}-requiring reactions in excitation.

Recent work clarified this issue in *Limulus* (Shin et al., 1993), asking whether light-induced excitation would be impeded by inhibiting Ca^{2+} release without lowering $[Ca^{2+}]$ below physiological levels. For a given $[Ca^{2+}]$ (determined by Ca^{2+}/Ca^{2+} buffer calculation), the activation rate of excitation slowed as more buffer was injected. Furthermore, the slowing of the activation rate varied smoothly as the amount of buffer injected increased. Since $[Ca^{2+}]$ was stabilized, this result suggests that the physiological effects of increasing buffer concentration were the result of its enhanced ability to prevent $[Ca^{2+}]$ elevation. This result suggests that excitation requires Ca^{2+} elevation in *Limulus*.

There are obstacles, however, to a clear interpretation of experiments that make use of intracellular buffers. First, it is often difficult to ascribe effects of $[Ca^{2+}]$ changes to a particular physiologically measured feature of excitation (e.g., response activation and deactivation kinetics, and adaptation and recovery kinetics), since these features possess complex Ca^{2+} dependencies. Various transduction components possess different, even multiple, Ca^{2+} dependencies imparting to cells very complex physiological behavior (e.g., Payne et al., 1988; Ehrlich, 1995). Thus, for example, the observation that prolonged low Ca^{2+} exposure diminishes test flash responses (Lisman, 1976) does not in itself justify the conclusion that activation processes are thereby inhibited. Rather, upstream activation processes may proceed at nearly normal rates, whereas incompletely terminated downstream events may mask the effects of activation. The fact that low Ca^{2+} exposure dramatically slows termination of the light-induced excitation and Ca^{2+} release (O'Day and Johnson, 1992) underscores this point.

Second, because of the variety of Ca^{2+}-dependent components in transduction and the differences in the time scales over which they respond to changes in $[Ca^{2+}]$, the Ca^{2+} dependence of a specific physiological feature (e.g., light adaptation) may vary. Thus, experimental manipulation of $[Ca^{2+}]$ may yield results that seem paradoxical if one assumes a unique Ca^{2+} dependence for any particular such feature. This may be particularly true under the extraphysiological conditions required by experiment. For example, in *Limulus*, Ca^{2+} deprivation uncouples light adaptation from its trigger, light-induced Ca^{2+} release (Brown and Blinks, 1974), and this uncoupling persists even when light elevates $[Ca^{2+}]$ to well within physiological range for several minutes (Srebro and Behbehani, 1974; Martinez and Srebro, 1976; O'Day and Johson, 1992). A mechanistic example might be the physiological effects of the gradual dephosphorylation of a key enzyme following its rapid, Ca^{2+}-dependent phosphorylation.

Third, experimental manipulation of Ca^{2+} affects Ca^{2+}-regulation processes, which themselves have electrophysiological effects that may be difficult to take into account. Several processes underlie Ca^{2+}-regulation, and each is affected differently by experimental manipulation of $[Ca^{2+}]$, making it difficult simply to estimate $[Ca^{2+}]$ or to assess physiological effects at any particular time. This problem may be minimized experimentally by stringent control of the intracellular environment by whole-cell pipet. However, in many cells (notably those with highly invaginated subcellular morphology [Hardie and Minke, 1995]), the extent to which agents can be successfully introduced to transducing regions by internal dialysis is quite uncertain.

Drosophila

The importance of Ca^{2+} in excitation in *Drosophila* phototransduction has been demonstrated (Hardie et al., 1993). Ca^{2+} activates both positive and negative feedback processes (Hardie, 1991; Hardie et al., 1993). Nonetheless, the underlying roles for Ca^{2+} in transduction remain unclear. The questions whether Ca^{2+} is an

obligatory intermediate, whether Ca^{2+} release from stores is necessary, and whether SOC function in excitation remain to be answered.

Examining whether Ca^{2+} is sufficient for excitation has been difficult. Rapid artificial release of intracellular Ca^{2+} by caged Ca^{2+} techniques failed to induce excitation (Hardie, 1995). In these experiments, it seems likely that the released Ca^{2+} reached the transducing regions, since under the same conditions, caged Ca^{2+} release was sufficient to inactivate the light-induced currents. This suggests Ca^{2+} alone is insufficient to induce excitation. Although results with intracellular dialysis of Ca^{2+} buffers by whole-cell pipet are consistent with a Ca^{2+} requirement for excitation, the uncertainties surrounding the use of buffers render the results difficult to interpret (Hardie, 1995).

Examination of Ca^{2+} release from intracellular stores has been difficult in execution and in interpretation (Peretz et al., 1994). Ca^{2+}-uptake inhibitor, thapsigargin, causes elevation of Ca, suggesting the presence of mobilizable stores (Ranganathan et al., 1994), and submicrovillar cisternae have been identified and proposed to be the light-labile Ca^{2+} stores (Suzuki and Hirogawa, 1992). However, since light-induced Ca^{2+} elevation has not been seen in low-Ca^{2+} conditions (Peretz et al., 1994; Ranganathan et al., 1994; Hardie, 1996b), it has been suggested that light-induced intracellular Ca^{2+} release is insignificant in transduction (Hardie and Minke 1995; Zuker, 1996). Such a conclusion seems premature, however, since intracellular Ca^{2+} release may require extracellular Ca^{2+}, if low-Ca^{2+} exposure quickly depletes light-labile stores. This possibility seems reasonable considering the rise in $[Ca^{2+}]$ induced simply by blocking Ca^{2+} uptake (Ranganathan et al., 1994).

Localization of Transduction Elements

The cellular transduction components underlying excitation are very highly localized (Fein and Lisman, 1975; Walz, 1982; Colman and Chamberlain, 1982; Stern et al., 1982; Payne et al., 1988; Nasi, 1991; Suzuki and Hirosawa, 1992) in regions that include the microvillar plasma and SMC. This gives rise to specialized microenvironments that support excitation. The proximity of these two membrane systems and the minuscule volume they delimit seem ideal for rapid and efficient performance of the multistep cascades that involve cytosolic and intramembranous diffusion of messenger molecules. Such localization may account for the very brief response latencies characteristic of invertebrate photoreceptors.

Electrophysiological experiments illustrate localization in *Limulus* photoreceptors (Fein and Lisman, 1975; Johnson et al., 1986). Intracellular injection of IP_3, of Ca^{2+} and of cGMP gives rise to excitation, but only when done in the transducing regions (Johnson et al., 1986). CGMP elicited conductance changes only when injected at discrete "hot spots" (Johnson et al., 1986) near the rhabdomeral plasma membrane. The idea that each microenvironment in the rhabdomeral region contains all elements necessary for transduction comes from recordings from "photoballs." These small vesicles pulled from the rhabdomeral lobe at the tip of a microelectrode respond to light with a receptor potential waveform nearly identical to the whole-cell response, differing by a scaling factor (Bacigalupo and Johnson, 1992). Photoballs have been observed in *Drosophila* as well as *Limulus* (Armstrong and O'Day, unpublished).

Molecular components of transduction in *Drosophila* that are localized to rhabdomeres include TRP and TRPL (Pollock et al., 1995; Lisman and Brown, 1972), G_q-protein (Lee et al., 1994), rhodopsin (O'Tousa et al., 1985; Suzuki and Hirosawa, 1991; Leonard et al., 1992), and PI-transfer protein, RDGB (Suzuki and Hirosawa, 1994). The fact that several polypeptides important in transduction have been shown to coimmunoprecipitate in *Calliphora* suggests (Heber et al., 1996) the presence of functioning multicomponent complexes. These considerations illustrate that rhabdomeres are modular assemblies of localized transducing elements.

Light-Activated Ion Conductances in Invertebrate Phototransduction

The identity of the light-activated channels in invertebrate phototransduction is a matter of great current interest. In *Limulus* photoreceptors, and in some scallop photoreceptors, it is clear that there is a cGMP-gated channel activated in the course of excitation. Whether other channel types are involved remains unclear (Nagy, 1991). In *Drosophila*, three retinally expressed genes for ion channel polypeptides have been described, and each remains a candidate for a light-activated channel component: *trp*, *trp-l*, and *DmCNGC* (Phillips et al., 1992; Baumann et al., 1994; Niemeyer et al., 1996).

Light Responses

Light-induced currents from invertebrate photoreceptors were first reported from *Limulus* ventral photoreceptors (Millecchia and Mauro, 1969) with two-microelectrode voltage clamp. Following a latency of tens of milliseconds, the response to a prolonged light stimulus rises rapidly, reaching a peak, and then quickly declines to a plateau current lasting until the end of illumination (Fig. 6). Following stimulus termination, the current falls to baseline. The decline from peak to plateau is owing to light adaptation caused by Ca^{2+} elevation (Lisman and Brown, 1975b). The response shows sharp outward rectification at positive voltages. Similar features are observed in *Drosophila* photoreceptors (Hardie, 1991; Ranganathan et al., 1991) with whole-cell voltage clamp. Also similar are scallop and squid photoreceptors (Cornwall and Gorman, 1983a,b, Nasi and Gomez, 1992; Gomez and Nasi, 1994), although the plateau current is not maintained (Fig. 6).

There is much physiological evidence for the involvement of TRP and TRPL as light-activated channels in *Drosophila* transduction. In null mutants of *trp*, the response to a prolonged light lacks the plateau phase. After the transient peak, the current falls completely to baseline, producing a transient response. The characteristic *trp* response can be mimicked in wild-type by Ca^{2+}-channel blocker, La^{3+}

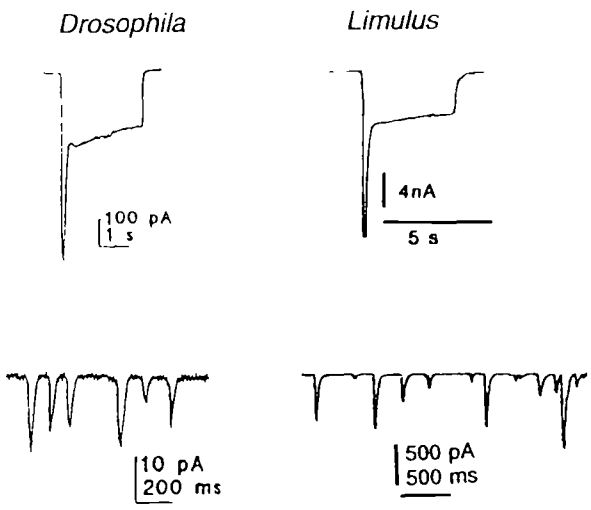

Fig. 6. Comparison of light responses from *Drosophila* and *Limulus* photoreceptors. **(Top)** Voltage-clamp responses to bright, prolonged illumination, showing transient and plateau phases. **(Bottom)** Quantum bumps recorded in voltage clamp. Data for *Drosophila* (Hardie and Minke, 1992) and *Limulus* are taken under similar experimental conditions. *Limulus* recording conditions are similar to those previously reported (Johnson and O'Day, 1995).

(Minke, 1982; Suss et al., 1989). This evidence suggested that *trp* codes for a Ca^{2+}-selective channel (Hardie and Minke 1992). If this is the case, however, it is puzzling why lowering extracellular Ca^{2+} fails to mimic the *trp* phenotype.

The notion that TRP and TRPL compose different light-dependent channels is supported by mutational analysis. The *trp* phenotype can be mimicked by La^{3+} in three additional ways. The ionic selectivity of the light-activated conductance is dramatically altered (*see* Ionic selectivities); the reversal voltage of the light response, E_{hv}, in the mutant is shifted to voltages negative of wild-type E_{hv}, and the complex current waveform near E_{hv} (multiphasic reversal; *see* Multiple Light Dependent Channels in Excitation) disappears. These observations support the notion (Hardie and Minke, 1994) that multiple light-activated channels contribute to excitation. Furthermore, the fact that *trp/trpl* double mutants exhibit only negligible light

responses (Niemeyer et al., 1996) lends considerable support to the notion that these are the only light-activated channels (assuming there are no significant pleiotropic effects in the double mutants).

Another possibility is that TRP normally serves as one subunit of a heterotetrameric channel, perhaps with TRPL as another, and that in *trp* mutants, other subunits can aggregate into abnormal but functional tetrameric channels. In this case, the physiological phenotype of *trp* mutants would arise from functional differences between normal and abnormal channel tetramers.

TRP and TRPL may operate as SOC channels. Heterologous expression of TRP and TRPL can give rise to Ca^{2+}-store-depletion-induced Ca^{2+} currents with thapsigargin (Vaca et al., 1994; Dong et al., 1995; Hu and Schilling, 1995; Gillo et al., 1996). The ability of Ca^{2+}-store depletion to excite *Drosophila* photoreceptors is supported by the observation (Hardie and Minke, 1995) that channel opening results from introduction of ionomycin, which drains Ca^{2+}-stores. However, thapsigargin did not induce significant membrane currents in whole-cell recordings from *Drosophila* photoreceptors (Ranganathan et al., 1994), despite significant $[Ca^{2+}]$ elevation. It may be that Ca^{2+}-stores coupled to the SOCs in the native preparation are less sensitive to thapsigargin than are those in the heterologous expression systems.

The overall results are consistent with roles of TRP and TRPL as SOC channel subunits, but other roles for TRP and TRPL and contributions from other channels are also possible. For instance, the data are consistent with the idea that TRP, TRPL, or both are required for the normal function of an additional light-dependent channel.

Single-Photon Responses

Invertebrate photoreceptors, like vertebrate rods, respond to single-photon absorptions (Fuortes and Yeandle, 1964). The macroscopic light-dependent current can be analyzed as a summation of numerous individual single-photon responses, called quantum bumps (Dodge et al., 1968). Quantum bumps have been observed in many species (Fuortes and Yeandle, 1964; Stieve, 1985; Hardie, 1991; Nasi, 1991; Nasi and Gomez, 1992). In *Limulus*, quantum bump amplitudes can reach 1 nA, whereas in *Drosophila*, they are typically 4–10 pA (Hardie, 1991). Thousands of light-dependent ion channel openings contribute to each quantum bump (Wong, 1978; Bacigalupo and Lisman, 1983). Their analysis has revealed several fundamental properties of phototransduction (e.g., Wong et al., 1982; Lisman et al., 1992; Scott et al., 1995). Adaptation takes place on the quantum bump level (Srebro and Behbehani, 1972; Wong et al., 1982), and as a result, bumps are too small to be distinguished in light-adapted cells (Adolph, 1964; Dodge et al., 1968). Underlying the fundamental physiological unit of excitation, the process of quantum bump generation is of great interest, but is not as yet understood.

Ionic Selectivities

Ionic selectivities of the light-dependent conductances vary widely among invertebrate species. In rhabdomeric photoreceptors of *Limulus* and scallops, the Na^+ permeability is roughly twice that of K^+, and Ca^{2+} permeability is very low (Brown and Mote, 1974; Gomez and Nasi, 1995). In contrast, the light-dependent conductances reported in *Drosophila* photoreceptors are principally Ca^{2+}-permeable, with respective P_{Ca}/P_{Na} values of about 40 (in wild-type) and 5 (in *trp* mutants) for the two putative channels (Hardie, 1991; Hardie and Minke, 1992). The light-dependent conductance from barnacle photoreceptors also exhibit a Ca^{2+} permeability (Brown et al., 1970).

The selectivities of the light-activated conductances have important consequences for understanding the orchestration of transduction. The light-induced $[Ca^{2+}]$ elevation in cells with low Ca^{2+} permeability (e.g., *Limulus*) arises principally from release from intracellular stores (Brown and Blinks, 1974). In cells with highly Ca^{2+}-permeable light-activated

channels, however (e.g., *Drosophila*,) the principal source for $[Ca^{2+}]$ elevation is likely through these channels themselves (however, *see* Calcium Ion in Excitation). In contrast to the depolarizing rhabdomeral photoreceptors, the light-dependent conductance of the hyperpolarizing, ciliary scallop photoreceptors is K^+-selective (Gomez and Nasi, 1994).

Unitary Conductances

Characteristics of ion channels in excitation can be determined from cell-attached patch-clamp recordings (Fig. 7). In *Limulus*, two light-dependent unitary (single-channel) events are generally observed, revealing respective conductances of 30–40 and 12–15 pS, with mean open times of 1.3 and 2.7 ms, respectively (Bacigalupo and Lisman, 1983; 1984; Bacigalupo et al., 1986; Nagy and Stieve, 1990; Johson et al., 1991; Nagy, 1991). A smaller, as yet uncharacterized component has also been detected (Nagy, 1991; Johnson et al., 1991).

Rhabdomeral photoreceptors from scallop exhibit unitary events of 48 and 17 pS (Nasi and Gomez, 1992). Their size and mean open time resemble those of *Limulus*, but their light-induced currents differ in activation kinetics and voltage dependence (Bacigalupo and Lisman, 1983; Johson et al., 1991; Nasi and Gomez, 1992). In addition, channel density appears to be 25-fold higher in scallop than in *Limulus* (Nasi and Gomez, 1992). Ciliary photoreceptors from scallop have a 27 pS, cGMP-gated K^+-conductance (Gomez and Nasi, 1995); however, detailed analysis of this channel is not yet available.

Unitary recordings of light-dependent channels from *Drosophila* have not been made to date, probably because of their inaccessibility to patch electrodes (Hardie and Minke, 1995; Bacigalupo and O'Day, 1996; O'Day et al., 1997). Nonetheless, an estimate of their unitary conductance has been made, based on the suggestion that a sustained inward "rundown current" (RDC), which often develops in whole-cell recordings, results from spontaneous activation of light-activated channels (Hardie and Minke, 1994). Values of ~3 and ~30 pS for the two putative channels are calculated from the variance/mean ratio of the RDC noise. Power spectra suggest mean open times of 0.2 and 2 ms, respectively.

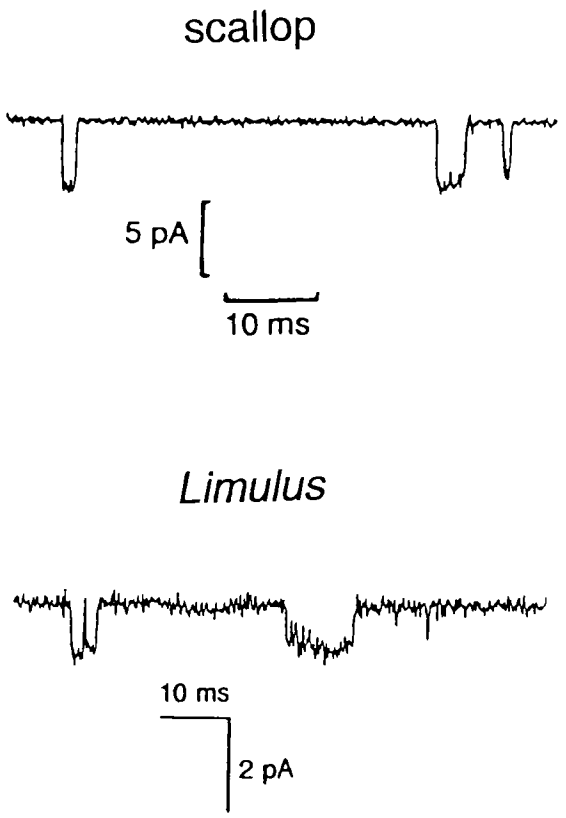

Fig. 7. Single-channel currents recorded in cell-attached patch configuration from light-sensitive regions of scallop (Nasi and Gomez, 1992) and *Limulus* (Bacigalupo and Lisman, 1983) photoreceptors. Experimental conditions are described in the original articles.

Different Conductance States vs Separate Channels

For *Limulus* and scallop photoreceptors, there is controversy over whether the different unitary conductances reflect two different channels gated by distinct internal transmitters (Nagy, 1990, 1991; Nasi and Gomez, 1992) or different subconductance states of a single type of channel (Johnson et al., 1991).

Supporting the latter idea in *Limulus* are the observations that both unitary currents have the

same reversal potential and that the mean open times of both single-channel events rise dramatically at more positive voltages (Johnson et al., 1991). More importantly, extensive examination of single-channel records reveal that large and small events never occur simultaneously, suggesting that they are not independent (Fig. 8A). This is inconsistent with the notion of two distinct channels, since statistical analysis predicts many such simultaneous events within the recording time examined. Further statistical analysis indicates that direct transitions from one open current level to the other without visiting the baseline should be extremely rare if the events are independent (Fig. 8A). However, such transitions occur frequently, as they should if the two events are related.

Interesting evidence supporting the opposite view, based on differences in deactivation kinetics between the two event types, has been reported (Nagy, 1990). However, such a difference would arise from a single channel type with two conductance states differentially sensitive to agonist (Fig. 8B). This issue remains to be explored.

The evidence supports the notion that the two most commonly observed events are substates of a unique channel type. Nonetheless, the possibility of additional light-dependent channel species is not ruled out. Such putative channels may be either too small to be clearly resolved or hidden within membrane infoldings (Stern et al., 1982; Calman and Chamberlain, 1982) inaccessible to the patch pipet.

Multiple Light-Dependent Channels in Excitation

Measurements of macroscopic currents support the view that invertebrate photoreceptors employ multiple classes of light-dependent channels. Key evidence includes the very complex waveforms of light-induced currents, particularly near the reversal potential, E_{hv}. In several species, light-induced currents can have multiple components that reverse at different potentials, creating a situation called "multiphasic reversal," where

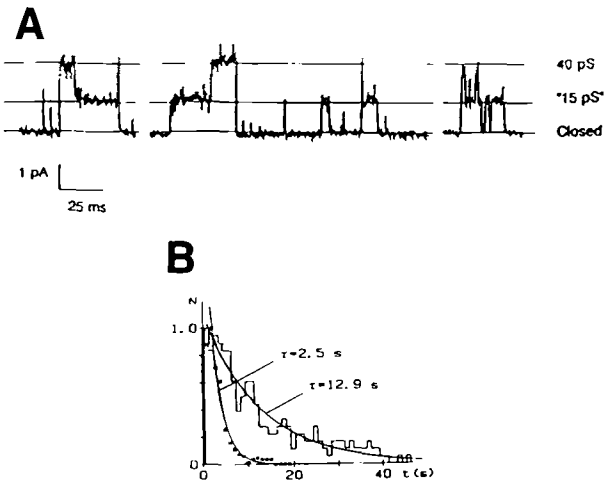

Fig. 8. Analysis of unitary events from *Limulus* photoreceptors. **(A)** Single-channel currents (Johnson et al., 1991) illustrating direct transitions between two conductance levels, 15 and 40 pS. **(B)** Plot of normalized estimate of number of open channels of the two putative types during deactivation, illustrating a difference in deactivation kinetics (Nagy, 1991).

there is no unique reversal potential (Nasi, 1991; Nagy, 1991; Hardie and Minke, 1992; Hardie et al., 1993).

A unique reversal potential generally, but not always, indicates a single class of light-dependent channel, whereas multiphasic reversal suggests multiple channel types. Multiphasic reversal, however, may arise from other sources than multiple species of light-activated channels (O'Day et al., 1993, 1997). These sources may be physiological, such as a contribution to the light-induced current from Ca^{2+}-induced acceleration of electrogenic Na/Ca exchange (Armon and Minke, 1983; O'Day and Gray-Keller, 1989; O'Day et al., 1991; Hardie and Minke, 1995; Hardie, 1995, 1996b). They may be nonphysiological, e.g., momentary clamp failure owing to membrane rearrangement induced by bright lights. In *Limulus* photoreceptors, a transient and extensive rearrangement of rhabdomeral membranes is induced by adapting illumination (Chamberlain and Barlow, 1979). In this regard, is interesting to note that it is common for a bright flash to cause an impaling microelectrode to pop suddenly

and spontaneously out of a *Limulus* photoreceptor (O'Day, unpublished). In addition, multiphasic reversal might arise in some cases from the combination of high Ca^{2+} selectivity of the light-dependent channel and large light-induced elevation. In such cases, there would be a shift in E_{hv} during the response itself, causing a shift $[Ca^{2+}]$ in the current near E_{hv}, assuming the $[Ca^{2+}]$ elevation could be sensed near the plasma membrane channels. This is consistent with the observation that multiphasic reversal is often labile, very intensity-dependent, and very Ca^{2+}-dependent.

The presence of multiple channel species could, in principle, be resolved directly by single-channel recordings, discussed above, but the issue has not yet been definitively addressed.

Channel Polypeptides

TRP and TRPL

The TRP and TRPL polypeptides each have six potential transmembrane segments and resemble voltage-gated Ca^{2+} channels, but they lack the repeated positive residues characteristic of voltage-gated channels in the S4 region of the primary sequence (Montell and Rubin, 1989; Phillips et al., 1992). TRP has an extra C-terminal "tail" lacking in TRPL. If these polypeptides operate as SOC components, the TRP tail may be involved in sensing Ca^{2+}-store depletion, since heterologous expression studies suggest that the tail confers thapsigargin sensitivity to channel gating (Sinkins et al., 1996).

DmCNGC

Evidence strongly suggests that a cGMP-activated channel is expressed in *Drosophila* retina. When cloned DmCNGC polypeptide is expressed heterologously, the resulting conductance exhibits cGMP sensitivity with a $K_{1/2}$ of 13 μM (Baumann et al., 1994), comparable with those of *Limulus* photoreceptors (Bacigalupo et al., 1991), vertebrate photoreceptors (Fesenko et al., 1985; Haynes and Yau, 1985); and olfactory receptors (Nakamura and Gold, 1987). The conductance is Ca^{2+}-selective with P_{Ca}/P_{Na} and P_{Ca}/P_K of about 30, contrasting with that of *Limulus* and scallop, but consistent with the macroscopic data from *Drosophila*. Like the vertebrate CNGC, it is blocked by extracellular Ca^{2+}. Single-channel recordings reveal a 39-pS channel with a distinctly cGMP-dependent mean open time, very similar to that of the larger open conductance state of *Limulus* and scallop rhabdomeral light-dependent channels (40 and 47 pS; Bacigalupo and Lisman, 1983; Nasi and Gomez, 1992). Although DmCNGC channels are localized in the *Drosophila* retina, information about their precise locations and direct evidence of a physiological role in phototransduction is lacking.

Conclusion

The available evidence illustrates the importance of intracellular second messengers, IP_3, cGMP, and Ca^{2+} in invertebrate excitation. However, many issues remain to be resolved to reach an understanding of the complete excitation pathway for any species. A description of the morphology and the biochemistry of photoreceptor transducing microenvironments and their architecture will be indispensable to a full understanding of phototransduction.

Acknowledgments

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