Cell

The Fly CAMTA Transcription Factor Potentiates Deactivation of Rhodopsin, a G Protein-Coupled Light Receptor

Junhai Han,[1,2] Ping Gong,[1,2] Keith Reddig,[1,2] Mirna Mitra,[1] Peiyi Guo,[1] and Hong-Sheng Li[1,*]
[1] Department of Neurobiology, University of Massachusetts Medical School, Worcester, MA 01605, USA
[2] These authors contributed equally to this work.
*Contact: hong-sheng.li@umassmed.edu
DOI 10.1016/j.cell.2006.09.030

SUMMARY

Control of membrane-receptor activity is required not only for the accuracy of sensory responses, but also to protect cells from excitotoxicity. Here we report the isolation of two noncomplementary fly mutants with slow termination of photoresponses. Genetic and electrophysiological analyses of the mutants revealed a defect in the deactivation of rhodopsin, a visual G protein-coupled receptor (GPCR). The mutant gene was identified as the *calmodulin-binding transcription activator* (*dCAMTA*). The known rhodopsin regulator Arr2 does not mediate this visual function of dCAMTA. A genome-wide screen identified five dCAMTA target genes. Of these, overexpression of the F box gene *dFbxl4* rescued the mutant phenotypes. We further showed that dCAMTA is stimulated in vivo through interaction with the Ca^{2+} sensor calmodulin. Our data suggest that calmodulin/CAMTA/Fbxl4 may mediate a long-term feedback regulation of the activity of Ca^{2+}-stimulating GPCRs, which could prevent cell damage due to extra Ca^{2+} influx.

INTRODUCTION

Membrane receptors mediate cell-cell communication and sensory responses to extracellular stimuli. As the largest family of membrane receptors, GPCRs receive signals from a variety of ligands including hormones, cytokines, and neurotransmitters as well as sensory stimuli and trigger distinct intracellular responses through heterotrimeric G proteins (Kristiansen, 2004; Pierce et al., 2002).

The $G_{q/11}$-type G proteins activate phospholipase Cβ (PLCβ) to produce diacylglycerol (DAG) and inositol 1,4,5-triphosphate (IP_3). IP_3 induces the release of Ca^{2+} from intracellular Ca^{2+} stores, including the endoplasmic reticulum. In addition, both DAG and IP_3 may trigger extracellular Ca^{2+} influx through ion channels on the cell membrane (Bird et al., 2004), such as the transient receptor potential (TRP) family of channels (Montell, 2001). Thus, $G_{q/11}$ signaling has a great impact on intracellular Ca^{2+} homeostasis.

As a versatile signaling molecule, Ca^{2+} stimulates a range of effectors and regulates various cellular functions. In many cases, Ca^{2+} signals cause long-term change of cellular activities by altering gene expression (Ikura et al., 2002), usually through the Ca^{2+} sensor calmodulin (Cruzalegui and Bading, 2000; West et al., 2001). A group of calmodulin-binding transcription activators (CAMTAs) have been identified in both plants and animals (Bouche et al., 2002; Yang and Poovaiah, 2002). However, it has yet to be demonstrated experimentally that calmodulin actually stimulates CAMTA activity.

A large body of work in the past decade has shown that GPCRs also activate the extracellular signal-regulated kinase (ERK) pathway (Luttrell, 2005) that promotes cell proliferation and/or differentiation. Therefore, the timely deactivation of stimulated GPCRs not only is required for cells to fine tune their signaling and avoid Ca^{2+}-dependent excitotoxicity but is also critical for the prevention of abnormal cell proliferation. Uncontrolled G protein or GPCR activity has been linked to a variety of tumors (Gudermann et al., 2000).

GPCR kinases (GRKs) (Krupnick and Benovic, 1998) and β-arrestins (Shenoy and Lefkowitz, 2003) are the primary regulators of many GPCRs. GRKs phosphorylate GPCRs and promote binding between receptors and β-arrestins, which uncouples G protein from the activated receptors. Prolonged GPCR stimulation may cause β-arrestin/clathrin-dependent receptor internalization/endocytosis (Claing et al., 2002) and may result in long-term cell desensitization to the ligand. However, GRKs and β-arrestins are not the only GPCR regulators. Many GPCRs, including endothelin type B, M2 muscarinic, vasoactive intestinal peptide type 1, and bradykinin type 2 receptors, are internalized independently of β-arrestin (Claing et al., 2002). Some other receptors undergo both β-arrestin-dependent and -independent internalization (Claing et al., 2002). Most importantly, the regulatory

machineries of GPCRs appear to possess activity-dependent and gene-expression-mediated plasticity. For instance, chronic stimulation of the μ-opioid or β-adrenergic receptors increases the expression levels of several GRKs and β-arrestins in the brain (Fan et al., 2003; Hurle, 2001) or lymphocytes (Oyama et al., 2005), respectively. Nevertheless, the transcription factors that confer plasticity to the potency of GPCR regulatory machineries have remained unknown.

The *Drosophila* phototransduction cascade is a genetic model system (Hardie and Raghu, 2001; Montell, 1999) for the study of GPCR signaling and regulation. This visual signaling cascade is localized in the rhabdomere (Hardie and Raghu, 2001), a highly packed microvillar structure that is analogous to the outer segment of mammalian photoreceptors. Through PLC and possibly another lipase (Chyb et al., 1999), the light receptor rhodopsin (O'Tousa et al., 1985), which couples to a G_q protein, opens the TRP-family Ca^{2+}/cation channels (Montell, 1999) to depolarize the photoreceptor cells. As speed is a key factor in visual function, the photoresponse terminates in less than 100 ms after the light is shut off (Ranganathan et al., 1991). The most important step in photoresponse termination is the deactivation of stimulated rhodopsin, in which a visual arrestin, Arr2, plays a pivotal role (Dolph et al., 1993). The phosphorylation of fly rhodopsin is important for its endocytosis (Satoh and Ready, 2005) but is not required for its deactivation or the termination of photoresponses (Scott and Zuker, 1997). Instead, the dephosphorylation of rhodopsin by a phosphatase RdgC appears to be critical for receptor deactivation (Vinos et al., 1997). In addition, eye-specific protein kinase C (INAC), the myosin III NINAC, and the scaffold protein INAD (Montell, 1999; Scott and Zuker, 1997), which do not act directly on rhodopsin, are also indispensable for the rapid termination of phototransduction.

Here we report the isolation of two noncomplementary mutants (*tes*[1] and *tes*[2], for t*ermination s*low) with defective photoresponse termination. The mutant gene is identified as *dCAMTA*, the sole fly homolog of the candidate human brain tumor-suppressor gene *CAMTA1* (Barbashina et al., 2005). Genetic and electrophysiological analyses indicate that the *tes* phenotype is due to insufficient deactivation of rhodopsin. Probably as a compensatory response to insufficient deactivation, the major rhodopsin Rh1 undergoes activity-dependent reduction in *tes* flies. As *dCAMTA* encodes a transcription factor, which is unlikely to be a direct regulator of rhodopsin, we performed microarray analyses to identify the target genes of dCAMTA. The overexpression of one target, the F box gene *dFbxl4*, rescued the *tes* mutant phenotype. We further showed that dCAMTA activity is stimulated in vivo by direct interaction with calmodulin. Our data suggest that calmodulin/CAMTA-mediated gene expression may potentiate the deactivation of rhodopsin and other Ca^{2+}-stimulating GPCRs in a feedback manner.

RESULTS

Mutation of *dCAMTA* Causes Defective Phototransduction in the Fly Eye

In an electroretinogram (ERG)-based chemical mutagenesis screen for additional genes in fly phototransduction, we isolated two noncomplementary mutant flies, *tes*[1] and *tes*[2] (for t*ermination s*low), with light responses terminating much more slowly than wild-type (Figure 1A). Whole-cell recording of isolated photoreceptor cells showed that the light-induced currents in *tes* cells deactivated significantly more slowly than those in wild-type (Figure 1B). This observation demonstrates that the defect in *tes* mutants is due to an abnormality in photoreceptor cells.

To identify the mutant gene in *tes* flies, we first mapped the mutations to the chromosomal region 45D5-45E1 based on the ERG phenotype uncovered by the deficiency chromosome w45-19g (missing the region 45C8-D10 to 45D9-E1) but not by w73-1 (missing 45A9-10 to 45D5-8). Six genes are annotated in this region of the *Drosophila* genome (Figure 1C). Using P element-mediated male recombination mapping (Chen et al., 1998), we narrowed the affected genomic region to three predicted genes (Figure 1C), including *dCAMTA*. According to a full-length mRNA sequence (GenBank accession number DQ902587) compiled using 5′RACE and PCR fragments, the actual *dCAMTA* gene, which encodes 2009 amino acid residues in 19 exons, occupies virtually the entire mapped region (Figure 1C). Another predicted gene, CG13952, actually encodes two exons of *dCAMTA*. Subsequent sequencing of *dCAMTA* exons revealed nonsense mutations in both *tes* alleles (Figure 1D).

Using antibodies raised against an N-terminal dCAMTA fragment, we confirmed the loss of dCAMTA protein in *tes* flies. A protein band slightly higher than 220 kDa was observed in wild-type, but not in *tes*, fly heads (Figure 1E). As we could not detect the presence of any truncated protein in *tes* flies, both mutants are likely to be dCAMTA protein null.

In heads of mutant flies that either lack eyes (sine oculis) or undergo retinal degeneration (*rdgA*), dCAMTA protein levels decreased dramatically (Figure 1E), suggesting that dCAMTA operates in visual function. To show that loss of dCAMTA is indeed responsible for the *tes* mutant phenotype, we generated transgenic flies expressing a wild-type *dCAMTA* cDNA in a *tes* mutant background using the *trp* gene promoter, which drives gene expression specifically in photoreceptors (*tes;P[trp-dCAMTA]*). As demonstrated both by ERG and whole-cell recordings of isolated photoreceptors, the light responses in *tes;P[trp-dCAMTA]* transgenic flies terminated as rapidly as those in wild-type (Figure 1F). Thus, *tes*[1] and *tes*[2] are two mutant alleles of the *dCAMTA* gene.

dCAMTA Functions as a Transcription Activator

To understand the visual role of dCAMTA, we first characterized the molecular function of this protein. dCAMTA has protein domain architecture (Figure 1D) very similar to its

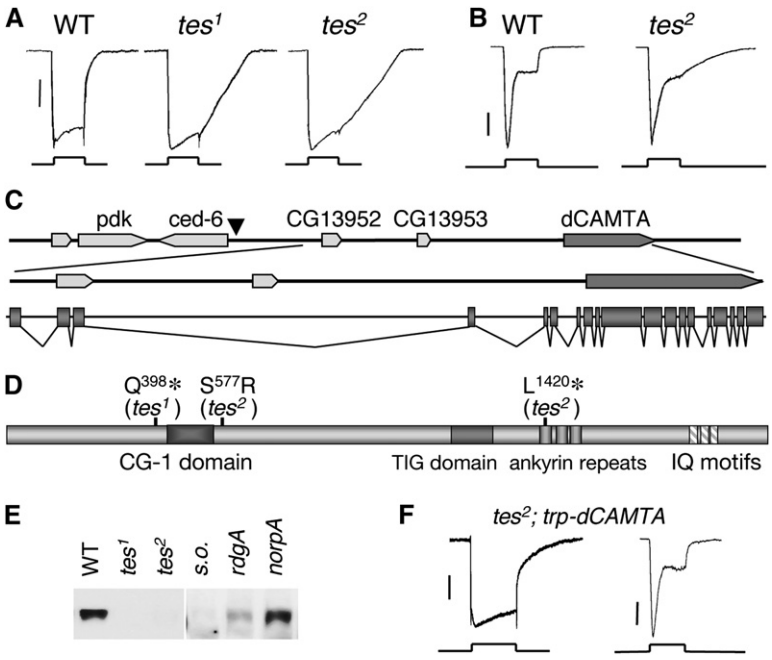

Figure 1. Mutation of *dCAMTA* Causes Defective Fly Photoresponse

All flies examined are <3 days old.

(A) ERG responses terminated slowly in *tes* flies. For all ERG traces, event markers represent 5 s orange light pulses, and scale bars are 5 mV. WT = wild-type.

(B) Whole-cell recordings of isolated *tes* photoreceptor cells revealed a defective termination of light response. The scale bar and light pulse are 200 pA and 500 ms, respectively, for all whole-cell currents.

(C) Annotated genes in chromosome region 45D5-45E1 (top). *tes* mutations were mapped to this region using deficiency chromosomes and further located distal to the insertion site (arrowhead) of P{EPgy2}EY02897 by male recombination mapping. The actual *dCAMTA* gene (bottom) occupies virtually the entire mapped region.

(D) Point mutations in *tes* alleles shown with respect to the functional domains of dCAMTA. * = stop codon.

(E) dCAMTA protein levels in dark-reared mutant flies. Each lane was loaded with four fly heads. *rdgA* and *norpA* are mutants for a DAG kinase and PLC, respectively. s.o. = sine oculis.

(F) A WT *dCAMTA* cDNA rescued the *tes* phenotype after being expressed in photoreceptors through a *trp* gene promoter. Both ERG (left) and whole-cell current (right) are shown.

human and plant homologs (Bouche et al., 2002): a CG-1 DNA-binding domain in the N-terminal region, three IQ motifs on the C-terminal tail, and a TIG domain and three ankyrin repeats in the midpart. As both human and plant CAMTAs activate gene transcription, it is highly likely that dCAMTA also functions as a transcription activator. This hypothesis was supported by the following observations. First, in immunostaining assays, dCAMTA proteins were localized in the nuclei of photoreceptor cells, but not in rhabdomeres or other subcellular regions (Figure 2A). Second, the CG-1 domain of dCAMTA bound specifically to a DNA fragment that contains a CGCG box (Figure 2B), which is the minimum recognition sequence for plant CG-1 domains (Yang and Poovaiah, 2002). This DNA/CG-1 interaction was disrupted by mutating CGCG to CGGG (Figure 2B) and was abolished by competition with 200-fold unlabeled probes containing CGCG, but not by those containing CGGG (Figure 2B). Third, in transfected 293T cells, dCAMTA proteins drove expression of a luciferase reporter gene through the same CGCG DNA fragment, while the CGGG DNA had no significant effect on dCAMTA-mediated luciferase expression (Figure 2C). Taken together, the above observations provide compelling evidence that dCAMTA functions as a transcription factor.

dCAMTA Is Dispensable for Development of Rhabdomeres

Given that many transcription factors are involved in morphogenesis of specific cell structures, the defective light responses in *tes* mutant photoreceptors might be due to abnormal rhabdomere development in the absence of dCAMTA. However, electron microscopy did not reveal any morphological differences between *tes* and wild-type rhabdomeres (Figure 2D). More importantly, expression of dCAMTA protein at the adult stage (1–2 day old) through a heat-shock promoter (*P[hs-dCAMTA];tes*) was sufficient to rescue the *tes* mutant phenotype in transgenic flies (Figures 2E and 2F). These data indicate that dCAMTA is not required for rhabdomeral morphogenesis. Instead, it may play a more direct role in the regulation of visual signaling—for example, by promoting the expression of a negative regulator of phototransduction.

In *P[hs-dCAMTA];tes* flies, dCAMTA protein reached a high level within 1 hr after the end of the heat shock. Nonetheless, the *tes* mutant phenotype was not fully rescued until at least 10 hr later (Figure 2F). This long rescue latency might be due to the time required for photoreceptor cells to express sufficient visual regulatory molecules through dCAMTA transcriptional activity and for their transport into rhabdomeres.

The Function of dCAMTA Is Critical for Rhodopsin Deactivation

In fly photoreceptors, activated rhodopsin triggers a G_q protein to stimulate PLC, which opens the TRP and TRPL channels to depolarize the cell. Based on the phenotypes of *tes* mutants and the above observations, it is very likely that one of these signaling molecules depends on the product of a dCAMTA target gene for rapid

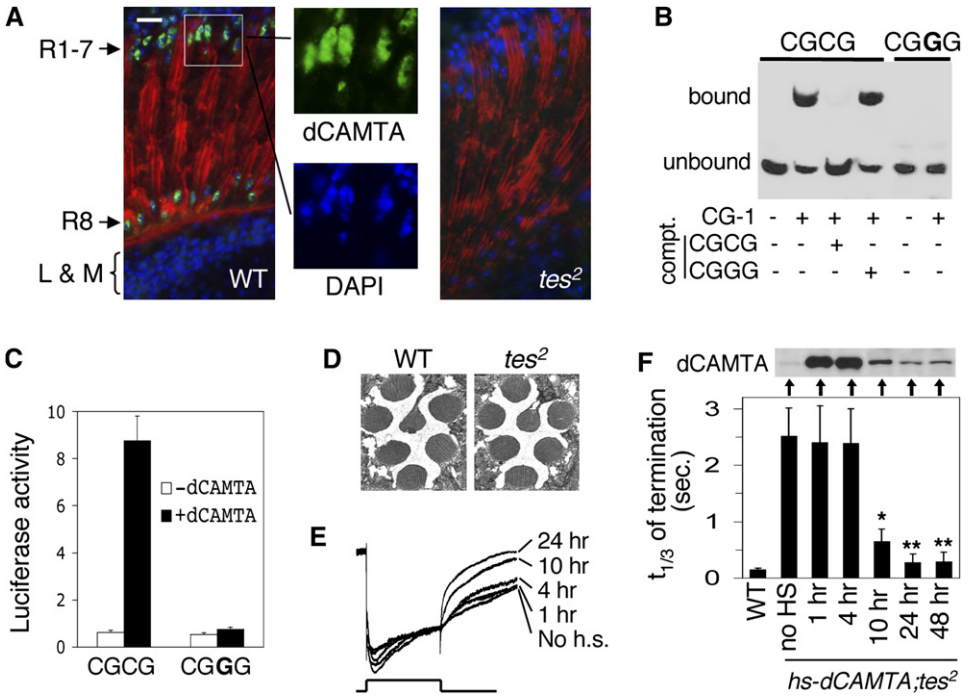

Figure 2. *dCAMTA* Encodes a Transcription Activator that Functions in the Adult Fly Eye

(A) dCAMTA proteins were detected in the nuclei of WT, but not *tes*, photoreceptors. Conical eye sections were costained with dCAMTA antibody (green), DAPI (blue, for nuclei), and an Rh1 rhodopsin antibody (red, showing rhabdomere bundles). The arrows point to the nuclei of photoreceptors (R1–R7 and R8) in the retina. The lamina (L) and medulla (M) are underneath the retina in the left panel. The boxed area is enlarged in the middle panels to show the same pattern of dCAMTA and DAPI signals. Scale bar = 10 μm.

(B) In electrophoresis mobility shift assays (EMSA), a CG-1 fragment of dCAMTA bound to a CGCG-box-containing, biotin-labeled DNA probe (CCAA CAGTCGCATGGGCAGCGTGCCCACGCGCACCATTGGCGCCAGTATGAG), but not after the CGCG was changed to CGGG. 200-fold unlabeled CGCG probes abolished the binding in the competition test (compt.).

(C) dCAMTA drove the expression of a luciferase reporter gene through the CGCG probe DNA in 293 cells. In this and all other figures, error bars represent mean ± SEM.

(D) EM pictures (9 × 10 μm, crossview of single ommatidia) show normal morphology of rhabdomeres (dark ovals) in 1-day-old *tes* flies.

(E) Overexpression of dCAMTA proteins in fully developed flies rescued the *tes* ERG phenotype. Two-day-old *P[hs-camta];tes*^{2} flies were heat shocked for 1 hr and examined at the indicated time after the shock. The amplitudes were normalized for comparison of the termination phase.

(F) The time courses of dCAMTA protein expression (top) and phenotype rescue (bottom) in *P[hs-camta];tes*^{2} flies after heat shock. The bars represent the time ($t_{1/3}$) required for a 1/3 recovery from the responses upon stimulation cessation.

deactivation. In an effort to identify this signaling molecule, we analyzed dim-light-stimulated quantum bumps in *tes* mutant photoreceptor cells.

Quantum bumps are unitary depolarization events that are triggered by single photons in photoreceptors. One bump represents the electrical activity resulting from activation of a single INAD macromolecular complex (Scott and Zuker, 1998), which contains multiple copies of PLC, TRP, INAC, INAD, and probably other regulatory proteins (Montell, 1999). The bump amplitude and shape are determined by the activities of all TRP channels in the complex and reflect the state of channel regulation. The activity level of PLC or upstream signaling molecules does not affect the amplitude but will change the frequency and latency of bumps (Scott and Zuker, 1998). In isolated *tes* mutant photoreceptors, the amplitude and shape of bumps were the same as in wild-type (Figure 3A), indicating that signaling downstream of PLC is controlled appropriately in the mutants. However, *tes* mutant cells generated multiple bumps when stimulated with a flash of dim light (log[I] = −6), in contrast to wild-type and rescued *tes*^{2}*;p[trp-dCAMTA]* photoreceptors, which produced only a single bump (Figure 3B). The extra bumps in *tes* cells must have been caused by prolonged activities of either PLC or an upstream molecule and could account for the slow termination of macroscopic currents observed in *tes* mutants.

An increased bump number has been previously observed in mutants defective in rhodopsin deactivation, including *arr2* mutants (Scott et al., 1997). To test whether rhodopsin deactivation was impaired in *tes* flies, we genetically reduced G_q protein levels by introducing a $G_{\alpha q}^{1}$ mutation into *tes* mutant flies and examined the effect on the bump numbers. In wild-type photoreceptors, an activated rhodopsin molecule stimulates one G_q protein, which triggers a single quantum bump (Scott and Zuker,

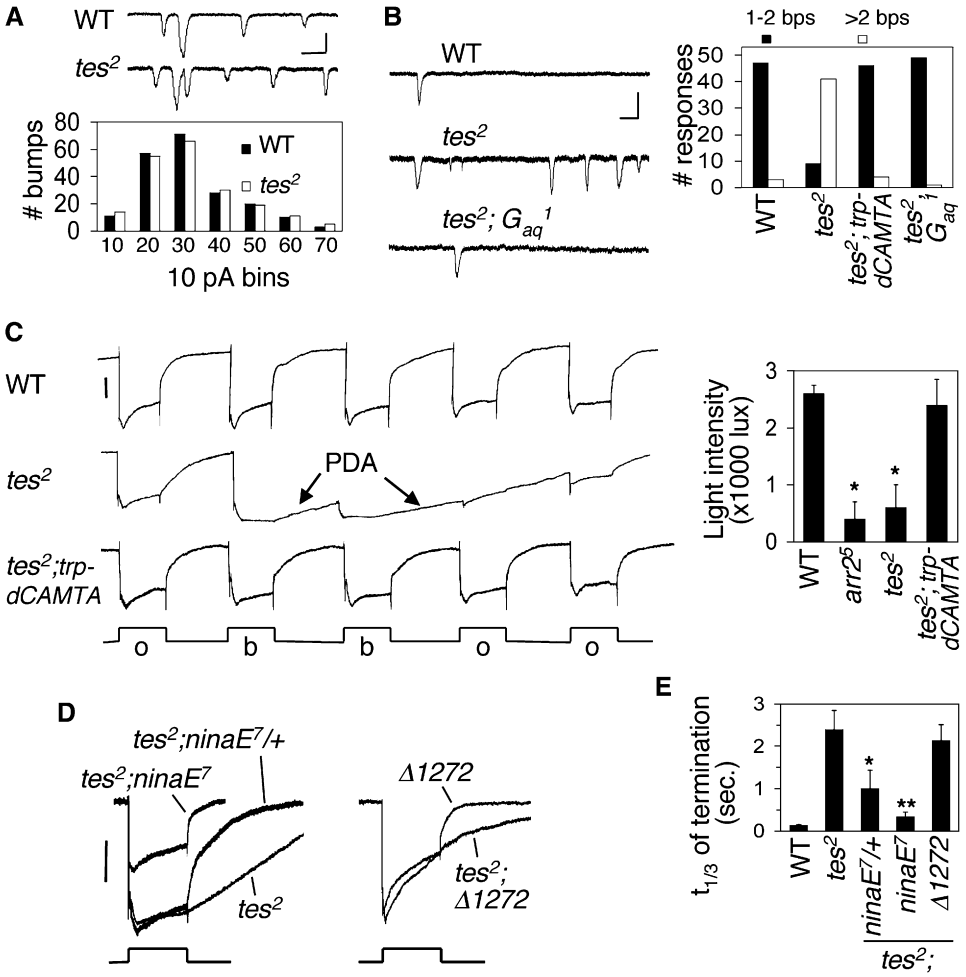

Figure 3. The Function of dCAMTA Is Critical for Rhodopsin Deactivation

(A) Quantum bumps in WT and *tes* photoreceptors have similar shapes (top) and amplitude distributions (bottom; total of 200 bumps were measured for each genotype). Photoreceptors were stimulated with constant light of log(I) = −6.5 (WT) or −7 (*tes*^{2}). Scale bars are 20 pA and 200 ms for all bump traces.

(B) A 10 ms light flash of log (I) = −6 triggered a single quantum bump in WT and multiple bumps in *tes* cells. A $G_{\alpha q}{}^1$ mutation (at log[I] = −4) and the expression of dCAMTA suppressed the extra bump activities in *tes* cells. Right panel: bump (bps) number categories of 50 positive responses from each genotype.

(C) Low-intensity blue light (650 lux) induced PDA in *tes* flies. All flies were dark reared. O = orange light, b = blue light. Right panel: minimum light intensities required for PDA production.

(D) *ninaE*^{7}, a hypomorphic Rh1 gene mutation, rescued the *tes* ERG phenotype completely, or partially as a heterozygote (left). The *TRP*$^{\Delta 1272}$ mutation had no effect on WT or *tes* response termination (right).

(E) Quantitative analyses of *ninaE*^{7} and *TRP*$^{\Delta 1272}$ effects on the termination speed of *tes* photoresponses.

1998). If rhodopsin regulation is normal in *tes* flies, the observed multiple bumps must have been triggered by a single G_q protein and should appear the same in *tes*,$G_{\alpha q}{}^1$ double mutants, except that the probability of response will be very low (Scott et al., 1997). Conversely, if the extra bumps are due to abnormal rhodopsin activity, the bump number should be reduced in *tes*,$G_{\alpha q}{}^1$ double-mutant photoreceptors because each bump is generated through a single G_q protein molecule. We found that the bump number was reduced to wild-type levels in the double mutants (Figure 3B). Thus, the results demonstrate that it is the deactivation of rhodopsin that is impaired in *tes* mutants.

To provide further evidence that *tes* mutants have defective rhodopsin deactivation, we examined blue-light-induced prolonged depolarization afterpotentials (PDA) in these flies. PDA is produced specifically when the number of activated rhodopsin molecules exceeds the number of available rhodopsin regulatory molecules (for example, Arr2) (Dolph et al., 1993). In mutants lacking rhodopsin regulators, since lower amounts of rhodopsin need to be activated, the light intensity required to produce PDA is

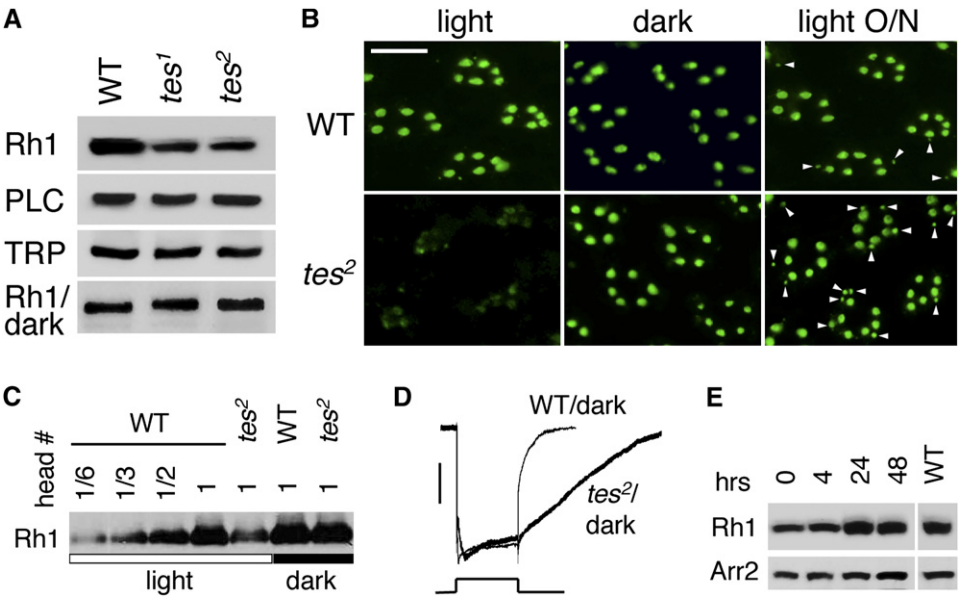

Figure 4. Rh1 Rhodopsin Undergoes Activity-Dependent Reduction in *tes* Flies

(A) Western blots showing reduced Rh1 levels in *tes* flies. All flies except the dark-reared ones were raised in an approximate 12 hr light/12 hr dark cycle.

(B) Immunostaining of eye cross-sections indicated that Rh1 proteins (in R1–R6 rhabdomeres) were reduced in light-exposed *tes* flies. Overnight (O/N) light stimulation of the dark-reared *tes* flies produced more Rh1 endocytotic vesicles (arrowheads) compared to WT. Scale bar = 10 μm.

(C) Rh1 level in 1-day old, light-exposed *tes* flies is 1/3 to 1/2 of that in WT. Dark-reared *tes* flies have an Rh1 level similar to WT.

(D) Despite the normal Rh1 level, the ERG responses terminated slowly in dark-reared *tes* flies.

(E) Rh1 level in *P[hs-dCAMTA];tes*^{2} flies recovered at 24 hr after heat shock. The Arr2 level is shown as a control.

much lower than that in wild-type (Vinos et al., 1997). We found that the situation was the same in *tes* mutants. The minimum light intensity needed to induce PDA was ~600 lux in *tes* versus ~2600 lux in wild-type and ~2400 lux in *tes;P[trp-dCAMTA]* flies (Figure 3C).

As the above experiments suggest a shortage of rhodopsin regulatory molecules in *tes* flies, we attempted to increase the termination speed of *tes* photoresponses by genetically reducing the levels of Rh1, the major rhodopsin in all outer (R1–R6) photoreceptor cells. For this purpose, we introduced *ninaE*^{7} (also known as *ninaE*^{P332}), a mutant allele of the Rh1 gene that expresses <1% of wild-type Rh1 protein levels (Washburn and O'Tousa, 1989), into the *tes*^{2} mutant background. In ERG recordings, the termination of photoresponses in *tes*^{2}*;ninaE*^{7} double mutants was as fast as wild-type (Figures 3D and 3E), although the amplitude of the responses was decreased. A similar effect was observed by introducing *ninaE*^{8}, another hypomorphic allele of the Rh1 gene, into the *tes* mutant background (data not shown). The decreased amplitude did not appear to be responsible for the improved termination of response since a deletion of the last four residues of the TRP protein (Δ1272), which reduces TRP levels and decreases the sustained response amplitude (Li and Montell, 2000), failed to improve the termination of *tes* responses (Figures 3D and 3E). In contrast, although the response amplitude was similar to wild-type, the termination speed in *tes*^{2}*;ninaE*^{7}/+ flies increased dramatically (Figures 3D and 3E). The time ($t_{1/3}$) required for a 1/3 recovery from the light response in the *tes*^{2}*;ninaE*^{7}/+ flies was 1.00 ± 0.42 s versus 2.40 ± 0.45 s for *tes*^{2} flies (Figure 3E). Thus, the *tes* mutant phenotype is rescued specifically by suppression of Rh1 activity, further confirming that the slowed photoresponse termination in *tes* mutants is due to a deficiency of rhodopsin-deactivating molecules.

Rh1 Rhodopsin Undergoes Activity-Dependent Reduction in *tes* Flies

Probably as a compensatory response to the uncontrolled rhodopsin activity, *tes* mutant photoreceptors downregulate the Rh1 protein level in a light-dependent manner. In the same ambient light conditions (~450 lux, in a cycle of 12 hr light/12 hr dark), *tes* mutants had significantly lower Rh1, but not PLC or TRP, levels than wild-type (Figures 4A–4C). This Rh1 reduction could have occurred through increased internalization and subsequent lysosomal degradation of Rh1 (Xu et al., 2004), as an overnight exposure of previously dark-reared flies to light produced significantly more Rh1-containing endocytotic vesicles in *tes* photoreceptors compared to wild-type (Figure 4B).

The reduction in Rh1 levels was not observed in dark-reared *tes* flies (Figures 4A–4C) in which Rh1 had not been activated. Despite normal Rh1 levels, the termination

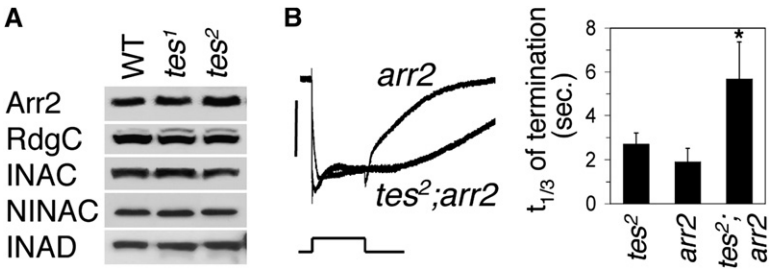

Figure 5. Known Visual Regulatory Proteins Do Not Mediate the dCAMTA Function

(A) Known regulatory molecules had normal protein levels in *tes* flies. Each lane was loaded with a single fly head.

(B) The photoresponse in $tes^2;arr2^5$ flies terminated much more slowly than that in each single mutant.

of light responses in these flies was at least as slow as that in light-exposed *tes* mutant flies (Figure 4D), indicating that the reduced Rh1 levels are not responsible for the slow-termination phenotype. On the contrary, the reduction in Rh1 levels could have occurred after the failure of Rh1 deactivation. Indeed, once the electrophysiological phenotype had been rescued in heat-shocked *P[hs-dCAMTA];tes*^{2} flies, Rh1 levels returned to normal (Figure 4E).

arr2 Is Not a Target Gene of dCAMTA

As Arr2 is the primary regulator of rhodopsin, a logical scenario for dCAMTA visual function could be that this transcription factor facilitates rhodopsin deactivation by promoting *arr2* gene expression. However, in western blots, we did not see any decrease in Arr2 protein levels in *tes* mutants (Figure 5A). Moreover, the termination speed of photoresponses in *tes;arr2*^{5} (a null allele of *arr2*) double mutants was much slower than that in either single mutant (Figure 5B), indicating that dCAMTA may function through a pathway different from Arr2. Thus, *arr2* is not a dCAMTA target gene in photoreceptors. In addition, the rhodopsin phosphatase RdgC and other proteins required for phototransduction termination, INAC, INAD, and NINAC, all had wild-type levels in *tes* flies (Figure 5A) and thus cannot be the mediators of dCAMTA visual function.

The F Box Protein dFbxl4 Mediates the Function of dCAMTA in Rhodopsin Regulation

To identify the dCAMTA target genes in the fly eye, we employed genome-wide microarray analyses to compare gene expression levels between wild-type and two *tes* mutant alleles, and also between heat-shocked *tes*^{2} and *P[hs-dCAMTA];tes*^{2} flies. At least five genes showed both decreased mRNA levels in *tes* alleles and recovered levels in *P[hs-dCAMTA];tes*^{2} flies. These genes encode an F box protein (CG1839), a cuticle protein (CG4784), a membrane protein (CG32372), a peptidase (CG32532), and a lipid-binding protein (CG7227). Using real-time RT-PCR, we confirmed that their expression was regulated in vivo by dCAMTA (Figure 6A), either directly or indirectly.

CG1839 is the fly homolog of the human F box and leucine-rich-repeat gene *Fbxl4* (Jin et al., 2004). We named it *dFbxl4* and further characterized its regulation by dCAMTA. We found that a CGCG-box-containing fragment within the *dFbxl4* promoter region bound to the CG-1 domain of dCAMTA, while another fragment immediately downstream of the CGCG box did not (Figure 6B). In addition, dCAMTA drove expression of a luciferase reporter gene in cultured cells through a 713 bp *dFbxl4* promoter sequence that contains the CGCG-box fragment, but not through a shorter one lacking the CGCG box (Figure 6C). The data indicate that the expression of *dFbxl4* is regulated directly by dCAMTA.

dFbxl4 has been previously reported to have high expression levels in photoreceptors (Xu et al., 2004). In situ hybridization experiments showed that the photoreceptor expression of *dFbxl4* decreased drastically in *tes* mutants (Figure 6D). To examine whether the loss of photoreceptor dFbxl4 was responsible for the *tes* mutant phenotype, we generated transgenic flies that express *dFbxl4* in *tes* photoreceptors using the *trp* promoter (Figure 6D). Both ERG and whole-cell recordings of photoreceptors showed that the slow-termination phenotype was fully rescued in *tes*^{2}*;P[trp-dFbxl4]* flies (Figure 6E). The phenotypic rescue was also observed in another transgenic fly that overexpresses dFbxl4 through the heat-shock promoter (Figure 6F). In contrast, heat-shock-driven expression of CG4784 and CG32372, another two dCAMTA target genes, had no significant effect on the *tes* phenotype (Figure 6F). Thus, *dFbxl4* is the target gene of dCAMTA that mediates its function in rhodopsin deactivation. Further evidence supporting the above model was provided by the observation that the light-dependent Rh1 reduction, which occurred in *tes* flies, was prevented by overexpression of dFbxl4 in *tes*^{2}*;P[trp-dFbxl4]* flies (Figure 6G).

Calmodulin Stimulates In Vivo dCAMTA Activity through Direct Protein Interactions

CAMTA transcription factors are characterized by their direct interaction with the Ca^{2+} sensor calmodulin (Bouche et al., 2002; Reddy et al., 2000; Yang and Poovaiah, 2002) and thus could be stimulated directly by Ca^{2+}/calmodulin. However, there is no experimental evidence that the calmodulin/CAMTA interaction is required for CAMTA activation.

To address this issue, we first mapped a calmodulin-binding site in dCAMTA with both calmodulin overlay (data not shown) and calmodulin-agarose binding assays. A glutathione S-transferase (GST)-fusion protein containing a 472 aa C-terminal fragment of dCAMTA bound to calmodulin-agarose beads, whereas GST alone or a GST-fused CG-1 fragment did not (see western blot beneath schematic in Figure 7A). This calmodulin-binding

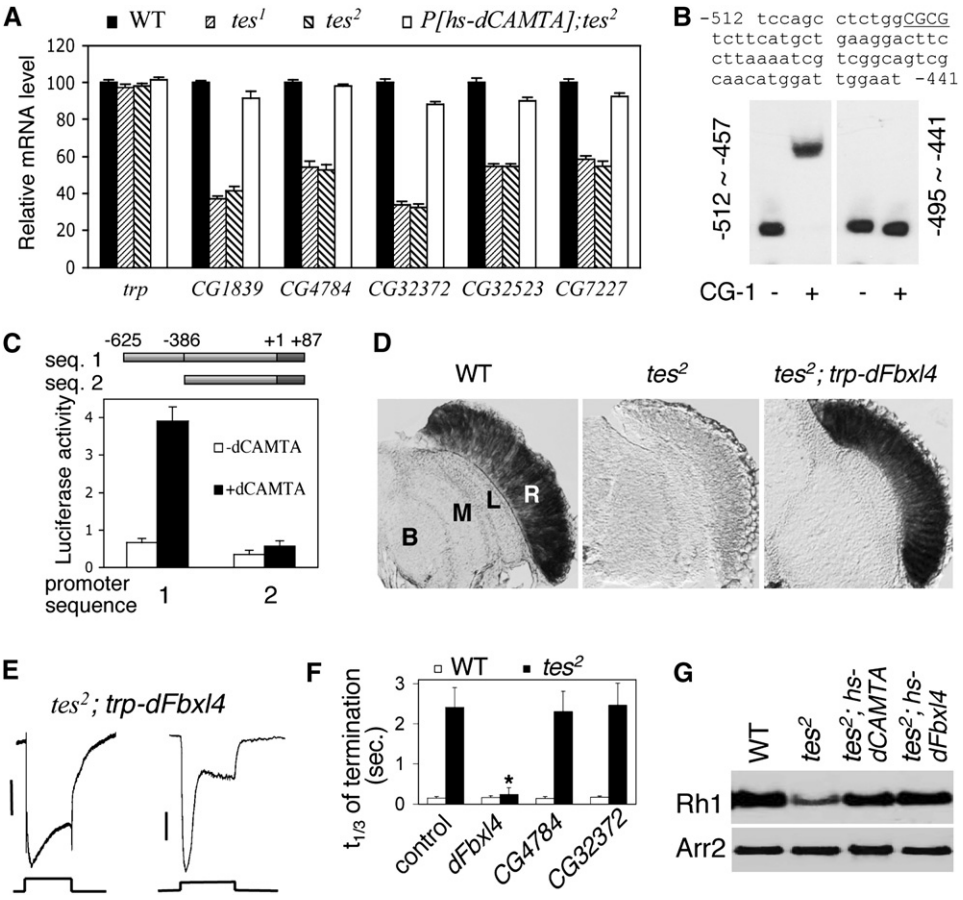

Figure 6. dFbxl4 Mediates the Function of dCAMTA in Rhodopsin Regulation

(A) RT-PCR comparison of gene-expression levels in *tes* mutants and heat-shocked *P[hs-dCAMTA];tes*^{2} flies with those in WT flies (normalized to 100%). *dFbxl4* is labeled as CG1839. Three data sets were averaged.

(B) In EMSA, the CGCG-containing fragment −512 ∼ −457 in the *dFbxl4* promoter region bound to the CG-1 domain of dCAMTA, while the fragment −495 ∼ −441 did not. The DNA sequence is shown on the top.

(C) In 293T cells, dCAMTA stimulated luciferase reporter-gene expression through a *dFbxl4* promoter sequence (seq. 1, −625 ∼ +87), but not through a shorter one (seq. 2, −386 ∼ +87) that lacks the CGCG box.

(D) In situ hybridization showing *dFbxl4* expression in photoreceptors of the marked genotypes. B = brain, L = lamina, M = medulla, R = retina.

(E) *trp* promoter-mediated photoreceptor expression of dFbxl4 rescued the *tes* phenotype in both ERG (left) and whole-cell (right) recordings.

(F) Effects of heat-shock-driven expression of dCAMTA target genes on the termination speed of WT and *tes* ERG responses. Controls are without transgene.

(G) Both *dFbxl4* and *dCAMTA* expression prevented Rh1 reduction in *tes* flies.

site appeared to be Ca^{2+} independent (data not shown) and might enable dCAMTA to interact with calmodulin even in the absence of nuclear Ca^{2+} ions after calmodulin translocates into the nuclei in response to Ca^{2+} (Deisseroth et al., 1998; Liao et al., 1999). Using smaller fragments, we further located the calmodulin-binding site to the region aa 1741–1923, which included three IQ motifs (Figure 7A).

As IQ motifs are usually involved in calmodulin binding (Rhoads and Friedberg, 1997), we generated I→N point mutations in each IQ motif of dCAMTA in an attempt to disrupt the dCAMTA/calmodulin interaction. In the calmodulin-agarose assay, mutation of the first IQ motif (I1803N), but not the second (I1829N) or third (I1852N), virtually abolished the binding between calmodulin and dCAMTA (Figure 7B).

Finally, to examine the functional significance of the dCAMTA/calmodulin interaction, we created transgenic flies expressing mutant dCAMTA proteins in a *tes* background. dCAMTA variants with mutations in the second or third IQ motif still rescued the *tes* mutant phenotype with effectiveness similar to a wild-type protein (Figure 7C). Although the dCAMTA variant lacking the first IQ motif also improved the termination of *tes* mutant light responses (from $t_{1/3}$ = 2.40 ± 0.45 s to $t_{1/3}$ = 1.27 ± 0.43 s), the termination speed was much slower compared with the expression of a wild-type protein ($t_{1/3}$ = 0.25 ± 0.11 s) (Figure 7C). The above data indicate that dCAMTA protein has

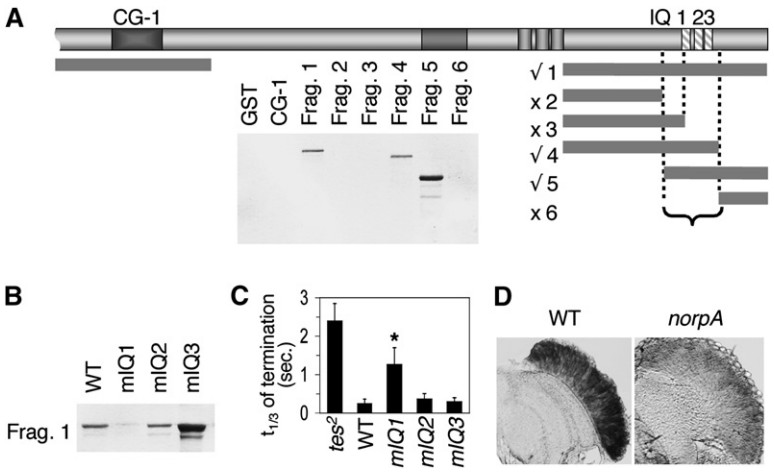

Figure 7. Calmodulin Stimulates In Vivo dCAMTA Activity through Direct Protein Interaction

(A) A calmodulin-agarose binding assay, which was performed in the presence of 2 mM Ca^{2+}, mapped a calmodulin-binding site of dCAMTA to a C-terminal IQ motif-containing region (bracket). GST was fused to all fragments for solubility and purification purposes.

(B) An I→N point mutation in IQ motif 1 (mIQ1) virtually abolished the calmodulin-binding capability of the longest C-terminal fragment of dCAMTA in (A). The fragments with a mutation in motif 2 or 3 had normal or enhanced binding, respectively.

(C) In transgenic flies, the mIQ1 dCAMTA protein had impaired capability of rescuing the *tes* phenotype.

(D) In situ hybridization showing decreased *dFbxl4* expression in *norpA* photoreceptors.

residual activity in the absence of calmodulin interaction. Nonetheless, higher dCAMTA activity, which is absolutely required for rapid rhodopsin deactivation, has to be stimulated by calmodulin. This calmodulin activation of dCAMTA is probably a process controlled by Ca^{2+}, as the expression of *dFbxl4* was significantly reduced in *norpA* photoreceptors that have virtually no rhodopsin-stimulated Ca^{2+} influx (Figure 7D).

DISCUSSION

dCAMTA-Dependent Rhodopsin/GPCR Regulation

The CAMTA protein family includes six members in plants, two in mammals, one in flies, and one in worms. They have been implicated in human tumor suppression (Barbashina et al., 2005; Katoh, 2003) and in plant responses to environmental stimuli (Reddy et al., 2000; Yang and Poovaiah, 2002). However, the specific signaling pathways regulated by CAMTAs had remained a mystery. In this work, we have shown that dCAMTA potentiates the deactivation of fly rhodopsin by promoting *dFbxl4* gene expression. It is plausible that other CAMTAs may also participate in the regulation of rhodopsin-related GPCRs in a similar manner.

As a transcription factor, dCAMTA may not function in a real-time manner like arrestins during rhodopsin deactivation. Once dCAMTA has induced the expression of sufficient dFbxl4, it could be temporarily dispensable for visual regulation. Indeed, significant rescue of *tes* mutant phenotypes persists for 2–3 days in heat-shocked *P[hs-dCAMTA];tes*^{2} flies even after dCAMTA protein levels have diminished (H.-S.L., unpublished data). Therefore, dCAMTA elicits a long-lasting effect on rhodopsin activity by strengthening the rhodopsin regulatory machinery at the gene-expression level. To our knowledge, dCAMTA is the first transcription factor identified to be involved in the plasticity of a GPCR regulatory machinery.

dFbxl4 contains an F box and at least ten leucine-rich repeats. Many F box proteins are subunits of the SCF-type E3 ubiquitin ligases and are responsible for substrate recruiting (Jin et al., 2004). Several GPCRs have been reported to undergo activity-dependent ubiquitination (Wojcikiewicz, 2004). It is possible that a dynamic, dFbxl4-mediated rhodopsin ubiquitination may abolish the rhodopsin-G_q interaction. Alternatively, given that both the F box and leucine-rich repeats are protein interaction domains, dFbxl4 could simply bind to rhodopsin to prevent G_q activation. Moreover, dFbxl4 may facilitate rhodopsin deactivation indirectly—for example, through the ubiquitination of an unknown rhodopsin-deactivating molecule in a manner similar to Mdm2-mediated β-arrestin ubiquitination (Shenoy et al., 2001). It will be interesting to determine exactly how dFbxl4 regulates the activity of rhodopsin.

Although dCAMTA is highly enriched in photoreceptor cells, it may function in other cells and tissues as well. Both *tes*^{1} and *tes*^{2} mutants have lower viability and fertility than wild-type, which are significantly improved by the leaky expression of dCAMTA in transgenic flies (J.H. and H.-S.L., unpublished data). In addition, we have detected moderate levels of *dCAMTA* mRNA at both embryonic and larval stages (H.-S.L., unpublished data). These observations may imply a more general role of dCAMTA in the regulation of GPCRs in all cell types. The photoreceptor cells might express much more dCAMTA protein merely to be accountable for the huge amount of rhodopsin protein and to ensure the high-speed deactivation of rhodopsin.

In mammalian cells, a similar CAMTA/Fbxl4 pathway could help prevent GPCRs from triggering the ERK cascade and thus avoid abnormal proliferation. A human CAMTA, CAMTA1, has been reported to be a candidate suppressor of oligodendrogliomas and neuroblastomas (Barbashina et al., 2005; Katoh, 2003).

Mechanisms of CAMTA-Mediated Transcription

Both human and plant CAMTAs activate transcription through a region next to the DNA-binding CG-1 domain (Bouche et al., 2002). The plant CG-1 domains recognize

DNA sequences that contain a CGCG box. Here we have demonstrated that the fly dCAMTA binds to the CGCG box to drive gene expression. Thus, stimulating gene transcription through the CGCG box could be a typical mode of CAMTA transcription activity.

Song et al. (2006) have recently reported that the human CAMTA2 functions as a coactivator of another transcription factor, Nkx2-5, to stimulate gene expression in COS cells (Song et al., 2006). This role of CAMTA could be a function that evolved later in animals, as no Nkx2-5-like transcription factor has been found in plants. In brief, although CAMTAs may stimulate transcription in different ways, binding to the CGCG box is more likely to be the primary mechanism.

Calmodulin Stimulation of CAMTAs

Through a series of calmodulin-dependent protein kinases (CaMKs) or calcineurin, Ca^{2+}/calmodulin stimulates various transcription factors in both neuron and immune cells (Cruzalegui and Bading, 2000; Rao et al., 1997; West et al., 2001). It had been suspected that certain transcription factors could be stimulated directly by calmodulin through protein-protein interaction. Without dependence on any mediator, these transcription factors would respond to Ca^{2+}/calmoduin in a more rapid and faithful manner. In this study, we have shown that mutation of the calmodulin-binding site in dCAMTA impairs its role in visual regulation. Our data have provided functional evidence that a CAMTA transcription factor is stimulated directly by calmodulin.

In the future, it will be important to characterize the detailed mechanism by which calmodulin stimulates CAMTA activity. Although we have been unable to detect a significant calmodulin signal in photoreceptor nuclei using an anti-calmodulin serum (J.H., and H.-S.L., unpublished data), it is still possible that a small amount of calmodulin may enter the nuclei to activate dCAMTA in response to Ca^{2+}. Alternatively, cytosolic calmodulin could stimulate nuclear transport of dCAMTA given that a nuclear localization sequence has been mapped next to the IQ motifs in the human CAMTA2 (Song et al., 2006). Interestingly, deletion of a large fragment that includes the IQ motifs did not significantly reduce in vitro transcription activity of CAMTA2 (Song et al., 2006), suggesting that an intramolecular inhibitory component, which is regulated through calmodulin binding, may also be located in the deleted region.

Potential Role of CAMTAs in the Maintenance of Intracellular Ca^{2+} Homeostasis

The activation of fly rhodopsin triggers PLC and TRP-channel-mediated Ca^{2+} influx (Hardie and Minke, 1992). Here we have shown that the Ca^{2+} sensor calmodulin stimulates dCAMTA and that the stimulated dCAMTA activity potentiates rhodopsin deactivation. Our results suggest that Ca^{2+}/calmodulin/dCAMTA may mediate a feedback regulation of rhodopsin activity through gene expression.

It is plausible that mammalian calmodulin/CAMTAs might also have such a feedback regulation on Ca^{2+}-stimulating GPCRs. This mechanism may enable the cell to remodel GPCR regulatory machineries according to altered cytosolic Ca^{2+} concentration and thus help to maintain intracellular Ca^{2+} homeostasis and prevent Ca^{2+}-dependent cell excitoxicity.

EXPERIMENTAL PROCEDURES

Fly Genetics

The genotype of wild-type flies is *cn,bw*. *tes* mutants were generated with the chemical mutagen EMS. The *tes* mutations in Figure 1F, Figures 2E and 2F, Figures 3D and 3E, Figure 4E, Figure 5B, Figures 6D–6G, and Figure 7C are *cn,tes*^{2}; all others are in the *cn,bw* background. All examined flies, except some in Figure 2F and Figure 4E, are <3 days old. The dark-reared flies were never exposed to light from the prepupal stage; all others were raised in an approximate 12 hr light (~450 lux, from regular fluorescent tubes)/12 hr dark cycle.

To generate *dCAMTA* transgenic flies, a wild-type *dCAMTA* cDNA or mutant ones with disrupted IQ motifs were subcloned into either a pCaSpeR-*trp(−400–+226)* or pCaSpeR-*hs* vector and injected into *w;cn,tes*^{2} flies. For *dFbxl4* and CG32372 transgenic flies, the GH11272 and RE09158 EST clones were inserted into the vectors, respectively. The CG4748 cDNA was obtained through PCR. To express proteins through heat shock, all flies (except those in Figure 2 and Figure 4E, which were shocked at ~2 days old) were shocked at the late pupal stage by immersing the fly vials in 37°C water bath for 1 hr and examined at 1 day old.

Electrophysiology

Electroretinograms were examined as previously described (Li and Montell, 2000). Fly eyes were stimulated with 5 s orange light pulses (4000 lux). To quantitate the speed of response termination, the time required for 1/3 recovery was measured. Prolonged depolarization afterpotentials were examined using the same setup, except with different color filters and light intensities. For each genotype, data from seven flies were averaged and the standard error of mean (SEM) was calculated.

For whole-cell recording of photoreceptor cells, the ommatidia of dark-reared flies were isolated in Ca^{2+}-free Ringer's solution according to a previously described protocol (Hardie et al., 1991). The pipette and bath solutions were (in mM) 100 potassium gluconate, 40 KCl, 2 $MgCl_2$, 0.1 EGTA, 5 ATP, 0.5 GTP, 10 HEPES (pH 7.15) and 130 NaCl, 5 KCl, 1.8 $CaCl_2$, 5 proline, 25 sucrose, 10 HEPES (pH 7.15), respectively. The resistance of recording pipettes was 5–6 MΩ. The series resistance was 15–20 MΩ, 75% of which was compensated. The seal resistance was >3 GΩ. Cells were clamped at −70 mV during recording. The stimulating light pulses (0.5 s) were delivered from a 100 W QTH light source (Oriel) to the cell after passing a high-speed shutter (76992, Oriel) and an orange filter. The signal was amplified using an Axopatch 200B, acquired at 1 kHz, and analyzed with pClamp 8 software (Axon Instruments, Inc.). Quantum bumps were recorded in the same configuration using either constant or 10 ms dim-light stimulations and filtered at 500 Hz. For each genotype, data from at least five different flies were recorded.

Antibodies

The dCAMTA antibodies were raised in rabbits against a GST-fused fragment (aa 275–653) that contains the CG-1 domain. An affinity column, which was created by coupling the same CG-1 fragment to Sepharose 4B, was used to purify the antibodies. The Arr2 antibodies were raised against a N-terminal fragment. The sources of other antibodies were DSHB (Rh1), C. Zuker (PLC), and C. Montell (all others).

Electron Microscopy and Immunohistochemistry

For EM and Rh1 labeling, fly heads were embedded in LR White resin, and eye cross-sections with a thickness of 200 nm or 1 μm, respectively, were cut to show the R1–R7 rhabdomeres of each ommatidium. A monoclonal Rh1 antibody (DSHB) and FITC-conjugated secondary antibodies were used for Rh1 labeling. For dCAMTA labeling, 10 μm head cryosections, which show a semilongitudinal view of photoreceptor cells, were costained with purified CG-1 antibodies, DAPI (for nuclei labeling), and Rh1 antibodies (to show rhabdomeres).

Electrophoresis Mobility Shift Assays

The DNA probes were created with PCR, and labeled with 3′ biotin (Pierce). Two micrograms of recombinant CG-1 fragment (aa 275–653) proteins was incubated with 20 fmol labeled probes, either with or without the presence of 4 pmol unlabeled probes, in 20 μl binding solution (Pierce LightShift Chemiluminescent EMSA Kit) for 20 min and then loaded onto 6% native PAGE gel.

Luciferase Assay

For the experiment in Figure 2, the probe DNAs for the EMSA were inserted upstream of a luciferase gene in a modified pGL3-basic vector (Promega), from which an intrinsic CGCG box in the multiple cloning region had been removed. In a same manner, the *dFbxl4* promoter sequences were fused to the luciferase gene. The constructs were introduced into 293T cells alone or together with a pcDNA3-*dCAMTA* DNA. After 24 hr, cells were harvested and examined with a luciferase assay system (Promega). Three sets of data were averaged.

Microarray Analyses and Real-Time RT-PCR

Total RNA was extracted from 1- to 3-day-old fly heads using Trizol reagent (Invitrogen). The probe syntheses and gene chip (*Drosophila* Genome 2.0 Array, Affymetrix) hybridization were carried out by Genome Explorations. For comparison between tes^2 and *P[hs-dCAMTA];*tes^2 flies, a 1 hr heat shock was applied to both types of fly 4 hr before RNA extraction.

Real-time RT-PCR was conducted using an ABI PRISM7700 and a SuperScript III Platinum One-Step kit (Invitrogen). Only the *P[hs-dCAMTA];*tes^2 flies were heat shocked, 24 hr before RNA extraction. The relative mRNA levels were calculated by setting the raw level of each gene in wild-type flies as 100%. See the Supplemental Data available with this article online for the primer sequences.

In Situ Hybridization

Digoxigenin (DIG)-labeled antisense RNA probe (for the last 600 bp of *dFbxl4*) was synthesized in vitro using SP6 polymerase and a pSPT18 vector (Roche) and hybridized to 10 μm horizontal cryosections of fly heads (<4 hr after eclosion) in DIG Easy Hyb buffer (Roche) at 65°C. After washes, the sections were incubated with AP-conjugated DIG antibodies, and the signals were developed with the AP substrates NBT and BCIP.

Calmodulin-Agarose Binding Assay

GST was fused to all protein fragments for solubility and purification purposes. Approximately 20 μg of each protein was incubated with 30 μl calmodulin agarose in Tris-buffered saline containing either 2 mM Ca^{2+} or 1 mM EDTA. After washes, proteins were eluted and subjected to SDS-PAGE and Coomassie staining.

Supplemental Data

Supplemental Data include Supplemental Experimental Procedures and can be found with this article online at http://www.cell.com/cgi/content/full/127/4/847/DC1/.

ACKNOWLEDGMENTS

We thank Craig Montell for support on the mutagenesis screen and valuable advice; Seung-Jae Lee and Hong Xu for assistance with the screen; Gregory Hendricks for help with tissue sections; Patrick J. Dolph for $arr2^5$ flies; Charles S. Zuker for $G_{\alpha q}{}^1$ flies; and Vivian Budnik, Marc R. Freeman, Jianwu Bai, and Zuoshang Xu for critical comments on the manuscript. This work was supported by NIH grant R01-AG022508 to H.-S.L.

Received: November 9, 2005
Revised: June 12, 2006
Accepted: September 4, 2006
Published: November 16, 2006

REFERENCES

Barbashina, V., Salazar, P., Holland, E.C., Rosenblum, M.K., and Ladanyi, M. (2005). Allelic losses at 1p36 and 19q13 in gliomas: correlation with histologic classification, definition of a 150-kb minimal deleted region on 1p36, and evaluation of CAMTA1 as a candidate tumor suppressor gene. Clin. Cancer Res. *11*, 1119–1128.

Bird, G.S., Aziz, O., Lievremont, J.P., Wedel, B.J., Trebak, M., Vazquez, G., and Putney, J.W., Jr. (2004). Mechanisms of phospholipase C-regulated calcium entry. Curr. Mol. Med. *4*, 291–301.

Bouche, N., Scharlat, A., Snedden, W., Bouchez, D., and Fromm, H. (2002). A novel family of calmodulin-binding transcription activators in multicellular organisms. J. Biol. Chem. *277*, 21851–21861.

Chen, B., Chu, T., Harms, E., Gergen, J.P., and Strickland, S. (1998). Mapping of Drosophila mutations using site-specific male recombination. Genetics *149*, 157–163.

Chyb, S., Raghu, P., and Hardie, R.C. (1999). Polyunsaturated fatty acids activate the Drosophila light-sensitive channels TRP and TRPL. Nature *397*, 255–259.

Claing, A., Laporte, S.A., Caron, M.G., and Lefkowitz, R.J. (2002). Endocytosis of G protein-coupled receptors: roles of G protein-coupled receptor kinases and beta-arrestin proteins. Prog. Neurobiol. *66*, 61–79.

Cruzalegui, F.H., and Bading, H. (2000). Calcium-regulated protein kinase cascades and their transcription factor targets. Cell. Mol. Life Sci. *57*, 402–410.

Deisseroth, K., Heist, E.K., and Tsien, R.W. (1998). Translocation of calmodulin to the nucleus supports CREB phosphorylation in hippocampal neurons. Nature *392*, 198–202.

Dolph, P.J., Ranganathan, R., Colley, N.J., Hardy, R.W., Socolich, M., and Zuker, C.S. (1993). Arrestin function in inactivation of G protein-coupled receptor rhodopsin in vivo. Science *260*, 1910–1916.

Fan, X.L., Zhang, J.S., Zhang, X.Q., Yue, W., and Ma, L. (2003). Differential regulation of beta-arrestin 1 and beta-arrestin 2 gene expression in rat brain by morphine. Neuroscience *117*, 383–389.

Gudermann, T., Grosse, R., and Schultz, G. (2000). Contribution of receptor/G protein signaling to cell growth and transformation. Naunyn Schmiedebergs Arch. Pharmacol. *361*, 345–362.

Hardie, R.C., and Minke, B. (1992). The trp gene is essential for a light-activated Ca2+ channel in Drosophila photoreceptors. Neuron *8*, 643–651.

Hardie, R.C., and Raghu, P. (2001). Visual transduction in Drosophila. Nature *413*, 186–193.

Hardie, R.C., Voss, D., Pongs, O., and Laughlin, S.B. (1991). Novel potassium channels encoded by the Shaker locus in Drosophila photoreceptors. Neuron *6*, 477–486.

Hurle, M.A. (2001). Changes in the expression of G protein-coupled receptor kinases and beta-arrestin 2 in rat brain during opioid tolerance and supersensitivity. J. Neurochem. *77*, 486–492.

Ikura, M., Osawa, M., and Ames, J.B. (2002). The role of calcium-binding proteins in the control of transcription: structure to function. Bioessays *24*, 625–636.

Jin, J., Cardozo, T., Lovering, R.C., Elledge, S.J., Pagano, M., and Harper, J.W. (2004). Systematic analysis and nomenclature of mammalian F-box proteins. Genes Dev. *18*, 2573–2580.

Katoh, M. (2003). Identification and characterization of FLJ10737 and CAMTA1 genes on the commonly deleted region of neuroblastoma at human chromosome 1p36.31-p36.23. Int. J. Oncol. *23*, 1219–1224.

Kristiansen, K. (2004). Molecular mechanisms of ligand binding, signaling, and regulation within the superfamily of G-protein-coupled receptors: molecular modeling and mutagenesis approaches to receptor structure and function. Pharmacol. Ther. *103*, 21–80.

Krupnick, J.G., and Benovic, J.L. (1998). The role of receptor kinases and arrestins in G protein-coupled receptor regulation. Annu. Rev. Pharmacol. Toxicol. *38*, 289–319.

Li, H.S., and Montell, C. (2000). TRP and the PDZ protein, INAD, form the core complex required for retention of the signalplex in Drosophila photoreceptor cells. J. Cell Biol. *150*, 1411–1422.

Liao, B., Paschal, B.M., and Luby-Phelps, K. (1999). Mechanism of Ca2+-dependent nuclear accumulation of calmodulin. Proc. Natl. Acad. Sci. USA *96*, 6217–6222.

Luttrell, L.M. (2005). Composition and function of g protein-coupled receptor signalsomes controlling mitogen-activated protein kinase activity. J. Mol. Neurosci. *26*, 253–264.

Montell, C. (1999). Visual transduction in Drosophila. Annu. Rev. Cell Dev. Biol. *15*, 231–268.

Montell, C. (2001). Physiology, phylogeny, and functions of the TRP superfamily of cation channels. Sci. STKE *2001*, RE1.

O'Tousa, J.E., Baehr, W., Martin, R.L., Hirsh, J., Pak, W.L., and Applebury, M.L. (1985). The Drosophila ninaE gene encodes an opsin. Cell *40*, 839–850.

Oyama, N., Urasawa, K., Kaneta, S., Sakai, H., Saito, T., Takagi, C., Yoshida, I., Kitabatake, A., and Tsutsui, H. (2005). Chronic beta-adrenergic receptor stimulation enhances the expression of G-Protein coupled receptor kinases, GRK2 and GRK5, in both the heart and peripheral lymphocytes. Circ. J. *69*, 987–990.

Pierce, K.L., Premont, R.T., and Lefkowitz, R.J. (2002). Seven-transmembrane receptors. Nat. Rev. Mol. Cell Biol. *3*, 639–650.

Ranganathan, R., Harris, G.L., Stevens, C.F., and Zuker, C.S. (1991). A Drosophila mutant defective in extracellular calcium-dependent photoreceptor deactivation and rapid desensitization. Nature *354*, 230–232.

Rao, A., Luo, C., and Hogan, P.G. (1997). Transcription factors of the NFAT family: regulation and function. Annu. Rev. Immunol. *15*, 707–747.

Reddy, A.S., Reddy, V.S., and Golovkin, M. (2000). A calmodulin binding protein from Arabidopsis is induced by ethylene and contains a DNA-binding motif. Biochem. Biophys. Res. Commun. *279*, 762–769.

Rhoads, A.R., and Friedberg, F. (1997). Sequence motifs for calmodulin recognition. FASEB J. *11*, 331–340.

Satoh, A.K., and Ready, D.F. (2005). Arrestin1 mediates light-dependent rhodopsin endocytosis and cell survival. Curr. Biol. *15*, 1722–1733.

Scott, K., and Zuker, C. (1997). Lights out: deactivation of the phototransduction cascade. Trends Biochem. Sci. *22*, 350–354.

Scott, K., and Zuker, C.S. (1998). Assembly of the Drosophila phototransduction cascade into a signalling complex shapes elementary responses. Nature *395*, 805–808.

Scott, K., Sun, Y., Beckingham, K., and Zuker, C.S. (1997). Calmodulin regulation of Drosophila light-activated channels and receptor function mediates termination of the light response in vivo. Cell *91*, 375–383.

Shenoy, S.K., and Lefkowitz, R.J. (2003). Multifaceted roles of beta-arrestins in the regulation of seven-membrane-spanning receptor trafficking and signalling. Biochem. J. *375*, 503–515.

Shenoy, S.K., McDonald, P.H., Kohout, T.A., and Lefkowitz, R.J. (2001). Regulation of receptor fate by ubiquitination of activated beta 2-adrenergic receptor and beta-arrestin. Science *294*, 1307–1313.

Song, K., Backs, J., McAnally, J., Qi, X., Gerard, R.D., Richardson, J.A., Hill, J.A., Bassel-Duby, R., and Olson, E.N. (2006). The transcriptional coactivator CAMTA2 stimulates cardiac growth by opposing class II histone deacetylases. Cell *125*, 453–466.

Vinos, J., Jalink, K., Hardy, R.W., Britt, S.G., and Zuker, C.S. (1997). A G protein-coupled receptor phosphatase required for rhodopsin function. Science *277*, 687–690.

Washburn, T., and O'Tousa, J.E. (1989). Molecular defects in Drosophila rhodopsin mutants. J. Biol. Chem. *264*, 15464–15466.

West, A.E., Chen, W.G., Dalva, M.B., Dolmetsch, R.E., Kornhauser, J.M., Shaywitz, A.J., Takasu, M.A., Tao, X., and Greenberg, M.E. (2001). Calcium regulation of neuronal gene expression. Proc. Natl. Acad. Sci. USA *98*, 11024–11031.

Wojcikiewicz, R.J. (2004). Regulated ubiquitination of proteins in GPCR-initiated signaling pathways. Trends Pharmacol. Sci. *25*, 35–41.

Xu, H., Lee, S.J., Suzuki, E., Dugan, K.D., Stoddard, A., Li, H.S., Chodosh, L.A., and Montell, C. (2004). A lysosomal tetraspanin associated with retinal degeneration identified via a genome-wide screen. EMBO J. *23*, 811–822.

Yang, T., and Poovaiah, B.W. (2002). A calmodulin-binding/CGCG box DNA-binding protein family involved in multiple signaling pathways in plants. J. Biol. Chem. *277*, 45049–45058.