Neuron, Vol. 18, 881–887, June, 1997, Copyright ©1997 by Cell Press

$InsP_3$ Receptor Is Essential for Growth and Differentiation but Not for Vision in Drosophila

Jairaj K. Acharya, Kees Jalink, Robert W. Hardy, Volker Hartenstein,* and Charles S. Zuker
Howard Hughes Medical Institute
and Departments of Biology and Neuroscience
University of California at San Diego
La Jolla, CA 92093
*Department of Biology
University of California at Los Angeles
Los Angeles, CA 90024

Summary

Phospholipase C (PLC) is the focal point for two major signal transduction pathways: one initiated by G protein-coupled receptors and the other by tyrosine kinase receptors. Active PLC hydrolyzes phosphatidylinositol bisphosphate (PIP_2) into the two second messengers inositol 1,4,5-trisphosphate ($InsP_3$) and diacyl glycerol (DAG). DAG activates protein kinase C, and $InsP_3$ mobilizes calcium from intracellular stores via the $InsP_3$ receptor. Changes in $[Ca^{2+}]_i$ regulate the function of a wide range of target proteins, including ion channels, kinases, phosphatases, proteases, and transcription factors (Berridge, 1993). In the mouse, there are three $InsP_3R$ genes, and type 1 $InsP_3R$ mutants display ataxia and epileptic seizures (Matsumoto et al., 1996). In Drosophila, only one $InsP_3$ receptor ($InsP_3R$) gene is known, and it is expressed ubiquitously throughout development (Hasan and Rosbash, 1992; Yoshikawa et al., 1992; Raghu and Hasan, 1995). Here, we characterize Drosophila $InsP_3R$ mutants and demonstrate that the $InsP_3R$ is essential for embryonic and larval development. Interestingly, maternal $InsP_3R$ mRNA is sufficient for progression through the embryonic stages, but larval organs show asynchronous and defective cell divisions, and imaginal discs arrest early and fail to differentiate. We also generated adult mosaic animals and demonstrate that phototransduction, a model PLC pathway thought to require $InsP_3R$, does not require $InsP_3R$ for signaling.

Introduction

Phosphoinositide-mediated signaling pathways are a ubiquitous mode of intracellular signal transduction in eukaryotic cells. The signals from many different receptors, including a variety of tyrosine kinase and G protein-coupled seven transmembrane receptors, converge on the activation of phospholipase C (PLC; Fain, 1990; Rhee and Choi, 1992; Berridge, 1993). PLC catalyzes the hydrolysis of the minor membrane phospholipid phosphatidylinositol-4,5-bisphosphate (PIP_2) into the second messengers inositol trisphosphate ($InsP_3$) and diacylglycerol (DAG; Fain, 1990; Rhee and Choi, 1992; Berridge, 1993). DAG activates members of the protein kinase C (PKC) family of proteins (Nishizuka, 1992), and $InsP_3$ mobilizes calcium from internal stores, which affect and modulate many cellular processes (Tsien and Tsien, 1990), including fertilization, development, and basic physiological signaling events. The molecular mechanisms of Ca^{2+} action are very diverse, and include the regulation of kinases and phosphatases to control protein phosphorylation; the formation of Ca^{2+}-calmodulin complexes, which regulate the activity of many proteins; and direct binding to calcium sensitive cellular targets. In many cases, Ca^{2+} binding to specific regulatory sites triggers release of additional stored Ca^{2+}, leading to regenerative waves and oscillations of Ca^{2+} (Cheng et al., 1993). Because of its fundamental role in cellular homeostasis, many aspects of Ca^{2+} regulation and spatiotemporal dynamics have been intensively studied. Of particular interest have been the mechanisms of Ca^{2+} release from internal stores via the $InsP_3$ receptor, which functions as the principal gate for the release of intracellular Ca^{2+} in excitable and nonexcitable cells (Berridge, 1993). In mammals, there are at least three different $InsP_3R$ genes, each of which, in turn, is spliced into a number of isoforms. Different cells express diferent combinations of receptors, with some expressing all three genes (Mikoshiba et al., 1994). In Drosophila, there is a single $InsP_3$ receptor. This provides an ideal system for the study of $InsP_3$ receptor function in processes as diverse as differentiation and cell physiology, without problems associated with functional redundancy or isoform-mediated compensation. We now demonstrate that the $InsP_3R$ is essential for cell growth and differentiation, including the earliest events, like maturation of the oocyte. We also examined the role of $InsP_3$ receptor in phototransduction, a model phosphoinositide-mediated signaling cascade. We use genetic mosaic strategies to generate adult, viable animals lacking $InsP_3R$ in photoreceptor cells and demonstrate, contrary to current models of photoreceptor cell function, that the $InsP_3R$ does not play a role in the activation of the light response.

Results and Discussion

The Drosophila $InsP_3R$ was previously cloned by homology to the mammalian genes (Hasan and Robash, 1992; Yoshikawa et al., 1992) and shown to function as an $InsP_3R$ in tissue culture cells (Yokishawa et al., 1992). The Drosophila gene is expressed throughout development in all tissues and is especially enriched in the larval head region, the adult brain, and the adult sensory organs, including antennae and eyes (Hasan and Rosbash, 1992; Yokashiwa et al., 1992; Raghu and Hasan, 1995).

We mapped the $InsP_3R$ gene to the third chromosome at position 83A4–5. A 60 kb chromosomal walk in this interval detected two transcriptional units: $InsP_3R$ (*$InsP_3R$*) and NMDA receptor (*Nmdar*; Ultsch et al., 1993; Figure 1). To generate mutants in the *Ins_3R*, we identified a larval lethal P-element insertion (j5B4) 5 kb upstream of the $InsP_3R$ initiator methionine codon. Precise excision of this P-element reverted the lethality, indicating that P-insertion was responsible for the lethal phenotype (data not shown). To isolate mutants with deletions in

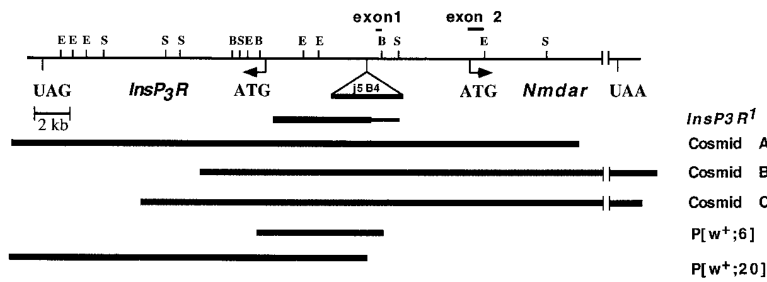


Figure 1. The *$InsP_3R1$* Flies Lack $InsP_3R$ Function

Shown is a 60 kb genomic interval indicating the location of *$InsP_3R$*, *Nmdar*, and j5B4 (P-element). *$InsP_3R$* and *Nmdar* refer to the Inositol 1,4,5-trisphosphate receptor (Yoshikawa et al., 1992) and the N-methyl-D-aspartate R1 receptor (Ultsch et al., 1993), respectively. The genes were mapped by sequence analysis and PCR amplification. Our sequence analysis revealed that the first 670 of the 842-nucleotide untranslated leader of the published *$InsP_3R$* cDNA sequence (Accession # D90403; Yoshikawa et al., 1992) codes for the Drosophila myosin light chain kinase, and likely reflects a cloning artifact in the original report. Instead, the Drosophila *$InsP_3R$* has a 327 bp untranslated leader. The *$InsP_3R1$* allele deletes all of the region between the P-insertion and position +139 of *$InsP_3R$*. This mutant was identified first by genomic Southern and subsequently confirmed by sequence analysis of the mutant chromosome. Cosmid-A, containing full-length *$InsP_3R$* and upstream elements (including partial coding sequence of *Nmdar*) rescues the lethality associated with j5B4 and *$InsP_3R^1$*. Cosmids -B and -C, coding for the entire *Nmdar*, do not rescue the mutants. The 6 kb Bam H1 genomic fragment (P[w^+; 6]), containing sequences between the *$InsP_3R$* and the *Nmdar*, also fails to rescue the mutants. However, a 20 kb construct, containing only the *$InsP_3R$* and 5 kb upstream sequences (P[w^+; 20]), rescues the lethality of *$InsP_3R1$* mutants.

$InsP_3R$, we used a strategy that relied on the high frequency of imprecise excisions following transposase-mediated mobilization of P-elements (Ashburner, 1989). We generated 1000 independent P-element jump-out lines by scoring for the loss of eye color (the P-element was marked with the *$white^+$* gene), and we screened by Southern blot analysis for imprecise excision events that deleted *$InsP_3R$* but not *Nmdar* sequences. We recovered 18 deletions that eliminated DNA from this region, one of which, *$InsP_3R^1$*, was specific for the $InsP_3R$ gene. *$InsP_3R^1$* is a larval lethal, 4.8 kb deletion mutant missing all of the DNA between the original P-element insertion site and nucleotide +139 in the *$InsP_3R$* transcriptional unit (i.e., all of the promoter sequences and up to 139 nucleotides downstream of the transcription start site). To demonstrate the specificty of this mutation, we performed P-element-mediated germ line transformations with a variety of cosmid constructs from this genomic region. The *$InsP_3R^1$* deletion eliminates $InsP_3R$ function, but not *Nmdar* or any other genes, because the lethality could be fully rescued with a cosmid clone containing the entire *$InsP_3R$* gene, but not with constructs containing the *Nmdar* gene or DNA sequences in between the two genes (Figure 1).

Multiple $InsP_3$ receptors have been identified in mammals (Mignery et al., 1989; De-Camilli et al., 1990; Mignery and Sudhof, 1990; Sudhof et al., 1991; Fischer et al., 1994; Furuichi and Mikoshiba, 1995; Khan et al., 1996), but only one of these, $InsP_3R1$ in mice, has been mutated to date. $InsP_3R1$ is the major neuronal member, expressed primarily in cerebellar Purkinje cells. $InsP_3R1$ mutants are viable and have no obvious electrophysiological deficiencies in Purkinje cell function, likely due to the presence of the other receptor isoforms (Matsumoto et al., 1996). In Drosophila, there is a single receptor gene; thus, an $InsP_3R$ mutant may provide fundamental insight into the requirement of $InsP_3R$ in processes as diverse as cell growth, differentiation, development, and cell physiology (although it is not possible to completely rule out the absence of a distantly related receptor until the characterization of the Drosophila genome is completed, repeated attempts to isolate other $InsP_3$ receptors have consistently failed). Indeed, homozygous *$InsP_3R^1$* mutants display extraordinary growth defects. Larvae hatch normally and initially show healthy behavior. However, $InsP_3R$ mutants fail to grow beyond the second larval instar and never metamorphose (Figure 2). Heterozygous larvae (Figure 2, right column), like wild-type controls, grow through each of the larval stages and develop normally. In contrast, the mutant larvae remain halted at the second larval stage even though they live longer than 7 days, leading to dramatic growth differences between control and mutant animals.

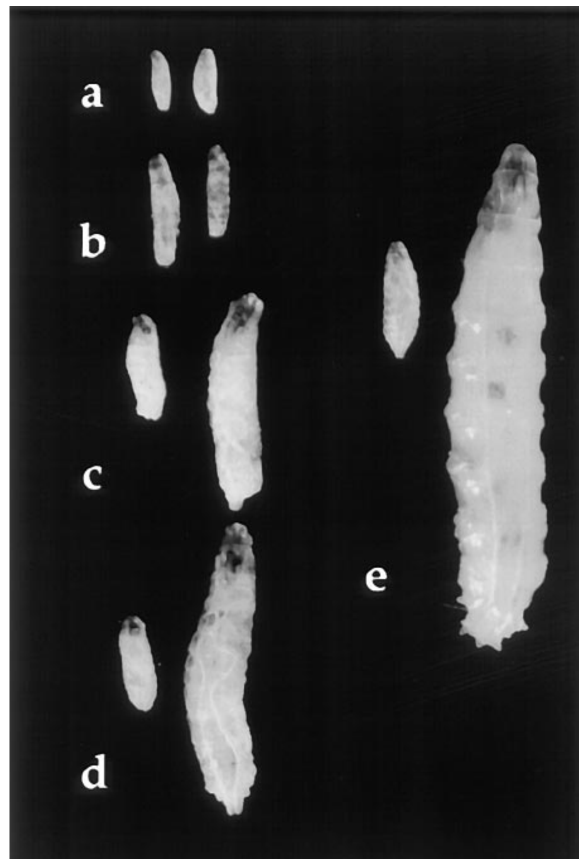


Figure 2. *$InsP_3R1$* Mutants Are Arrested at Second Instar

The figure shows *$InsP_3R1$/$InsP_3R1$* on the left and heterozygous *$InsP_3R^1$/+* larvae on the right at (a) first, (b) second, (c) early-, (d) mid-, and (e) late third instar stages.

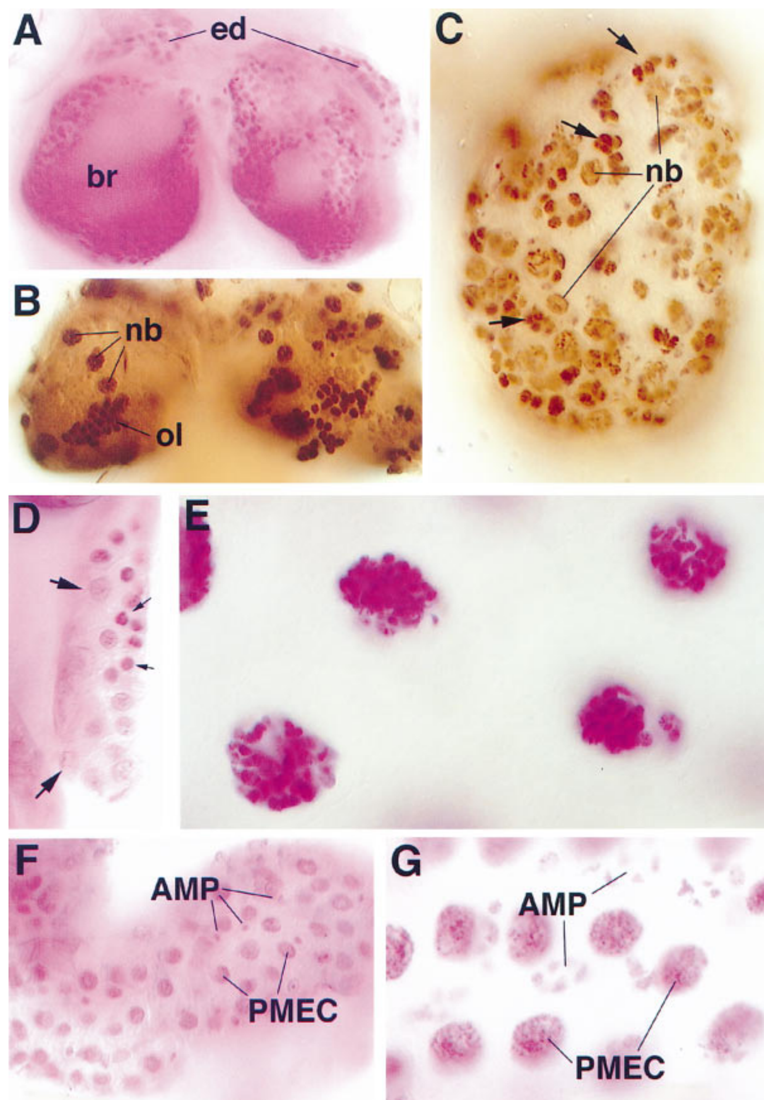


Figure 3. *$InsP_3R^1$* Larvae Are Defective in Cell Division and Differentiation

Growth defects in 4-day-old *$InsP_3R^1$* homozygous larvae (A, B, D, and F), compared with wild-type controls of the same stage (C, E, and G). All panels were taken at the same magnification (×500).

(A) shows a dorsal view of brain hemispheres (br) labeled with basic fuchsin. Eye imaginal discs (ed), each numbering less than 100 cells, are anteriorly attached to the brain.

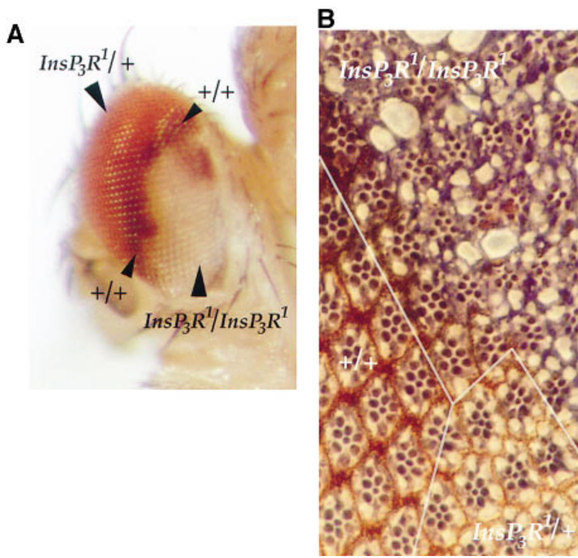
(B) and (C) show brain neuroblasts (nb) of larvae labeled with anti-BrdU antibody. BrdU, which is incorporated into nuclei of proliferating neuroblasts, had been fed to the larva during day 2 (B) and day 3 (C) post hatching. In the control, clones of 4–8 labeled ganglion mother cells accompany each neuroblast (arrows in C). In *$InsP_3R^1$* homozygote, many neuroblasts are labeled, demonstrating that they at least replicate once during the BrdU pulse. However, there are little, if any, ganglion mother cells, resulting in the reduced brain size compared to the control (ol = optic lobe cells).

(D) and (E) show part of salivary gland of *$InsP_3R^1$* homozygote and heterozygous control, respectively. Control nuclei outmeasure, in volume, mutant nuclei by at least two orders of magnitude. Also note the variable nuclear size in mutants (arrows in D).

(F) and (G) compare larval midgut epithelial cells (PMECs) in mutant and control midgut, respectively. Adult midgut progenitors (AMPs) appear in the embryo as individual cells interspersed at regular intervals among the PMECs. During larval stages, they undergo 2–3 mitotic divisions, resulting in small "islands" of 4–8 AMPs (G). In *$InsP_3R^1$* homozygotes, AMPs apparently fail to proliferate and impose as single, small cells scattered in between the PMECs.

The larvae of holometabolous insects are composed of two types of tissues: the larval cells, which do not themselves proliferate but grow by enlargement and polytenization, and the imaginal discs, consisting of diploid cells that proliferate extensively and give rise to most of the adult structures. Histological examination of various tissues from 4-day-old *$InsP_3R^1$* homozygous mutant larvae (corresponding to late third larval instar of control larvae) revealed a pronounced defect in the growth of larval tissues and imaginal progenitor cells. For instance, brain neuroblasts and midgut progenitor cells can be recognized but do not undergo any significant proliferation (Figures 3A, 3B, and 3F). As a result, the size of the brain corresponds to that of control late-first or early-second instar larvae. Also, salivary glands (Figure 3D) and principal midgut epithelial cells (Figure 3F) undergo little endoreplication of the DNA. During normal development, larval cells perform up to 10 rounds of endoreplication, resulting in large cells with highly polytenic chromosomes (see the salivary gland nuclei in Figure 3E). Nuclei in homozygous *$InsP_3R^1$* larvae are exceptionally smaller, with at most 1–2 rounds of endoreplication. *$InsP_3R^1$* mutants also show dramatic defects in the development of imaginal discs; they are rudimentary at best, with little if any cell proliferation or differentiation (Figure 3A; data not shown).

Within some larval tissues, nuclei are of different sizes (e.g., Figure 3D), suggesting a variable number of endoreplications. This could be most easily explained by assuming that $InsP_3R$ is essential for cell growth and proliferation, but some $InsP_3R$ mRNA is provided maternally (the mothers are heterozygous for *$InsP_3R^1$*, and the ooplasm is largely provided by nurse cells). Therefore, different larval cells may have differences in the amount of $InsP_3R$ message (or protein) they received from the egg during cleavage and would arrest growth at different times. If this is true, then making germ line clones lacking all $InsP_3R$ should lead to early lethality. We generated zygotic clones homozygous for *$InsP_3R^1$*, using the dominant female sterile (DFS; Chou et al., 1993) technique in an *$InsP_3R1$*/P[*ovo^{D1}*]^{c13X3} female, and did not recover any viable eggs or embryos. In contrast, many clones heterozygous for *$InsP_3R^1$*/+ were obtained and served as internal controls (number of *$InsP_3R1$*/P[*ovo^{D1}*]^{c13X3}



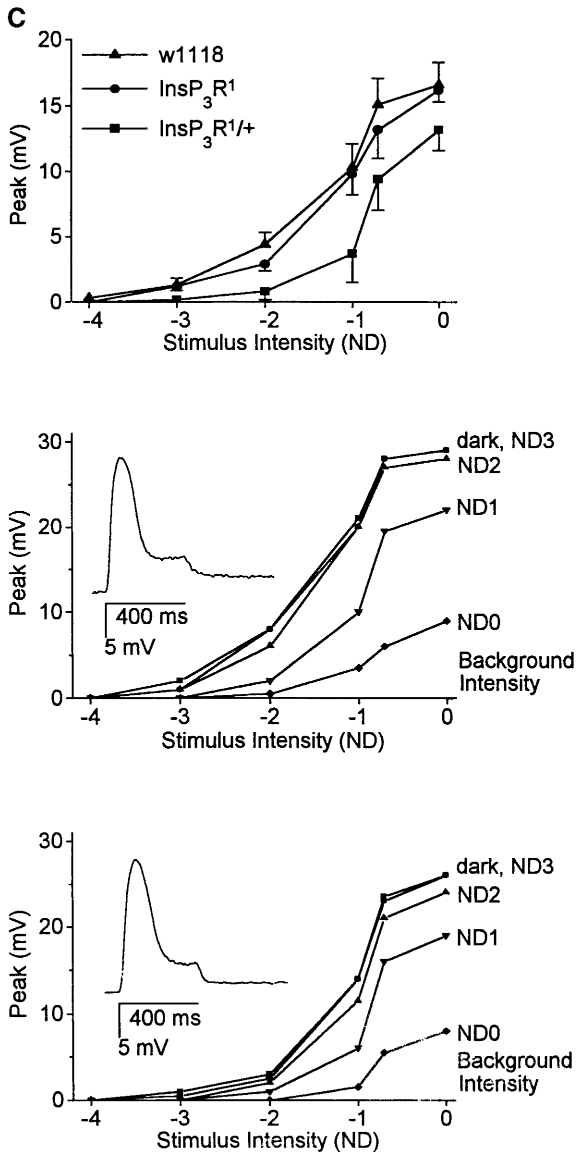


Figure 4. $InsP_3R$ Is Not Required for Phototransduction

(A) *$InsP_3R^1$* somatic clone in the eye. The *$InsP_3R^1$/$InsP_3R^1$* mutant patch is white, the control P[w^+]/P[w^+] homozygous patch is red, and the heterozygous P[w^+]/*$InsP_3R^1$* background is orange.

(B) The *$InsP_3R^1$* mutant clones undergo degeneration. Shown is a 1 μm section through a mosaic similar to the one shown in (A). The mutant *$InsP_3R^1$* photoreceptors are *w* and hence do not have the red pigment granules in the supporting cells. The *$InsP_3R^1$* mutant ommatidia show retinal degeneration with disruptions in the ommatidial architecture. However, note the nearly intact rhabdomeric structure in the mutant ommatidia. The adjacent heterozygous and the homozygous wild-type tissue are morphologically intact.

(C) The upper panel shows electrophysiological responses to pulses of light in control and *$InsP_3R^1$* mutant photoreceptor cells. Shown are the intensity-response relationships of dark-adapted cells (mean ± SEM; $n \geq 6$) for green test stimuli of the indicated intensities. The *$InsP_3R^1$* mutant photoreceptors display normal light responses. The middle (w^{1118}) and lower (*$InsP_3R^1$*) panels demonstrate that adaptation to background light is similar in wild-type and mutant flies. For each cell, intensity-response relations to green test pulses were recorded in the presence of different intensities of 570 nm background illumination at ND 3 (circles), ND 2 (triangles), ND 1 (inverted triangles), and ND 0 (diamonds). Insets show responses to 530 nm, 400 ms light pulses at ND 0, demonstrating that the kinetics of activation and deactivation are similar between the wild type and mutant cells.

tested = 226; see Experimental Procedures). Thus, we conclude that $InsP_3R$ is required zygotically and *$InsP_3R^1$* larval lethality is a maternal-effect phenotype due to maternally contributed message. Taken together, these results demonstrate that $InsP_3R$ is essential for cell proliferation, growth, and differentiation (Figure 2). The availability of this *$InsP_3R$* mutant, together with the use of tissue specific mosaicism (i.e., FLP/FRT), allows now for a comprehensive study of $InsP_3R$ function and regulation in a wide variety of tissues and developmental stages.

In addition to our work on $InsP_3R$ function during embryonic and larval development, we also examined the role of $InsP_3R$ in a well-studied PLC-based signaling pathway (Zuker, 1996). In particular, we addressed one of the outstanding questions in the field of invertebrate photoreceptor biology, namely the gating mechanism of the light-activated channels. In Drosophila photoreceptor neurons, light activation of rhodopsin activates a Gq_α, which in turn activates a PLC encoded by the *norpA* gene. PLC catalyzes the breakdown of PIP_2 into $InsP_3$ and DAG; these are thought to lead to the eventual opening (and modulation) of the TRP and TRPL membrane channels and the generation of a receptor potential (Pak, 1991; Hardie and Minke, 1995; Ranganathan et al., 1995; Zuker, 1996;). Because the principal light-activated channel (TRP) is highly permeable to calcium, and because TRP mutants display only a transient response to light, several investigators have proposed that this channel functions as a store-operated channel that responds to a capacitative calcium entry signal (Hardie and Minke, 1993; Vaca et al., 1994; Hardie and Minke, 1995; Hu and Schilling, 1995; Wes et al., 1995). According to this model, light-activation of rhodopsin results in the production of IP_3, which would lead to the emptying of $InsP_3$-sensitive internal calcium stores and the subsequent gating of plasma membrane channels, via either conformational coupling (Irvine, 1990; Berridge, 1995) or a diffusible signal (Putney, 1986; Randriamampita and Tsien, 1993; Berridge, 1995). Several in-

Table 1. Response Kinetics of Control w1118 and *InsP3R1* Photoreceptors

	Latency (ms)	Time-to-Peak (ms)	50% Decay from Peak (ms)	50% Decay from Steady-State (ms)	Steady-State Level (% of Peak)
w^{1118}	18.6 ± 0.6	50.0 ± 2.4	36.2 ± 2.2	23.1 ± 2.9	27.6 ± 2.6
IP_3R^1	17.2 ± 0.9	53.5 ± 2.6	42.9 ± 3.3	19.6 ± 2.3	25.6 ± 3.0

Shown are the mean values (±SEM) for latency (defined as the time from the light stimulus to onset of the response), time-to-peak (time from onset of the stimulus to peak amplitude), 50% decay from the peak (time from peak to 50% of transient response), 50% decay from steady-state (time to 50% deactivation), and the steady-state level (expressed as a percentage of the peak value). Values for $InsP_3R^1$ cells do not show statistically significant differences from w^{1118} control values when tested with the t test.

vestigators have further proposed that the TRP channel and its vertebrate homologs represent examples of the elusive store-operated channels (i.e., Icrac = calcium-release-activated-channel; see, for example, Vaca et al., 1994; Berridge, 1995; Hardie and Minke, 1995; Hu and Schilling, 1995; Wes et al., 1995). Recently, we showed that the TRP and TRPL light-activated channels do not localize in close proximity to the internal calcium stores (Niemeyer et al., 1996), thus eliminating the possibility of gating by protein–protein interaction via conformational coupling (Hardie and Minke, 1995; Pollock et al., 1995). In addition, we used fluorescent calcium indicators to study the dynamics of $[Ca^{2+}]_i$ in intact photoreceptors and showed that all light-induced increases in $[Ca^{2+}]_i$ are entirely dependent on the influx of extracellular Ca^{2+} (Ranganathan et al., 1994; see also Peretz et al., 1994). These results suggested that internal $InsP_3$-sensitive stores are not involved in activation of the light response; however, because small, highly localized changes may not have been detected, this study could not rigorously eliminate an involvement of $InsP_3$-mediated calcium release in the activation of the light response. To categorically define the requirement of the $InsP_3R$ in this pathway, we have now taken advantage of $InsP_3R^1$ mutants and generated viable, adult mosaic animals containing homozygous $InsP_3R^1$ mutant photoreceptors in an otherwise wild-type genetic background. The experiment was designed so that the $InsP_3R^+$ cells were *white*$^+$ and pigmented (e.g., $white^+ InsP_3R^+ / white^+ InsP_3R^+$ or $white^+ / InsP_3R^1$), while the $InsP_3R^1$ cells were *white*$^-$ and unpigmented, thus allowing for the unambiguous identification of mutant cells (see Experimental Procedures; Figure 4A). Structural analysis of the mutant patches revealed that photoreceptors and surrounding tissues undergo retinal degeneration, consistent with the phenotypes observed in the larvae (Figure 4B). We used in situ intracellular recordings to study light responses in control and mutant photoreceptor cells. Using this strategy, we could reliably identify the genotype of the cells and perform high-resolution electrophysiological recordings of light-induced photoreceptor activity. In Drosophila photoreceptor cells, the response to a pulse of light is characterized by a short latency, followed by a fast initial transient depolarization that rapidly reaches a sustained adapted state. Upon termination of the stimulus, the response decays exponentially back to baseline (see insets of Figure 4). We measured sensitivity by examining light responses over a 5 log range of light intensities (Figure 4C). To compensate for differences in eye color between wild type and mutant patches (Figure 4A), we also recorded from control w^{1118} flies. Possible defects in response kinetics were examined by comparing latencies, time-to-peak, steady-state levels, and decay time in control and $InsP_3R^1$ mutant cells (Table 1). In addition, we studied adaptation by recording responses to test stimuli in the presence of background light of various intensities (Figure 4C). Remarkably, $InsP_3R^1$ homozygous mutant photoreceptors are indistinguishable from wild-type controls in sensitivity, kinetics of activation and deactivation, and adaptation. Thus, we find no evidence for a role of the $InsP_3R$ in Drosophila phototransduction, and models invoking $InsP_3$-induced calcium release in the activation of this pathway must be seriously reexamined.

Experimental Procedures

Identification, Isolation, and Rescue of $InsP_3R$ Mutants

A 3 kb cDNA clone consisting of the 3′ end of the $InsP_3R$ was used to initiate a 60 kb genomic walk. The genomic organization of the *InsP3R* and *Nmdar* genes within this interval was determined by sequence analysis and restriction mapping. The precise site of insertion of the 5jB4 P-element (Bier et al., 1989) was determined by plasmid rescue and direct sequencing of the junction with genomic DNA. Imprecise excision lines were generated by mobilizing the P-element using a stable source of a transposase, Δ2–3, exactly as previously described (Robertson, 1988). Jump-out lines were analyzed for deletions between +6 kb and −1 kb of $InsP_3R$ by Southern blot analysis using DNA probes from the +6000 to −1000 position (Yoshikawa et al., 1992). The cosmids used for the P-element mediated germ line transformations were isolated from a cosmid library kindly provided by the Tamkun laboratory (Tamkun et al., 1992). P-element transformations were performed as previously described (Spradling and Rubin, 1982).

Histological Examination of the Larva and Mosaic Retinas

Larvae from control and heterozygous mutant animals were hand dissected, fixed in 4% paraformaldehye, and stained with basic fuchsin as previously described (Wieschaus, 1986). BrdU staining of dividing neuroblasts was performed as previously described (Truman, 1988).

Heads from mosaic flies were fixed in phosphate buffer containing 4% formaldeyde, 1% glutaraldehyde, and 2% sucrose. Postfixation was done in 1% osmium tetroxide containing 3% sucrose; after rapid dehydration, the heads were embedded in spurs, and 1μm thick sections were cut, stained with 1% methylene blue, and photographed under phase contrast.

Somatic Recombination Experiments

To generate germ line clones of $InsP_3R^1$ mutants, $P[ovo^{D1}]^{c13X3}$/Balancer males were crossed to $InsP_3R^1$/Balancer virgins, and the first instar larval progeny were exposed to 1 krad of X-rays (Chou et al., 1993). The heterozygous $P[ovo^{D1}]^{c13X3}/InsP_3R^1$ females were crossed to $InsP_3R^1$/Balancer male flies, and the vials were examined during the next twenty days for eggs, embryos, and larvae. As controls, we scored the same flies for $+/InsP_3R^1$ progeny resulting from germ

line clones derived from recombination events occurring between the *InsP₃R* and the P[*ovo*^{D1}]^{c13X3} insertion. We recovered five such lines from 226 individual crosses.

To generate homozygous clones of *InsP₃R*^{1} cells in the retina, *InsP₃R*^{1}/AT64 heterozygous first instar larvae were subjected to 1 krad of X-rays, and the adult eye was examined for white patches in an orange background. AT64 is a P[w^+] insertion located at 82F12.

Electrophysiology

For intracellular recordings, we used mosaic flies containing mutant patches that ranged from a few ommatidia up to 50% of the compound eye. Heads were cut coronally, adjacent to the mutant patch, and photoreceptor cells were penetrated with sharp microelectrodes (70–150 MΩ) filled with 2 M KCl. Responses were low-pass filtered at 1 kHz. For stimulation, light from a 75W Xenon lamp was passed through a 530 nm bandpass filter (Oriel 53875) and attenuated with neutral density filters as indicated. All other manipulations were done exactly as previously described (Niemeyer et al., 1996). In all animals, we recorded from homozygous wild-type, heterozygous, and homozygous mutant cells. As controls for the specificity and cell-autonomy of the intracellular recordings, we also generated *norpA* mutants patches in an otherwise wild-type background and recorded from the normal and the mutant patches. As expected, mutant cells did not respond, while normal cells produced robust responses (data not shown).

Acknowledgments

We thank Dr. Michael Rosbash for providing us with a cDNA probe for $InsP_3R$ and Dr. J. W. Tamkun for the cosmid library. We especially thank Yumei Sun and Michael Socolich for outstanding technical assistance. We also thank Dr. Usha Acharya and members of the Zuker lab for critical reading of the manuscript. K. J. is an American Cancer Society Junior fellow. J. K. A. and R. W. H. are associates of the Howard Hughes Medical Institute. C. S. Z. is an investigator of the Howard Hughes Medical Institute. This work was supported in part by a grant from the National Eye Institute.

Received May 22, 1997; revised May 27, 1997.

References

Ashburner, M. (1989). Drosophila: A Laboratory Handbook (Cold Spring Harbor: Cold Spring Harbor Laboratory Press), pp. 1017–1063.

Berridge, M.J. (1993). Cell signalling. A tale of two messengers. Nature *365*, 388–389.

Berridge, M.J. (1995). Capacitative calcium entry. Biochem. J., 1–11.

Bier, E., Vaessin, H., Shepherd, S., Lee, K., McCall, K., Barbel, S., Ackerman, L., Carretto, R., Uemura, T., Grell, E., et al. (1989). Searching for a pattern and mutation in the Drosophila genome with a P-lacZ vector. Genes Dev. *3*, 1273–1287.

Cheng, H., Lederer, W., and Cannell, M. (1993). Calcium sparks: elementary events underlying excitation-contraction coupling in heart muscle. Science *262*, 740–742.

Chou, T.B., Noll, E., and Perrimon, N. (1993). Autosomal P[ovoD1] dominant female-sterile insertions in Drosophila and their use in generating germ line chimeras. Development *119*, 1359–1369.

De-camilli, P., Takei, K., Mignery, G.A., and Sudhof, T.C. (1990). InsP3 receptor turnaround. Nature *344*, 495.

Fain, J.N. (1990). Regulation of phosphoinositide-specific phospholipase C. Biochim. Biophys. Acta *1053*, 81–88.

Fischer, G.A., Clementi, E., Raichman, M., Sudhof, T., Ullrich, A., and Meldolesi, J. (1994). Stable expression of truncated inositol 1,4,5-trisphosphate receptor subunits in 3T3 fibroblasts. Coordinate signaling changes and differential suppression of cell growth and transformation. J. Biol. Chem. *269*, 19216–19244.

Furuichi, T., and Mikoshiba, K. (1995) Inositol 1,4,5-trisphosphate receptor-mediated Ca^{2+} signaling in the brain. J. Neurochem. *64*, 953–960.

Hardie, R.C., and Minke, B. (1993). Novel Ca^{2+} channels underlying transduction in Drosophila photoreceptors: implications for phosphoinositide-mediated Ca^{2+} mobilization. Trends Neurosci. *16*, 371–376.

Hasan, G., and Rosbash, M. (1992). Drosophila homologs of two mammalian intracellular Ca^{2+}-release channels: identification and expression patterns of the inositol 1,4,5-triphosphate and the ryanodine receptor genes. Development *116*, 967–975.

Hu, Y., and Schilling, W.P. (1995). Receptor-mediated activation of recombinant Trpl expressed in Sf9 insect cells. Biochem. J., 605–611.

Irvine, R.F. (1990). 'Quantal' Ca^{2+} release and the control of Ca^{2+} entry by inositol phosphates—a possible mechanism. FEBS Lett. *263*, 5–9.

Khan, A.A., Soloski, M.J., Sharp, A.H., Schilling, G., Sabatini, D.M., Li, S.H., Ross, C.A., and Snyder, S.H. (1996). Lymphoctye apoptosis: mediation by increased type 3 inositol 1,4,5-trisphosphate receptor. Science *273*, 503–507.

Matsumoto, M., Makagawa, T., Inoue, T., Nagata, E., Tanaka, K., Takano, H., Minowa, O., Kuno, J., Sakakibara, S., Yamada, M., Yoneshima, H., Miyawaki, A., Fukuuchi, Y., Furuichi, T., Okano, H., Mikoshiba, K., and Noda, T. (1996). Ataxia and epileptic seizures in mice lacking type 1 inositol 1,4,5-trisphosphate receptor. Nature *379*, 168–171.

Mignery, G.A., and Sudhof, T.C. (1990). The ligand binding site and transduction mechanism in the inositol-1,4,5-triphosphate receptor. EMBO J. *9*, 3893–3898.

Mignery, G.A., Sudhof, T.C., Takei, K., and De, C.P. (1989). Putative receptor for inositol 1,4,5-trisphosphate similar to ryanodine receptor. Nature *342*, 192–195.

Mikoshiba, K., Furuichi, T., and Miyawaki, A. (1994). Structure and function of IP_3 receptors. Semin. Cell Biol. *5*, 273–281.

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

Nishizuka, Y. (1992). Intracellular signaling by hydrolysis of phospholipids and activation of protein kinase C. Science *258*, 607–614.

Pak, W.L. (1991). Molecular genetic studies of photoreceptor function using Drosophila mutants. Prog. Clin. Biol. Res. *362*, 1–32.

Peretz, A., Suss-Toby, E., Rom-Glas, A., Arnon, A., Payne, R., Minke, B. (1994). The light response of Drosophila photoreceptors is accompanied by an increase in cellular calcium: effects of specific mutations. Neuron *12*, 1257–1267.

Pollock, J.A., Assaf, A., Peretz, A., Nichols, C.D., Mojet, M.H., Hardie, R.C., and Minke, B. (1995). TRP, a protein essential for inositide-mediated Ca^{2+} influx, is localized adjacent to the calcium stores in Drosophila photoreceptors. J. Neurosci. *15*, 3747–3760.

Putney, J.J. (1986). A model for receptor-regulated calcium entry. Cell Calcium *7*, 1–12.

Raghu, P., and Hasan, G. (1995). The inositol 1,4,5-triphosphate receptor expression in Drosophila suggests a role for IP_3 signalling in muscle development and adult chemosensory functions. Dev. Biol. *171*, 564–577.

Randriamampita, C., and Tsien, R.Y. (1993). Emptying of intracellular Ca^{2+} stores releases a novel small messenger that stimulates Ca^{2+} influx. Nature *364*, 809–814.

Ranganathan, R., Bacskai, B.J., Tsien, R.Y., and Zuker, C.S. (1994). Cytosolic calcium transients: spatial localization and role in Drosophila photoreceptor cell function. Neuron *13*, 837–848.

Rhee, S.G., and Choi, K.D. (1992). Multiple forms of phospholipase C isozymes and their activation mechanisms. Adv. Second Messenger Phosphoprotein Res. *26*, 35–61.

Robertson, H.M., Preston, C.R., Phillis, R.W., Johnson-Schlitz, D., Benz, W.K., and Engels, W.R. (1988). A stable source of P-element transposase in Drosophila melanogaster. Genetics *118*, 461–470.

Spradling, A.C., and Rubin, G.M. (1982). Transposition of cloned P elements into Drosophila germ line chromosomes. Science *218*, 341–347.

Sudhof, T.C., Newton, C.L., Archer, B.D., Ushkaryov, Y.A., and Mignery, G.A. (1991). Structure of a novel InsP3 receptor. EMBO J. *10*, 3199–3206.

Tamhun, J.W., Deuring, R., Scott, M.P., Kissinger, M., Pattatucci, A.M., Kaufman, T.C., and Nennison, J.A. (1992). Brahma: a regulator of Drosophila homeotic genes structurally related to the yeast transcriptional activator SNF2/SW12. Cell *68*, 561–572.

Truman, J.W., and Bate, C.M. (1988). Spatial and temporal patterns of neurogenesis in the central nervous system of Drosophila melanogaster. Dev. Biol. *125*, 145–157.

Tsien, R.W., and Tsien, R.Y. (1990). Calcium channels, stores, and oscillations. Annu. Rev. Cell Biol. *6*, 715–760.

Ultsch, A., Schuster, C.M., Laube, B., Betz, H., and Schmitt, B. (1993). Glutamate receptors of Drosophila melanogaster. Primary structure of a putative NMDA receptor protein expressed in the head of the adult fly. FEBS Lett. *324*, 171–177.

Vaca, L., Sinkins, W.G., Hu, Y., Kunze, D.L., and Schilling, W.P. (1994). Activation of recombinant trp by thapsigargin in Sf9 insect cells. Am. J. Physiol. C1501–1505.

Wes, P.D., Chevesich, J., Jeromin, A., Rosenberg, C. Stetten, G., and Montell, C. (1995). TRPC1, a human homolog of a Drosophila store-operated channel. Proc. Natl. Acad. Sci. USA *92*, 9652–9656.

Wieschaus, E., and Nusslein-Volhar,C. (1986). Looking at Embryos in Drosophila: A Practical Approach, D.B. Roberts, ed. (Oxford: IRL Press), pp. 199–227.

Yoshikawa, S., Tanimura, T., Miyawaki, A., Nakamura, M., Yuzaki, M., Furuichi, T., and Mikoshiba, K. (1992). Molecular cloning and characterization of the inositol 1,4,5-trisphosphate receptor in Drosophila melanogaster. J. Biol. Chem. *267*, 16613–16619.

Zuker, C.S. (1996). The biology of vision of Drosophila. Proc. Natl. Acad. Sci. USA *93*, 571–576.