Dissecting independent channel and scaffolding roles of the *Drosophila* transient receptor potential channel

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Drosophila transient receptor potential (TRP) serves dual roles as a cation channel and as a molecular anchor for the PDZ protein, INAD (inactivation no afterpotential D). Null mutations in *trp* cause impairment of visual transduction, mislocalization of INAD, and retinal degeneration. However, the impact of specifically altering TRP channel function is not known because existing loss-of-function alleles greatly reduce protein expression. In the current study we describe the isolation of a set of new *trp* alleles, including trp^{14} with an amino acid substitution juxtaposed to the TRP domain. The trp^{14} flies stably express TRP and display normal molecular anchoring, but defective channel function. Elimination of the anchoring function alone in $trp^{\Delta 1272}$, had minor effects on retinal morphology whereas disruption of channel function caused profound light-induced cell death. This retinal degeneration was greatly suppressed by elimination of the Na^+/Ca^{2+} exchanger, CalX, indicating that the cell death was due primarily to deficient Ca^{2+} entry rather than disruption of the TRP-anchoring function.

Introduction

The transient receptor potential (TRP) superfamily is comprised of a large group of related cation channels that function in sensory processes ranging from phototransduction to touch, hearing, taste, olfaction, osmosensation, and thermosensation (for review see Montell, 2005b). As such, dissecting the specific contributions of TRP channels is central to understanding each of these sensory modalities. It is generally accepted that the roles of mammalian TRP proteins is to mediate influx of cations, such as Ca^{2+} and Na^+. However, in the case of *Drosophila* TRP, which is the founding member of the superfamily that is required for phototransduction (Montell and Rubin, 1989), the protein functions both as a Ca^{2+}-permeable channel and as a molecular anchor (Li and Montell, 2000; Tsunoda et al., 2001).

Many of the proteins that are essential for fly visual transduction are organized into a large macromolecular complex, referred to as the signalplex (for review see Montell, 2005a). The molecular scaffold that nucleates the signalplex, inactivation no afterpotential D (INAD; Shieh and Zhu, 1996), consists of multiple PDZ protein interaction modules and appears to be constantly associated with TRP as well as two other important signaling proteins, PLC (encoded by *norpA* [no receptor potential A, encodes PLC]) and PKC (encoded by *inaC* [*inactivation no afterpotential C*, encodes PKC]). These three core binding proteins depend on INAD for localization in the phototransducing compartment of the fly photoreceptor cells, the rhabdomeres (Chevesich et al., 1997; Tsunoda et al., 1997). In addition, at least five other signaling proteins appear to associate with INAD (Huber et al., 1996; Chevesich et al., 1997; Xu et al., 1998; Wes et al., 1999; Goel et al., 2001) and these latter proteins may interact dynamically with INAD. However, none of these noncore binding proteins requires interaction with INAD for normal localization.

A surprising finding is that there is a reciprocal requirement for association of TRP and INAD for concentration of these two proteins in the rhabdomeres (Li and Montell, 2000; Tsunoda et al., 2001). Deletion of the COOH-terminal four residues in TRP destroys the PDZ binding site and results in mislocalization of INAD (Li and Montell, 2000). In turn, the rhabdomeral distributions of PKC and PLC are also disrupted. The interaction between TRP and INAD is not necessary for targeting of these proteins, but rather for subsequent retention in the rhabdomeres. Also, unexpected was the finding that

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Abbreviations used in this paper: Arr2, arrestin2; CalX, Na^+/Ca^{2+} exchanger; ERG, electroretinogram; hsp70, heat shock protein 70; INAC, inactivation no afterpotential C, encodes PKC; INAD, inactivation no afterpotential D; *ninaE, neither inactivation nor afterpotential E* (encodes Rh1); *norpA, no receptor potential A*, encodes PLC; RDGC, retinal degeneration C; Rh1, rhodopsin 1; TRP, transient receptor potential; TRPγ, transient receptor potential γ; TRPL, transient receptor potential-like.

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interference with the direct interactions between TRP and INAD had no major impact on activation of the TRP channels (Li and Montell, 2000), which in *Drosophila* photoreceptor cells is very rapid and occurs within milliseconds. These data demonstrate that the TRP channel functions as a molecular anchor, in addition to its more appreciated role as a cation channel.

The dual roles of TRP raise the question as to the impact of altering the channel activity independent of effects on the anchoring function. Null mutations in TRP result in light-dependent retinal degeneration, in addition to causing a transient response to bright light (Cosens and Perry, 1972; Chevesich et al., 1997). Retinal degeneration in fly photoreceptor cells is a common phenomenon that occurs as a result of mutations in nearly any protein important for phototransduction (for review see Pak, 1994; Montell, 1999). However, in most cases the mechanism underlying the retinal degeneration has not been clarified. In some mutants retinal degeneration occurs as a result of formation of stable rhodopsin–arrestin complexes, which in turn lead to endocytosis of rhodopsin (Alloway et al., 2000; Kiselev et al., 2000; Orem and Dolph, 2002). Ca^{2+} overload due to expression of a constitutively active TRP channel can also lead to rapid cell death in fly photoreceptor cells (Yoon et al., 2000; Wang et al., 2005). However, the mechanism underlying the retinal degeneration in *trp*-null mutant flies is not known. In particular, it is not clear whether the light-dependent retinal degeneration due to loss of *trp* function results from disruption of the anchoring role, or from lower Ca^{2+} influx during light stimulation. This question has not been possible to address because the existing loss-of-function mutations in *trp* have major impacts on protein levels and consequently disrupt both TRP functions (Montell and Rubin, 1989).

In addition to TRP, there are two related cation channels expressed in photoreceptors, transient receptor potential-like (TRPL), and transient receptor potential γ (TRPγ; Phillips et al., 1992; Xu et al., 2000). Currently, there are no loss-of-function mutations in TRPγ and elimination of TRPL has only subtle effects on the photoresponse (Niemeyer et al., 1996; Reuss et al., 1997; Leung et al., 2000). Nevertheless, TRPL contributes to phototransduction as flies that are missing both TRP and TRPL are blind (Niemeyer et al., 1996; Reuss et al., 1997).

In the current report, we describe the isolation of multiple new *trp* alleles, including one (trp^{14}) that specifically affected the channel function, but not the molecular anchoring role. In contrast to the wild-type light response, we found that in trp^{14} photoreceptor cells, the light response was transient. This phenotype resulted from a missense mutation in TRP juxtaposed to the highly conserved TRP domain (for review see Montell, 2005b). In addition, we found that the light-induced retinal degeneration was as severe in trp^{14} flies as in *trp*-null flies, trp^{P343}. Conversely, elimination of the TRP–INAD interaction had relatively minor effects on the morphology of the photoreceptor cells. Finally, the retinal degeneration associated with either trp^{14} or trp^{P343} was suppressed by a loss-of-function mutation in the Na^+/Ca^{2+} exchanger, CalX. These results demonstrate that the cell death in *trp* mutant photoreceptor cells is due primarily to disruption of TRP channel activity and decreased light-dependent Ca^{2+} influx, rather than elimination of the TRP anchoring role.

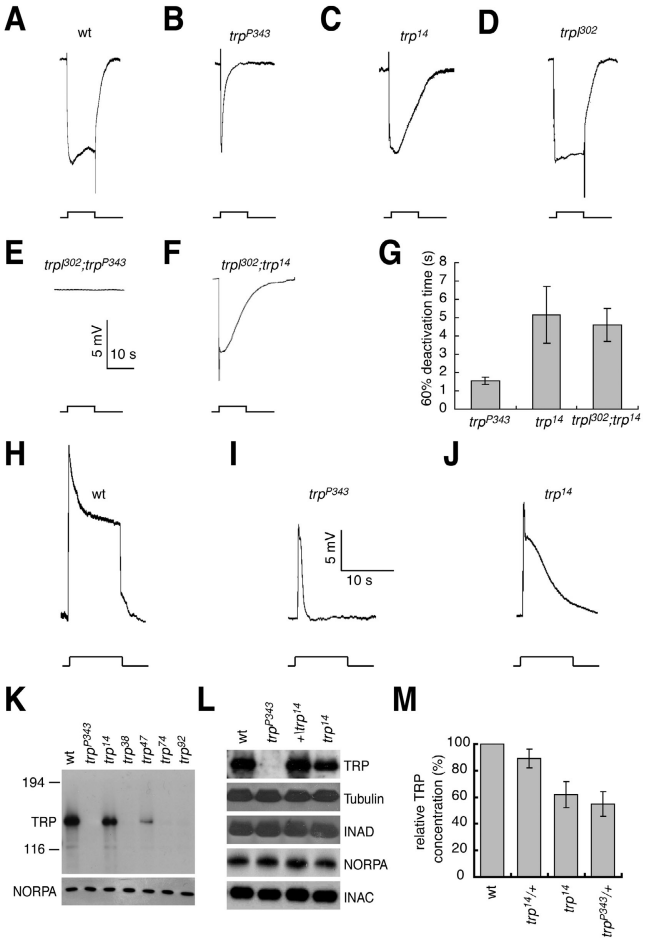

Figure 1. **ERG phenotype and expression of TRP in trp^{14} flies.** (A–F) ERG recordings. Flies <1 d after eclosion were dark-adapted for 2 min before exposure to a 10-s pulse of orange light (indicated by the event marker below the ERGs). (A) Wild type (wt); (B) trp^{P343}; (C) trp^{14}; (D) $trpl^{302}$; (E) $trpl^{302};trp^{P343}$; (F) $trpl^{302};trp^{14}$. (G) Time required for 60% return to the dark state. Shown are the mean times in seconds (s) based on ERGs obtained from ≥20 flies of the indicated genotypes. SDs are indicated. (H–J) Photoreceptor cell responses assayed by intracellular recordings. 1-d-old flies were dark adapted for 2 min before being exposed to a 10-s pulse of orange light. Scale bars are provided indicating the amplitude (mV) and timescale (s). (H) wt; (I) trp^{P343}; (J) trp^{14}. (K) Expression of TRP in mutant alleles assayed on Western blots. Head extracts were prepared from flies <1 d after eclosion and a Western blot was first probed with anti-TRP antibodies and subsequently with anti-NORPA antibodies to provide a loading control. The positions of protein size markers (kD) are indicated. (L) Expression levels of core members of the signalplex in trp^{14}. The Western blot was first probed with anti-TRP antibodies and then reprobed with anti-INAD, anti-NORPA, anti-INAC, and anti-tubulin antibodies. (M) Relative TRP protein levels in trp^{14} flies. The TRP protein level in trp^{14} heads was compared with wt, trp^{P343}, and trp^{14} heterozygotes. The intensities of the bands were determined using a phosphoimager. SDs are indicated.

Results

Identification of new *trp* alleles

To identify new alleles of the *trp* locus, we screened the recently isolated collection of chemically induced third chromosome mutations, which display defects in the electroretinogram (ERG) recording (for details see Wang et al., 2005). Exposure of wild-type flies to light results in two discriminable components in the ERG (Fig. 1 A). These include a sustained corneal

negative maintained component arising from responses of all retinal cells (photoreceptor cells and pigment cells) and on- and off-transients emanating from activity in the second-order neurons in the optic lobes (for review see Montell, 1999). The classic *trp* phenotype is characterized by a transient response to light (Fig. 1 B), resulting from rapid light-dependent inactivation of the remaining TRPL cation channel.

We crossed each of the third chromosome mutations to the strong trp^{P343} allele and identified five that failed to complement the recessive TRP phenotype and therefore represented new *trp* alleles. Four of the new *trp* alleles exhibited a transient ERG phenotype indistinguishable from trp^{P343} (unpublished data), whereas the phenotype of the fifth, trp^{14} was distinct in that the decline in the light response was much slower than in trp^{P343} or other alleles isolated in this or previous studies (Fig. 1, C and G; 60% deactivation time: trp^{P343}: 1.5 ± 0.2 s, trp^{14}: 5.2 ± 1.6 s). The trp^{14} phenotype was due to an autonomous defect in the photoreceptor cells, rather than the pigment cells, as the slower decline in the receptor potential was evident in single photoreceptor cells assayed by performing intracellular recordings (Fig. 1, H–J).

Table I. **Molecular defects in *trp* alleles**

Allele	Type of mutation	Location	Specific change	TRP protein level
trp^{P343}	Splice site	Intron 7; first base of 5′ splice site	GT to AT	ND
trp^{14}	Two missense	Pore loop; between TM6 and TRP box 1	L612F; R671N	60% wild-type level
trp^{38}	Promoter[1]			ND
trp^{47}	Two missense	Adjacent to TRP box 1; TRP domain, between boxes 1 and 2	T674A; W684R	5% wild-type level
trp^{74}	Missense	Fourth ankyrin repeat	G167E	ND
trp^{92}	Deletion/ frameshift	Transmembrane domain 1	Deletion of G in codon 336; stop after adding three residues	ND

The *trp* genomic DNA encoding the transcribed region was sequenced for each *trp* allele. ND, not detected.
[1]No mutation was found in trp^{38}, suggesting that there was a mutation affecting expression of the mRNA.

Molecular defects and TRP expression in *trp* alleles

Currently, all of the existing loss-of-function mutations cause large reductions in protein levels, although the molecular lesions have not been defined. Among the extant *trp* alleles, the one with the strongest phenotype is trp^{P343}. We sequenced the trp^{P343} genomic region and found that the TRP protein coding region was identical to wild type. Rather, there was a mutation in a conserved 5′ splice site that was essential for mRNA splicing (Table I). The mutation presumably results in instability of the mRNA, as no trp^{P343} mRNA is detected (Montell and Rubin, 1989). Given the strong phenotype and lack of mRNA or TRP protein expressed in trp^{P343} (Montell and Rubin, 1989), this allele would appear to represent a null.

To examine the levels of TRP protein expressed in the new alleles described here, we performed Western blots. Among those alleles that displayed a phenotype typical of trp^{P343}, three did not express any detectable TRP protein (Fig. 1 K, trp^{38}, trp^{74}, and trp^{92}), whereas a fourth expressed very low levels of TRP (Fig. 1 K, trp^{47}). This was in contrast to trp^{14} flies in which TRP was expressed at ∼60% the level as in wild type (Fig. 1, K–M). Other rhabdomeral proteins including INAD, INAC, NORPA, and Rh1 (rhodopsin 1) were expressed at comparable levels in trp^{14}, trp^{P343}, and wild-type flies (Fig. 1 L).

To determine the molecular defects associated with the five new *trp* alleles, we sequenced the genomic DNA and compared the sequences to that of the original isogenized stock used to conduct the mutagenesis. Among the four alleles that expressed very low or no detectable TRP, one had a frameshift mutation resulting in premature translation termination (trp^{92}), two had missense mutations (trp^{47} and trp^{74}), and one had no mutation in the transcribed region and therefore may contain a mutation affecting the *trp* promoter (Table I, trp^{38}). The allele (trp^{14}) expressing TRP at 60% wild-type levels, and which exhibited a phenotype distinct from other *trp* alleles, had two missense mutations. One of these mutations changed an amino acid in the pore loop between transmembrane domains five and six (residue 612; leucine to phenylalanine), whereas the other was situated between the sixth transmembrane segment and a highly conserved sequence referred to as TRP box 1 (residue 671; arginine to glutamine).

Normal anchoring function in trp^{14} mutant

Given that TRP has dual functions as a molecular anchor and as a cation channel (Li and Montell, 2000; Tsunoda et al., 2001), we considered whether the TRP phenotype in trp^{14} was a consequence of perturbation of the anchoring role. *Drosophila* compound eyes consist of ∼800 repetitive units, referred to as ommatidia, each of which includes six outer photoreceptor cells (R1-6) and a central R7 or R8 cell in the distal region of the retina. Each photoreceptor cell contains a microvillar segment, the rhabdomere, where most of the proteins that function in phototransduction, such as the core members of the signalplex, are concentrated. These include TRP, protein kinase C (INAC), phospholipase C (NORPA), and INAD (Fig. 2 A). Mutations that eliminate or disrupt the anchoring role of TRP result in mislocalization of these core members such that they are present in both the rhabdomeres and cell bodies (Fig. 2 B; Chevesich et al., 1997; Tsunoda et al., 1997; Li and Montell, 2000; Tsunoda et al., 2001). However, other INAD binding partners, such as Rh1, do not depend on TRP for normal localization (Fig. 2 B; Li and Montell, 2000). We found that in trp^{14} photoreceptor cells, each of the core and other rhabdomeral proteins examined displayed a rhabdomere localization pattern indistinguishable from wild type (Fig. 2 C). Consistent with these data, we found that INAD coimmunoprecipitated effectively with the TRP^{14} protein (Fig. 2 E). These data indicate that the TRP phenotype in trp^{14} flies is not due to an alteration

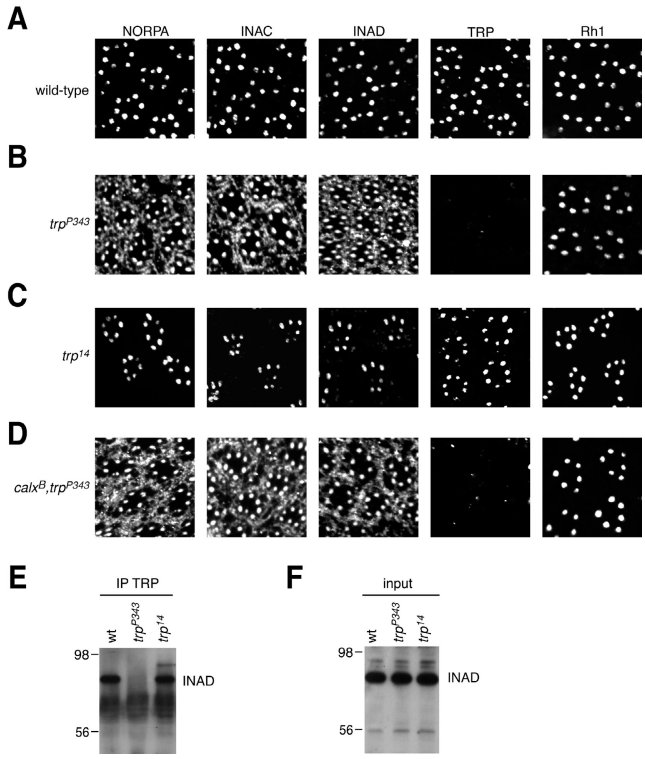

Figure 2. **Normal anchoring role of TRP in *trp*^{14} flies.** (A–D) Examination of the spatial distributions of INAD and the core INAD binding proteins. The cross sections of compound eyes were obtained from flies 5 d after eclosion maintained under a 12-h light–12-h dark cycle. Sections from flies of the following genotypes were probed with anti-NORPA, anti-INAC, anti-INAD, anti-TRP, and anti-Rh1 antibodies: (A) wild type; (B) *trp*^{P343}; (C) *trp*^{14}; and (D) *calx*^{B},*trp*^{P343}. (E) Normal interaction between INAD and TRP in *trp*^{14}. Fly head extracts were prepared and immunoprecipitations (IPs) were performed with anti-TRP antibodies. A Western blot containing the IPs was probed with anti-INAD antibodies. The positions of two protein size markers are indicated. (F) A Western blot containing 2% of the total extracts used for the coIPs was probed with anti-INAD antibodies.

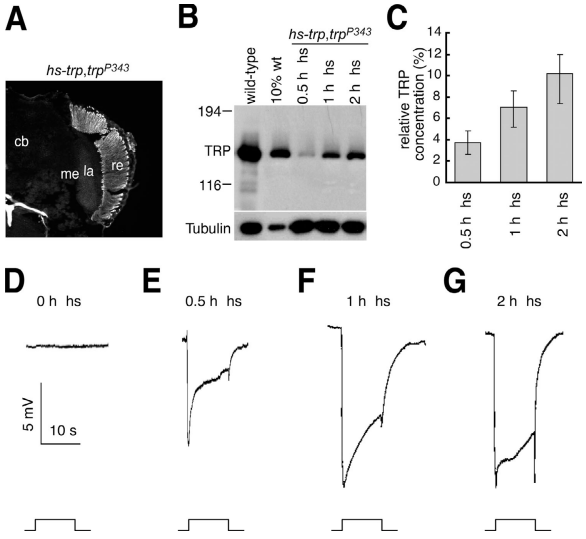

Figure 3. **Expression of low levels of TRP rescues the *trp* ERG phenotype.** (A) Expression of *hs-trp* results in expression primarily in the retina. Newly eclosed *hs-trp,trp*^{P343} flies were heat shocked for 2 h at 37°C and frozen sections, which were prepared 24 h later, were stained with anti-TRP antibodies. cb, central brain; la, lamina; me, medulla; re, retina. (B) Western blot showing TRP protein levels in *hs-trp,trp*^{P343} flies exposed to heat shock (hs) treatment of the indicated durations. The heat shocks were performed on newly eclosed flies and the head extracts were prepared 24 h later. The second lane from the left contains 10% the quantity of extracts (from wild-type fly heads) loaded in the other lanes. The same blot was reprobed with anti-tubulin antibodies. (C) Relative levels of TRP expressed in *trpl;hs-trp,trp*^{P343} flies exposed to different heat shock durations. The TRP protein levels after different heat shock times were compared with the band intensity obtained using 10% as much wild-type extracts. The quantification was performed using a phosphoimager. SDs are indicated. (D–G) ERG recordings obtained in *trpl;hs-trp,trp*^{P343} flies exposed to heat shock treatments of different lengths. Flies were heat shocked for either 0.5, 1, or 2 h at 37°C immediately after eclosion and the ERGs were performed 1 d later by dark adapting the flies for 2 min and exposing them to a 10-s pulse of orange light.

in the TRP anchoring function. Rather, they raise the possibility that the phenotype is due to a defect in TRP channel function.

Transient light response in *trp*^{14} was due to mutation in *trp* rather than low levels of expression or mislocalization of TRP

In *trp* flies such as *trp*^{P343} the transient potential ERG phenotype is a consequence of inactivation of the TRPL channel during constant light stimulation. However, in *trpl* mutant flies, which express TRP but not TRPL, the ERG response is maintained during a typical 5–10-s light pulse (Fig. 1 D). To examine TRP^{14} channel function independent of TRPL, we introduced the *trp*^{14} allele in a *trpl*-null mutant (*trpl*^{302}) background (Niemeyer et al., 1996). As previously shown, *trpl*^{302};*trp*^{P343} flies are blind as they do not express TRPL or TRP (Niemeyer et al., 1996; Reuss et al., 1997). Flies harboring just the *trpl*^{302} mutation show a response to 10 s of light similar to wild type, because these flies express wild-type TRP. In contrast, *trpl*^{302};*trp*^{14} flies displayed a TRP phenotype similar to *trp*^{14} (Fig. 1, F and G; 60% deactivation time: *trp*^{P343} 1.5 ± 0.2 s, *trp*^{14}: 5.2 ± 1.6 s, *trpl*^{302};*trp*^{14}: 4.2 ± 0.8 s). These data indicate that the transient light response in *trp*^{14} flies was due to disruption of TRP channel function.

To exclude that the *trp*^{14} phenotype was a consequence of mislocalization of TRP^{14}, we spatially localized the mutant protein. We performed immunostaining experiments and found that TRP^{14} was detected exclusively in the rhabdomeres, as was the case for wild-type TRP (Fig. 2, A and C). Therefore, the transient light response in *trp*^{14} was not due to mislocalization of the TRP protein.

Given that *trp*^{14} flies express a 40% lower concentration of TRP, we tested whether a transient light response could be induced by expression of low levels of TRP. Therefore, we generated transgenic flies that expressed varying levels of wild-type TRP under the control of the heat-shock promoter (*hs-trp*) and placed the transgene in a *trpl*^{302};*trp*^{P343} genetic background. Even though use of the *heat shock protein 70* (*hsp70)* promoter typically results in widespread expression, the TRP protein expressed under the control of the *hsp70* promoter was found exclusively in the retina and not in the optic lobes or elsewhere in the adult head (Fig. 3 A). To induce different low levels of TRP, we exposed the *hs-trp* flies to 30, 60, and 120 min heat-shock treatments, which resulted in the

production of ~4%, 7%, and 10% of the wild-type levels of TRP, respectively (Fig. 3, B and C). Expression of only ~10% the normal concentration of TRP restored an ERG in $trpl^{302}$;trp^{P343} flies, which was not transient (Fig. 3 G). Even 4% the normal levels of TRP did not cause a transient light response similar to trp^{14}, although the amplitude of the ERG was reduced (Fig. 3 E). These data indicate that the trp^{14} mutant phenotype was due to the mutation of the TRP^{14} protein, rather than simply due to expression of low levels or mislocalized TRP protein.

Channel activity critical to prevent light-dependent retinal degeneration

Strong loss-of-function mutations in *trp*, such as in trp^{P343}, result in light dependent retinal degeneration (Fig. 4). Considering TRP has dual channel and nonchannel roles, disruption of either function could potentially cause retinal degeneration. To address which of these two roles is more critical to prevent retinal cell death, we examined the morphology of $trp^{\Delta 1272}$ (Li and Montell, 2000) and trp^{14} ommatidia, which display specific defects in the scaffold and TRP channel functions, respectively. Wild-type ommatidia contain eight photoreceptor cell rhabdomeres, seven of which are present in any given plane regardless of their age or whether the flies were maintained in the dark or under a light–dark cycle (Fig. 4).

We found that the retinal degeneration in trp^{14} and trp^{P343} was much more severe than that in $trp^{\Delta 1272}$ flies. In both trp^{P343} and trp^{14} flies, the rhabdomeres began to disappear between 7 and 10 d after eclosion and almost no rhabdomeres remained after 14 d of exposure to a 12-h light–12-h dark cycle (Fig. 4, A and B). By contrast most $trp^{\Delta 1272}$ flies maintained a full complement of seven rhabdomeres after 14 d of a light–dark cycle (Fig. 4 A). Nevertheless, the size of the rhabdomeres was typically smaller than in similarly aged wild-type and large intracellular vacuoles were present inside the cell bodies indicating that retinal degeneration had initiated (Fig. 4 B). By 30 d after eclosion, most of the rhabdomeres in these flies had degenerated (unpublished data). Thus, the retinal degeneration was much more severe in trp^{P343} and trp^{14} than $trp^{\Delta 1272}$, indicating that the TRP channel function rather than the scaffold function was more critical to prevent the retinal cell death.

The retinal degeneration resulting from defects in TRP function was suppressed by maintaining the flies in the dark, which keeps the TRP channels in an inactive state. To assess the extent of suppression, we maintained the flies in the dark for 30 d, which was more than twice as long the 14-d light–dark cycle that caused elimination of almost all rhabdomeres in trp^{14} or trp^{P343} flies. In dark-maintained $trp^{\Delta 1272}$ flies, seven rhabdomeres were present in ommatidia, although the size of the rhabdomeres was reduced (Fig. 4, C and D). Indistinguishable results were obtained with trp^{P343}. The suppression of retinal degeneration was even more complete with trp^{14}, as all ommatidia contained a full set of seven rhabdomeres of normal size (Fig. 4, C and D). This result was striking as the retinal degeneration occurring under a light–dark cycle was significantly more rapid in trp^{14} than in $trp^{\Delta 1272}$.

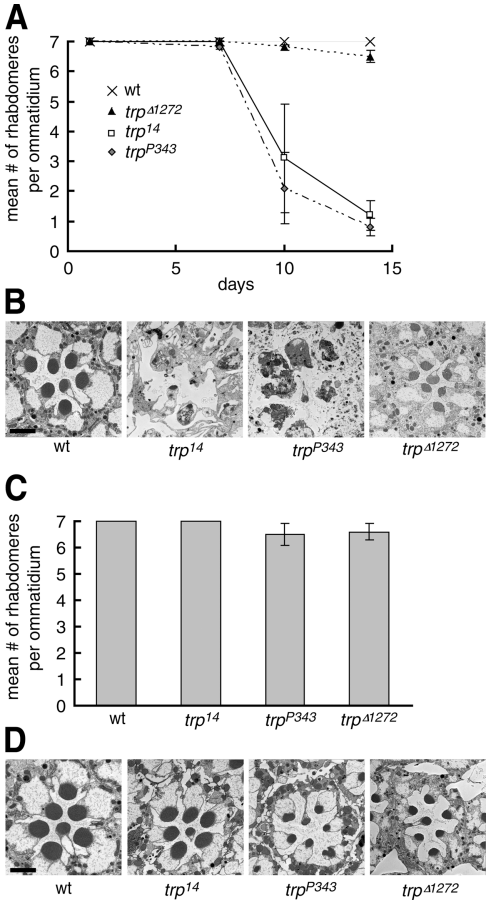

Figure 4. **Light-dependent retinal degeneration in *trp* mutant flies.** (A) The time-course of retinal degeneration of flies maintained under a 12-h light–12-h dark cycle. The mean numbers of rhabdomeres per ommatidium were determined by examining EM cross sections through the distal regions number of the compound eyes. Each point is based on ≥80 ommatidia from two separate flies each. Error bars indicate SDs. (B) Examination of the morphology of wt, trp^{14}, trp^{P343}, and $trp^{\Delta 1272}$ by transmission EM. Cross sections were obtained from 14-d-old flies kept under a 12-h light–12-h dark cycle. (C) Mean number of rhabdomeres per ommatidium from 30-d-old dark-reared flies. SDs are indicated. (D) Morphology of 30-d-old dark-reared wt, trp^{14}, trp^{P343}, and $trp^{\Delta 1272}$ flies examined by transmission EM. Bars, 2 μm.

Rapid retinal degeneration of *trp* photoreceptors suppressed by mutations in *calx* and *arr2*

To explore the mechanism underlying the retinal degeneration in *trp* mutants, we used a genetic approach. The combination of results described above indicates that a defect in TRP channel function underlies the retinal degeneration in *trp* flies. If the basis of the retinal degeneration in the *trp* mutant was due to diminished Ca^{2+} influx during light stimulation, then the cell death might be reduced by mutations in the Na^{+}/Ca^{2+} exchanger, CalX, which functions in Ca^{2+} extrusion in photoreceptor cells.

To test whether *calx* can suppress the retinal degeneration in trp^{P343} and trp^{14}, we examined the morphology of $calx^{B}$,trp^{P343}, and $calx^{B}$,trp^{14} compound eyes. As described above, trp^{14} or trp^{P343} flies maintained under a light–dark cycle for 14 d displayed nearly complete loss of the rhabdomeres (Fig. 5, A–D). The retinal degeneration in $calx^{B}$ flies was even

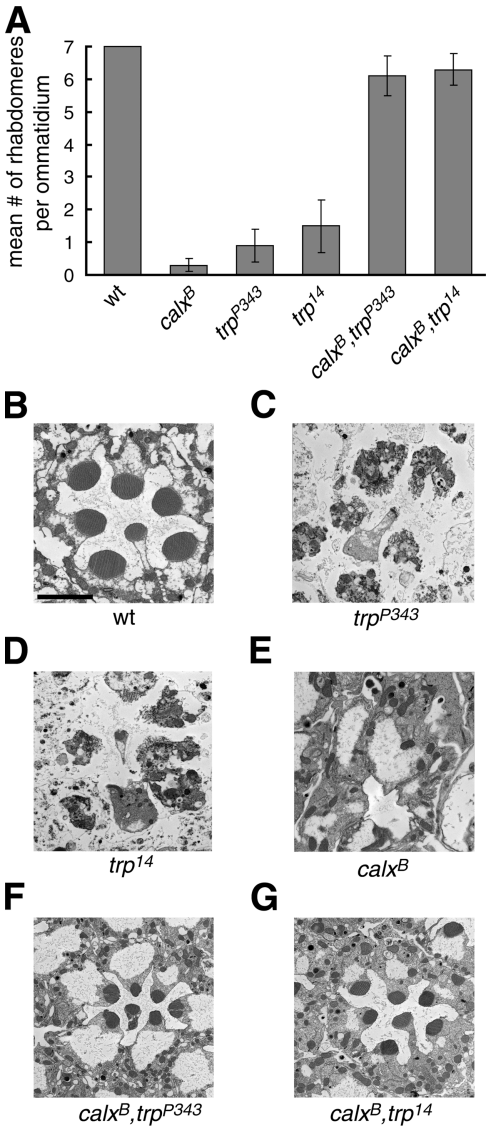

Figure 5. **Suppression of retinal degeneration in *trp* mutant flies by the calx mutation.** (A) Histogram of the mean number of rhabdomeres per ommatidium from 14-d-old flies held under a 12-h light–12-h dark cycle. The quantification is based on examination of EM cross sections from the distal region of the compound eyes from at least 80 ommatidia (two separate flies each). The error bars indicate SDs. (B–G) EM images of cross sections from: (B) wt; (C) trp^{P343}; (D) trp^{14}; (E) $calx^{B}$; (F) $calx^{B},trp^{P343}$; and (G) $calx^{B},trp^{14}$ flies. Bar, 2 μm.

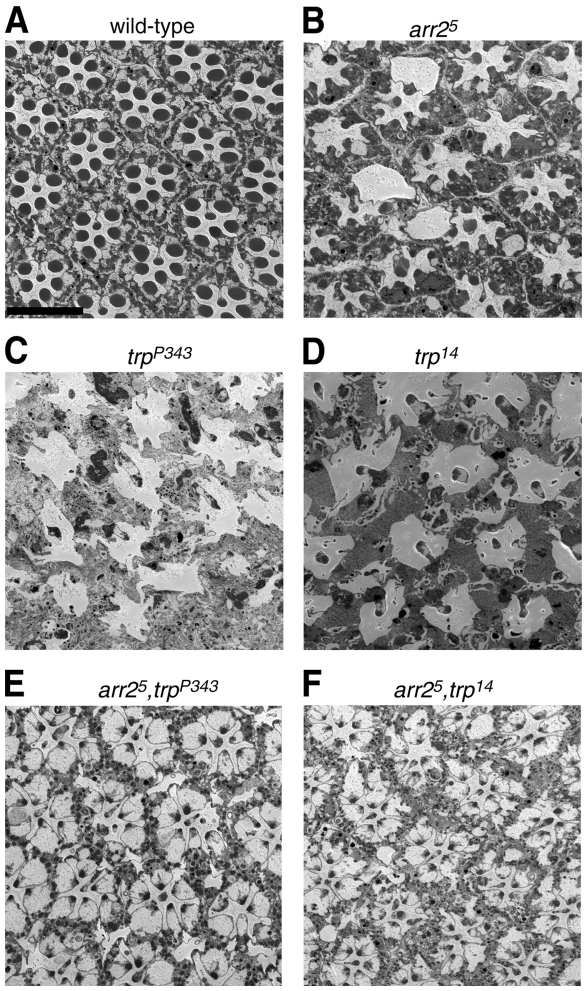

Figure 6. **Suppression of retinal degeneration in *trp* flies by the *arr2* mutation.** EM images of cross sections from 14-d-old flies maintained under a 12-h light–12-h dark cycle. (A) Wild type; (B) $arr2^{5}$; (C) trp^{P343}; (D) trp^{14}; (E) $arr2^{5},trp^{P343}$; (F) $arr2^{5},trp^{14}$. Bar, 10 μm.

more severe as there were few rhabdomeres left after a 7 d light–dark cycle (unpublished data) and almost no rhabdomeres left after a 14-d light–dark cycle (Fig. 5, A and E). In contrast, most ommatidia from either $calx^{B},trp^{P343}$ or $calx^{B},trp^{14}$ double mutant flies contained all the rhabdomeres after 14 d under a light–dark cycle (Fig. 5, A, F, and G). Moreover, the core signalplex proteins, NORPA, INAC, and INAD were mislocalized in $calx^{B},trp^{P343}$ flies (Fig. 2 D), indicating that introducing the *calx* mutation did not prevent loss of the TRP scaffold function in trp^{P343}. The effect of *calx* on *trp* was specific as the $calx^{B}$ mutation did not suppress the cell death resulting from mutations in other phototransduction genes such as *inaC*, which encodes an eye-enriched PKC (unpublished data). These results indicated that the retinal degeneration in trp^{14} or trp^{P343} was a consequence of decreased intracellular Ca^{2+} levels, whereas the photoreceptor cell death in the *calx* mutant resulted from Ca^{2+} overload.

To explore further the mechanism of the retinal degeneration in trp^{14} and trp^{P343}, we considered whether it could be suppressed by mutations in the gene encoding the major arrestin (*arrestin2* [*arr2*]; Dolph et al., 1993). Elimination of Arr2 reduces the retinal degeneration associated with certain mutations (Alloway et al., 2000; Kiselev et al., 2000), such as *norpA* (disrupts phospholipase C), which prevents light-dependent activation of TRP channels. The retinal degeneration in *norpA* results from formation of stable rhodopsin–arrestin complexes and subsequent endocytosis of rhodopsin (Orem and Dolph, 2002).

We found that strong mutations in *arr2* partially suppressed the retinal degeneration in trp^{14} and trp^{P343} flies. Whereas a 14-d exposure to a light–dark cycle resulted in extensive loss of rhabdomeres in $arr2^{5},trp^{P343}$ or trp^{14} eyes (Fig. 6, B–D), most ommatidia in $arr2^{5},trp^{P343}$ or $arr2^{5},trp^{14}$ double mutants con-

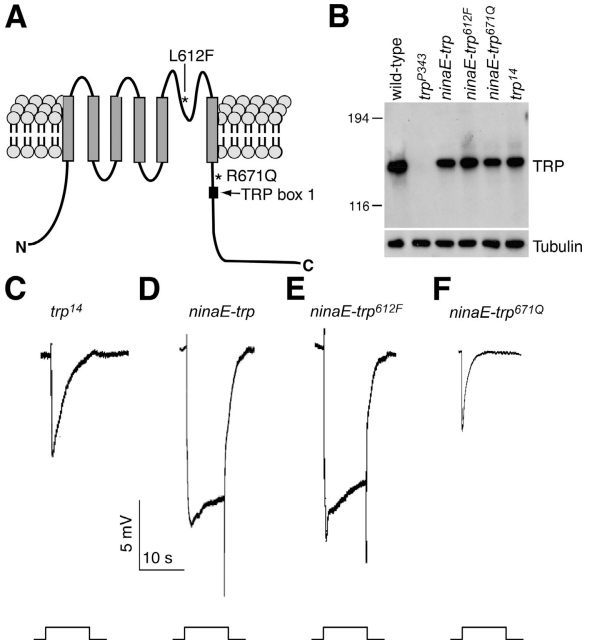

Figure 7. **R671Q mutation disrupts TRP channel function.** (A) Locations of L612F and R671Q missense mutations in TRP[14] indicated on a cartoon of the TRP protein. The gray rectangles represent the six transmembrane segments and the black box stands for TRP box 1. (B) Western blot showing expression of TRP in *ninaE-trp*, *ninaE-trp*^{612F}, and *ninaE-trp*^{671Q} transgenic flies. All three transgenes were in a *trpl*^{302};*trp*^{P343} background. The fly head extracts used for the Western blot were prepared from flies <1 d after eclosion and probed with anti-TRP antibodies. The same blot was reprobed with anti-tubulin antibodies. (C–F) ERG recordings obtained from *trpl*^{302};*trp*^{14} (C) and flies containing the following transgenes in a *trpl*^{302};*trp*^{P343} background: (D) *ninaE-trp*; (E) *ninaE-trp*^{612F}; and (F) *ninaE-trp*^{671Q}. To perform the ERGs, flies <1 d after eclosion were dark adapted for 2 min before being exposed to a 10-s pulse of orange light.

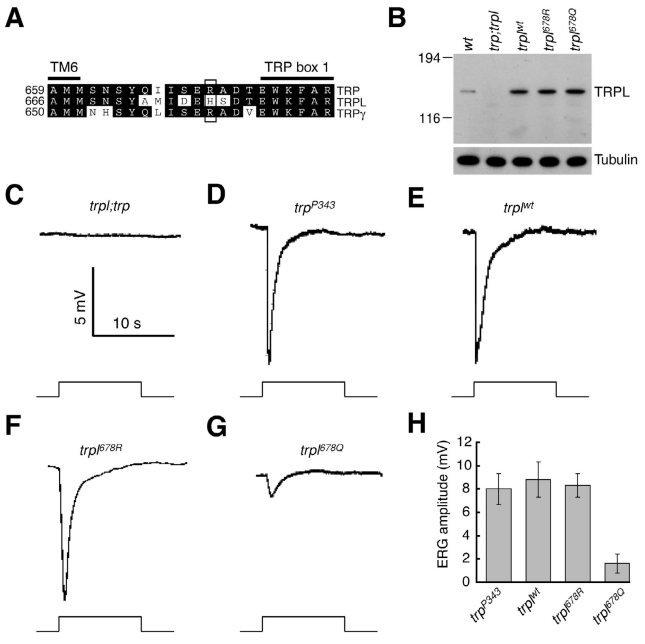

Figure 8. **TRPL channel function depends on a basic residue at position 678, which corresponds to arginine 671 in TRP.** (A) Alignment of the TRP, TRPL, and TRPγ amino acid sequences between the sixth transmembrane domain (TM6) and TRP box 1. The amino acids in TRPL (residue 678) and TRPγ (residue 662) corresponding to arginine 671 in TRP are indicated by the box. (B) TRPL protein expression in flies containing the *trpl* transgenes. A wild-type *trpl* transgene and mutant transgenes containing the H678R and H678Q missense changes (*trpl*^{678R} and *trpl*^{678Q}, respectively) were fused to the *ninaE* promoter and introduced into a *trpl*^{302};*trp*^{P343} background. The Western blot containing head extracts prepared from flies <1 d after eclosion was probed with anti-TRPL antibodies and reprobed with anti-tubulin antibodies. (C–G) ERG recordings were performed on flies <1 d after eclosion. The flies were dark adapted for 2 min before being exposed to a 10-s pulse of orange light. (C) *trpl;trp*^{P343}; (D) *trp*^{P343}. (E–G) The ERGs performed on the following transgenic flies were performed in a *trpl*^{302};*trp*^{P343} background: (E) *ninaE-trpl*^{wt} (*trpl*^{wt}); (F) *ninaE-trpl*^{H678R} (*trpl*^{678R}); and (G) *ninaE-trpl*^{H678Q} (*trpl*^{678Q}). (H) Quantification of the ERG amplitudes obtained in the indicated *trpl* transgenic flies in a *trpl*^{302};*trp*^{P343} background. SDs are indicated.

tained seven rhabdomeres (Fig. 6, E and F). However, the sizes of the rhabdomeres were significantly reduced.

Substitution of a basic residue adjacent to the TRP domain causes a defect in channel activity

The two mutations in *trp*^{14} alter residues in the pore loop or immediately NH_2 terminal to TRP box 1 (residues 612 and 671, respectively; Fig. 7 A). Given the potential effects of a pore–loop mutation on ion selectivity and the highly conserved nature of the region between the sixth transmembrane segment (TM6) and the TRP domain (Montell, 2005b; Fig. 8 A), both of these mutations were in intriguing positions that could potentially be responsible for the *trp*^{14} phenotype.

To determine whether one or both mutations were responsible for the transient light response phenotype in *trp*^{14}, we generated and tested transgenic flies expressing TRP isoforms with just the L612F or R671Q amino acid substitution (*trp*^{612F} and *trp*^{671Q}, respectively). The wild-type or mutant *trp* cDNAs were fused to the *ninaE* (*neither inactivation nor afterpotential E* [encodes Rh1]) promoter and introduced into the *trpl*^{302};*trp*^{P343} double mutant background. We subsequently performed Western blots on the transgenic flies demonstrating that wild-type TRP, TRP^{612F}, and TRP^{671Q} were all expressed at similar levels (Fig. 7 B).

We found that the missense mutation juxtaposed to the TRP domain was responsible for the phenotype in *trp*^{14}. The *trpl*;*ninaE-trp*^{612F},*trp*^{P343} flies displayed a wild-type ERG response (Fig. 7 E) indistinguishable from *trpl*;*ninaE-trp*^{wt},*trp*^{P343} (Fig. 7 D). Conversely, the *trpl*;*ninaE-trp*^{671Q},*trp*^{P343} flies showed a transient ERG phenotype (Fig. 7 F). Moreover, the *trp*^{671Q} flies exhibited an ERG phenotype with a more rapid decline typical of *trp*^{P343} suggesting that the 612F mutation resulted in a slight suppression of the TRP phenotype. These data demonstrate that the missense mutation at residue 671 situated between the sixth transmembrane domain and TRP box 1 (Montell, 2005b) was the key mutation responsible for the *trp*^{14} phenotype.

Mutation of TRPL in same position as TRP^{14} causes channel defect

The residues in TRPL and TRPγ, corresponding to the required arginine 671 in TRP, are also basic amino acids (histidine 678 and arginine 662, respectively; Fig. 8 A) suggesting that a basic residue at this position flanking the TRP domain is essential in the *Drosophila* TRPC channels. Therefore, we generated trans-

genic flies expressing derivatives of TRPL in which histidine 678 was replaced either with a conservative arginine substitution ($trpl^{678R}$) or with an uncharged glutamine ($trpl^{678Q}$). The mutant and wild-type *trpl* cDNAs were fused to the *ninaE* promoter and introduced into a $trpl^{302}$;trp^{P343} background. The transgenic TRPL proteins were all expressed at similar levels, though at an approximate sevenfold higher level than in wild-type due to the strong *ninaE* promoter (Fig. 8 B).

To determine the consequences of the mutations in TRPL, we performed ERGs after introducing the transgenes in a $trpl^{302}$;trp^{P343} background. Whereas the double $trpl^{302}$;trp^{P343} mutant was blind, overexpression of the wild-type TRPL in this genetic background ($trpl^{wt}$) restored a transient response to light indistinguishable from trp^{P343} (Fig. 8, C–E). Furthermore, introduction of the $trpl^{678R}$ transgene into the genome of $trpl^{302}$;trp^{P343} flies resulted in an ERG response similar to $trpl^{wt}$ (Fig. 8, E, F, and H). Thus, replacing histidine 678 with an arginine did not disrupt TRPL function. However, the amplitude of the ERG was significantly reduced in $trpl^{302}$;trp^{P343} flies expressing the $trpl^{678Q}$ transgene with the histidine to glutamine substitution in residue 678 (Fig. 8, G and H). The combination of these results demonstrates a critical role of a basic residue at the corresponding positions in TRP and TRPL, flanking the highly conserved TRP box 1.

Discussion

Drosophila TRP is a multifunctional protein as it serves both as a cation channel and a molecular anchor required for the retention of the scaffold protein, INAD, in the rhabdomeres. The TRP scaffold function is critical because the consequent mislocalization of INAD in turn causes instability and mislocalization of TRP, PLC, and PKC. Thus, over time the core proteins in the signalplex are lost from the rhabdomeres and the visual response is reduced. In addition to TRP, other related proteins may also have dual roles as several vertebrate TRPs, such as TRPM2 (Perraud et al., 2001; Sano et al., 2001), TRPM6, and TRPM7 (Nadler et al., 2001; Runnels et al., 2001; Schlingmann et al., 2002; Walder et al., 2002), consist of channel domains fused to enzyme domains. In the case of *Drosophila* TRP, we have previously characterized the specific role of the anchoring function on the photoresponse, using transgenic flies expressing a derivative of TRP that is missing the INAD binding site ($trp^{\Delta 1272}$; Li and Montell, 2000). Surprisingly, young $trp^{\Delta 1272}$ flies display a normal photoresponse, although as the flies age, INAD and the core binding proteins are not retained in the rhabdomeres.

Null mutations in *trp* have at least three major consequences in photoreceptor cells. These include the inability to maintain a light response, mislocalization of INAD, PLC, and PKC, and light-induced retinal degeneration. However, it has not been possible to determine the physiological consequences resulting from specifically disrupting the TRP channel function independent of the anchoring role, as all of the previously described loss-of-function mutations (with the exception of $trp^{\Delta 1272}$) virtually eliminate the TRP protein. The trp^{14} allele expressed relatively high levels of the TRP protein and exhibited a normal anchoring role as INAD coimmunoprecipitated with the TRP^{14} protein as effectively as with wild-type TRP. Furthermore, the spatial distributions of the core members of the signalplex were normal in trp^{14} photoreceptor cells.

Rather than affecting the anchoring role, the mutation of the basic residue situated between the sixth transmembrane segment and the TRP domain, disrupted TRP channel function such that the response to light stimulation was transient. Though the molecular basis for the defect in TRP channel function is unclear, mutation of the corresponding basic residue in TRPL also disrupted the activity of this latter channel. Thus, this region would appear to play a critical role in TRPC channel function in vivo. The transient light response in trp^{14} is not a simple consequence of the slightly lower expression of the mutant protein (60% of wild-type levels) as we found that expression of wild-type TRP at 4% the normal levels did not cause a transient light response, though the amplitude of the ERG was reduced. The TRP^{14} protein also displayed a wild-type rhabdomeral expression pattern, so that the phenotype was not due to mislocalization of the protein.

Of significance here, we found that the retinal degeneration associated with loss-of-function mutations in *trp* was due primarily to defects in channel function, rather than disruption of the anchoring role. This finding is surprising because elimination of the TRP scaffold function causes time-dependent instability and mislocalization of all four core proteins in the signalplex. Thus, low levels of TRP, INAD, PLC, and PKC result in less pronounced cell death than an amino acid substitution in TRP that disrupts channel function, but has no impact on the concentrations of the core proteins in the signalplex.

The basis for the retinal degeneration was decreased light-dependent Ca^{2+} influx because the cell death in either trp^{14} or *trp*-null mutant flies (trp^{P343}) was greatly reduced by strong loss-of-function mutations in the gene encoding the Na^+/Ca^{2+} exchanger, CalX. This effect was not a consequence of suppression of the anchoring defect because the core signalplex proteins were still mislocalized in *calx;*trp^{P343} double mutant flies. Given that the strong light-dependent retinal degeneration in *calx* was reciprocally suppressed by the trp^{P343} or trp^{14} mutations, these data also indicated that the cell death in *calx* resulted from Ca^{2+} overload.

The mechanism through which decreased Ca^{2+} influx causes cell death in *trp* appears to be due at least in part from increased rhodopsin–arrestin complexes. Stable rhodopsin–arrestin complexes and endocytosis of rhodopsin has been associated with degeneration resulting from mutations in the PLC and rhodopsin phosphatase (Alloway et al., 2000; Kiselev et al., 2000; Orem and Dolph, 2002). In the current study, we found that the *trp*-dependent retinal degeneration was partially suppressed by mutations in *arr2*. Because Ca^{2+}/calmodulin-dependent phosphorylation of arrestin promotes the release of arrestin from rhodopsin (Alloway and Dolph, 1999), we suggest that a consequence of decreased light-dependent Ca^{2+} influx in trp^{14} is reduced phosphorylation of arrestin, which in turn results in increased stability of arrestin–rhodopsin complexes. Alternatively, the reduced Ca^{2+} influx could result in increased arrestin–rhodopsin complexes due to affects on the rhodopsin

phosphatase, RDGC (retinal degeneration C). The activity of RDGC is dependent on Ca^{2+}/calmodulin (Lee and Montell, 2001) and loss of function mutations in *rdgC* result in stable rhodopsin–arrestin complexes and retinal degeneration (Kiselev et al., 2000).

The observation that decreased TRP-dependent Ca^{2+} influx underlies retinal degeneration in fly photoreceptor cells has potential implications in terms of the possible effects on cell survival resulting from loss-of-function mutations in vertebrate TRPs. It appears that constitutive activity of *Drosophila* and mammalian TRP leads to cell death due to Ca^{2+} overload (Yoon et al., 2000; Hara et al., 2002; Wehage et al., 2002; Aarts et al., 2003; Wang et al., 2005). Moreover, constitutive activity of TRPs by anoxic conditions has been proposed to underlie the massive cell death in the mammalian brain that can occur under anoxic conditions, such as occurs as a result of stroke (Agam et al., 2000; Yoon et al., 2000; Hara et al., 2002; Aarts et al., 2003; Wang et al., 2005).

The opposite of constitutive activation is elimination of TRP channel function and whether loss of vertebrate TRP-dependent Ca^{2+} influx leads to cell death has not been addressed. However, the results of the current analysis indicate that this is a likely possibility. Elimination TRPM7 from chicken DT40 cells results in cell death (Nadler et al., 2001), but the basis for the requirement for TRPM7 is not known. Given that TRPM7 consists of a TRP channel domain, fused to a COOH-terminal protein kinase domain, the cell death due to loss of TRPM7 could reflect a requirement for either the channel or kinase functions. Moreover, as TRPM7 is highly permeable to both Mg^{2+} and Ca^{2+} (Nadler et al., 2001), it is unclear the Mg^{2+} or Ca^{2+} influx is most important for viability. It will be of interest to determine whether the TRPM7-dependent cell death can be suppressed by inhibition of the Na^+/Ca^{2+} exchanger, as we observed for *Drosophila* TRP and CalX.

Materials and methods

Fly stocks

The trp^{14}, trp^{38}, trp^{47}, trp^{74}, and trp^{92} alleles were isolated by performing ethylmethylsulfonate mutagenesis and screening for third chromosome mutations affecting the ERG as we have described recently (Wang et al., 2005). The trp^{P343} (Montell and Rubin, 1989), $calx^{B}$ (Wang et al., 2005), $trp^{\Delta 1272}$ (Li and Montell, 2000), $trpl^{302}$ (Niemeyer et al., 1996), and $arr2^{5}$ (Alloway and Dolph, 1999) mutations were previously described.

Generation of transgenic flies

To express TRP under the control of the *hsp70* promoter, the full-length *trp* cDNA was excised from pBluescriptKS+ (pBSNot-*ctrp9*) with NotI and XbaI and subcloned between the NotI and XbaI sites of pCaspeR-hs (Thummel and Pirrotta, 1992). To express the wild-type or mutated *trp* genes under the control of the major rhodopsin (*ninaE*) promoter, the corresponding cDNAs were subcloned between the NotI and XbaI sites of the pNX vector and the inserts were subsequently excised and introduced into the KpnI site of pCaspeR4. To express the wild-type or mutated TRPL under the control of *ninaE* promoter, the full-length wild-type or mutated *trpl* cDNAs were subcloned into the NotI site of the pNX vector and the inserts were subsequently excised and introduced into the KpnI site of pCaspeR4 (Thummel and Pirrotta, 1992). The constructs were injected into w^{1118} embryos and transformants were identified on the basis of eye pigmentation. The third chromosome transgenes were selected and put into the trp^{P343} background by recombination.

ERG and intracellular recordings

ERG recordings were performed as previously described (Wes et al., 1999). In brief, two glass microelectrodes filled with Ringer's solution were inserted into small drops of electrode cream, which were placed on the surfaces of the compound eye and the thorax. A light projector (model 765; Newport Corp.) was used for stimulation. The ERGs were amplified with a electrometer (IE-210; Warner Instruments) and recorded with an analogue-to-digital converter (MacLab/4s) and the Chart v3.4/s program (A/D Instruments). All recordings were performed at room temperature. Intracellular recordings on photoreceptor cells were performed by placing a low-resistance microelectrode filled with 3 M KCl through a small hole on the compound eye and a reference electrode on the thorax as described (Xiong et al., 1994).

Western blots

Fly heads were homogenized in SDS sample buffer with a pellet pestle (Kimble-Kontes) and the proteins were fractionated by SDS-PAGE. The proteins were transferred overnight at 25V to Immobilon-P transfer membranes (Millipore) in Tris-glycine buffer. The blots were probed with the appropriate rabbit or mouse primary antibodies and subsequently with either IgG peroxidase conjugate (anti–rabbit or mouse, respectively; Sigma-Aldrich) or ^{125}I-labeled protein A (NEN Life Science Products). In those cases in which mouse primary antibodies were used, the blots were probed with rabbit anti–mouse IgG (Sigma-Aldrich) before using the ^{125}I-labeled protein A. The signals were detected using either ECL reagents (GE Healthcare) or a phosphoimager. Polyclonal rabbit anti-TRP (Chevesich et al., 1997), anti-INAD (Wes et al., 1999), and anti-NORPA antibodies (Wang et al., 2005) were previously described. Monoclonal anti-Rh1 and anti-tubulin antibodies were purchased from the Developmental Studies Hybridoma Bank (University of Iowa, Iowa City, IA). Rabbit anti-TRPL antibodies were purchased from Chemicon.

Co-immunoprecipitations

Fly heads (2 mg) were homogenized in 200 μl of buffer A (20 mM Tris-HCl, pH 7.5, 100 mM NaCl, 5 mM $MgCl_2$, 10% sucrose, 1 mM EDTA, and complete protease inhibitor (Brakeman et al., 1997) containing 1% CHAPS with a pellet pestle. The extracts were centrifuged at 15,000 *g* for 5 min at 4°C to remove cellular debris. Homogenates were diluted with 800 μl buffer A to achieve a final concentration of 0.2% CHAPS. 1 μl of anti-TRP antibodies were added and incubated for 1 h at 4°C, followed by 10 min centrifugation at 4°C. To reduce nonspecific binding, the protein A–Sepharose beads (GE Healthcare) were first incubated with buffer A containing 0.2% CHAPS and 1% BSA for 1 h at 4°C. The immunocomplexes were then incubated for 30 min at 4°C with 50 μl of a 50% slurry of the blocked protein A–Sepharose beads in buffer A with 0.2% CHAPS, followed by 1 min centrifugation at 5,000 *g* to pellet the protein A–Sepharose beads and associated immunocomplexes. After washing twice with buffer A, the beads were resuspended in SDS sample buffer, fractionated by SDS-PAGE, and a Western blot was probed with anti-INAD antibodies.

Immunolocalizations

Hemisected fly heads were fixed with paraformaldehyde and embedded in LR White resin as described previously (Porter and Montell, 1993). Cross sections (0.5 μm) of compound eyes were cut through the distal region of the retina, which included the R7 cells, and stained with primary antibodies (1:200) and FITC-labeled secondary antibodies (1:200) as described previously (Porter and Montell, 1993). Samples were examined with a Axioplan microscope (Carl Zeiss MicroImaging, Inc.) using a Plan-Apochromat 63× objective and the images were acquired using a SensiCam camera (Cooke) and IPLab 3.6.5 software. The images were then transferred into Adobe Photoshop 7.0 for assembling the figures.

Transmission EM

Heads were dissected from flies reared under a 12-h light–12-h dark cycle or in constant darkness and embedded for transmission EM as described previously (Porter et al. 1992) except that 0.1 M sodium phosphate (pH 7.4) was used as the buffer. Thin sections (85 nm) prepared at a depth of 30 μm were examined by transmission EM using an electron microscope (FEI Tecnai 12; Carl Zeiss MicroImaging, Inc.). The images were acquired using a camera (model 794; Gatan) and Digital Micrograph software (Gatan, Inc.) and converted into tiff files.

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References

Aarts, M., K. Iihara, W.L. Wei, Z.G. Xiong, M. Arundine, W. Cerwinski, J.F. MacDonald, and M. Tymianski. 2003. A key role for TRPM7 channels in anoxic neuronal death. *Cell.* 115:863–877.

Agam, K., M. von Campenhausen, S. Levy, H.C. Ben-Ami, B. Cook, K. Kirschfeld, and B. Minke. 2000. Metabolic stress reversibly activates the *Drosophila* light-sensitive channels TRP and TRPL *in vivo*. *J. Neurosci.* 20:5748–5755.

Alloway, P.G., and P.J. Dolph. 1999. A role for the light-dependent phosphorylation of visual arrestin. *Proc. Natl. Acad. Sci. USA.* 96:6072–6077.

Alloway, P.G., L. Howard, and P.J. Dolph. 2000. The formation of stable rhodopsin-arrestin complexes induces apoptosis and photoreceptor cell degeneration. *Neuron.* 28:129–138.

Brakeman, P.R., A.A. Lanahan, R. Obrien, K. Roche, C.A. Barners, R.L. Huganir, and P.F. Worley. 1997. Homer: a protein that selectively binds metabotropic glutamate receptors. *Nature.* 386:284–288.

Chevesich, J., A.J. Kreuz, and C. Montell. 1997. Requirement for the PDZ domain protein, INAD, for localization of the TRP store-operated channel to a signaling complex. *Neuron.* 18:95–105.

Cosens, D., and M.M. Perry. 1972. The fine structure of the eye of a visual mutant, A-type, of *Drosophila melanogaster*. *J. Insect Physiol.* 18:1773–1786.

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

Goel, M., R. Garcia, M. Estacion, and W.P. Schilling. 2001. Regulation of *Drosophila* TRPL channels by immunophilin FKBP59. *J. Biol. Chem.* 276:38762–38773.

Hara, Y., M. Wakamori, M. Ishii, E. Maeno, M. Nishida, T. Yoshida, H. Yamada, S. Shimizu, E. Mori, J. Kudoh, et al. 2002. LTRPC2 Ca^{2+}-permeable channel activated by changes in redox status confers susceptibility to cell death. *Mol. Cell.* 9:163–173.

Huber, A., P. Sander, A. Gobert, M. Bähner, R. Hermann, and R. Paulsen. 1996. The transient receptor potential protein (Trp), a putative store-operated Ca^{2+} channel essential for phosphoinositide-mediated photoreception, forms a signaling complex with NorpA, InaC and InaD. *EMBO J.* 15:7036–7045.

Kiselev, A., M. Socolich, J. Vinos, R.W. Hardy, C.S. Zuker, and R. Ranganathan. 2000. A molecular pathway for light-dependent photoreceptor apoptosis in *Drosophila*. *Neuron.* 28:139–152.

Lee, S.J., and C. Montell. 2001. Regulation of the rhodopsin protein phosphatase, RDGC, through interaction with calmodulin. *Neuron.* 32:1097–1106.

Leung, H.T., C. Geng, and W.L. Pak. 2000. Phenotypes of *trpl* mutants and interactions between the transient receptor potential (TRP) and TRP-like channels in *Drosophila*. *J. Neurosci.* 20:6797–6803.

Li, H.S., and C. Montell. 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.

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

Montell, C. 2005a. TRP channels in *Drosophila* photoreceptor cells. *J. Physiol.* 567.1:45-51.

Montell, C. 2005b. The TRP superfamily of cation channels. *Sci. STKE.* 2005:re3.

Montell, C., and G.M. Rubin. 1989. Molecular characterization of the *Drosophila trp* locus: a putative integral membrane protein required for phototransduction. *Neuron.* 2:1313–1323.

Nadler, M.J., M.C. Hermosura, K. Inabe, A.L. Perraud, Q. Zhu, A.J. Stokes, T. Kurosaki, J.P. Kinet, R. Penner, A.M. Scharenberg, and A. Fleig. 2001. LTRPC7 is a Mg.ATP-regulated divalent cation channel required for cell viability. *Nature.* 411:590–595.

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

Orem, N.R., and P.J. Dolph. 2002. Loss of the phospholipase C gene product induces massive endocytosis of rhodopsin and arrestin in *Drosophila* photoreceptors. *Vision Res.* 42:497–505.

Pak, W.L. 1994. Retinal degeneration mutants of *Drosophila*. *In* Molecular Genetics of Inherited Eye Disorders. A.F. Wright and B. Jay, editors. Harwood Academic Publishers, Chur, Switzerland. 29–52.

Perraud, A.L., A. Fleig, C.A. Dunn, L.A. Bagley, P. Launay, C. Schmitz, A.J. Stokes, Q. Zhu, M.J. Bessman, R. Penner, et al. 2001. ADP-ribose gating of the calcium-permeable LTRPC2 channel revealed by Nudix motif homology. *Nature.* 411:595–599.

Phillips, A.M., A. Bull, and L.E. Kelly. 1992. Identification of a *Drosophila* gene encoding a calmodulin-binding protein with homology to the *trp* phototransduction gene. *Neuron.* 8:631–642.

Porter, J.A., J.L. Hicks, D.S. Williams, and C. Montell. 1992. Differential localizations of and requirements for the two *Drosophila ninaC* kinase/myosins in photoreceptor cells. *J. Cell Biol.* 116:683–693.

Porter, J.A., and C. Montell. 1993. Distinct roles of the *Drosophila ninaC* kinase and myosin domains revealed by systematic mutagenesis. *J. Cell Biol.* 122:601–612.

Reuss, H., M.H. Mojet, S. Chyb, and R.C. Hardie. 1997. In vivo analysis of the *Drosophila* light-sensitive channels, TRP and TRPL. *Neuron.* 19: 1249–1259.

Runnels, L.W., L. Yue, and D.E. Clapham. 2001. TRP-PLIK, a bifunctional protein with kinase and ion channel activities. *Science.* 291:1043–1047.

Sano, Y., K. Inamura, A. Miyake, S. Mochizuki, H. Yokoi, H. Matsushime, and K. Furuichi. 2001. Immunocyte Ca^{2+} influx system mediated by LTRPC2. *Science.* 293:1327–1330.

Schlingmann, K.P., S. Weber, M. Peters, L. Niemann Nejsum, H. Vitzthum, K. Klingel, M. Kratz, E. Haddad, E. Ristoff, D. Dinour, et al. 2002. Hypomagnesemia with secondary hypocalcemia is caused by mutations in TRPM6, a new member of the TRPM gene family. *Nat. Genet.* 31:166–170.

Shieh, B.-H., and M.-Y. Zhu. 1996. Regulation of the TRP Ca^{2+} channel by INAD in *Drosophila* photoreceptors. *Neuron.* 16:991–998.

Thummel, C.S., and V. Pirrotta. 1992. New pCaSpeR P element vectors. *Drosoph. Inf. Serv.* 71:150.

Tsunoda, S., J. Sierralta, Y. Sun, R. Bodner, E. Suzuki, A. Becker, M. Socolich, and C.S. Zuker. 1997. A multivalent PDZ-domain protein assembles signalling complexes in a G-protein-coupled cascade. *Nature.* 388:243–249.

Tsunoda, S., Y. Sun, E. Suzuki, and C. Zuker. 2001. Independent anchoring and assembly mechanisms of INAD signaling complexes in *Drosophila* photoreceptors. *J. Neurosci.* 21:150–158.

Walder, R.Y., D. Landau, P. Meyer, H. Shalev, M. Tsolia, Z. Borochowitz, M.B. Boettger, G.E. Beck, R.K. Englehardt, R. Carmi, and V.C. Sheffield. 2002. Mutation of TRPM6 causes familial hypomagnesemia with secondary hypocalcemia. *Nat. Genet.* 31:171–174.

Wang, T., H. Xu, J. Oberwinkler, Y. Gu, R.C. Hardie, and C. Montell. 2005. Light activation, adaptation, and cell survival functions of the Na^{+}/Ca^{2+} exchanger CalX. *Neuron.* 45:367–378.

Wehage, E., J. Eisfeld, I. Heiner, E. Jungling, C. Zitt, and A. Lückhoff. 2002. Activation of the cation channel long transient receptor potential channel 2 (LTRPC2) by hydrogen peroxide. A splice variant reveals a mode of activation independent of ADP-ribose. *J. Biol. Chem.* 277:23150–23156.

Wes, P.D., X.-Z.S. Xu, H.-S. Li, F. Chien, S.K. Doberstein, and C. Montell. 1999. Termination of phototransduction requires binding of the NINAC myosin III and the PDZ protein INAD. *Nat. Neurosci.* 2:447–453.

Xiong, W.-C., H. Okano, N.H. Patel, J.A. Blendy, and C. Montell. 1994. *repo* encodes a glial-specific homeo domain protein required in the *Drosophila* nervous system. *Genes Dev.* 8:981–994.

Xu, X.Z., F. Chien, A. Butler, L. Salkoff, and C. Montell. 2000. TRPγ, a *Drosophila* TRP-related subunit, forms a regulated cation channel with TRPL. *Neuron.* 26:647–657.

Xu, X.Z., A. Choudhury, X. Li, and C. Montell. 1998. Coordination of an array of signaling proteins through homo- and heteromeric interactions between PDZ domains and target proteins. *J. Cell Biol.* 142:545–555.

Yoon, J., H.C. Ben-Ami, Y.S. Hong, S. Park, L.L. Strong, J. Bowman, C. Geng, K. Baek, B. Minke, and W.L. Pak. 2000. Novel mechanism of massive photoreceptor degeneration caused by mutations in the *trp* gene of *Drosophila*. *J. Neurosci.* 20:649–659.