Translocation of the Drosophila Transient Receptor Potential-like (TRPL) Channel Requires Both the N- and C-terminal Regions Together with Sustained Ca\(^{2+}\) Entry

Received for publication, July 1, 2011, and in revised form, July 29, 2011 Published, JBC Papers in Press, August 4, 2011, DOI 10.1074/jbc.M111.278564

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**Background:** Equipment of the neuronal plasma membrane with ion channels is regulated by translocation of ion channel subunits.

**Results:** Analysis of chimeric ion channels composed of Drosophila channels TRP and TRPL revealed Ca\(^{2+}\)-dependent translocation of TRPL and chimera containing N- and C-terminal regions of TRPL.

**Conclusion:** The translocation of the TRPL channel requires both the N- and C-terminal regions together with sustained Ca\(^{2+}\) entry.

**Significance:** The results have implications for understanding subcellular trafficking of TRP family members.

In Drosophila photoreceptors the transient receptor potential-like (TRPL), but not the TRP channels undergo light-dependent translocation between the rhabdomere and cell body. Here we studied which of the TRPL channel segments are essential for translocation and why the TRP channels are required for inducing TRPL translocation. We generated transgenic flies expressing chimeric TRP and TRPL proteins that formed functional light-activated channels. Translocation was induced only in chimeras containing both the N- and C-terminal segments of TRPL. Using an inactive trp mutation and overexpressing the Na\(^{+}/\)Ca\(^{2+}\) exchanger revealed that the essential function of the TRP channels in TRPL translocation is to enhance Ca\(^{2+}\)-influx. These results indicate that motifs present at both the N and C termini as well as sustained Ca\(^{2+}\) entry are required for proper channel translocation.

The physiological properties of cells are largely determined by a specific set of ion channels at the plasma membrane. Besides regulation at the gene expression level, trafficking of ion channels into and out of the plasma membrane has been established as an important mechanism for manipulating the number of channels at a specific cellular site (for reviews see Refs. 1, 2). For instance, the translocation of AMPA-type glutamate receptors from endosomal membranes into the synapses of hippocampal and lateral amygdala neurons underlies the formation of long-term potentiation and is thus involved in associative learning (3, 4). Regulation by controlled insertion and internalization of ion channels has also been studied for a number of vertebrate transient receptor potential (TRP) \(^{4}\) channels and for the Drosophila TRPL channel (see Refs. 5, 6). TRP channels function in sensory systems such as invertebrate photoreceptors, mechanoreceptors, pheromone receptors, taste receptors, pain receptors or receptors for detection of hot and cold temperature, but also as regulators of ion homeostasis in non-neuronal cells (see Refs. 7–11). The TRP channel superfamily is classified into seven related subfamilies designated TRPC (canonical or classical), in which the Drosophila TRP and TRPL are members, TRPM (Melastatin), TRPN (NompC), TRPV (Vanilloid receptor), TRPA (ANKTM1), TRPP (Polycystin), and TRPML (Mucolipin, for reviews see Refs. 12, 13). Regulated subcellular translocation has been reported for TRPV1 and TRPV2 that are translocated from an internal compartment to the plasma membrane upon hormonal stimulation with nerve growth factor, insulin-like growth factor-I, or neuropeptide head activator (14–17). The cell surface delivery of another TRPV channel, TRPV5, is stimulated by the serine protease tissue kallikrein in a protein kinase C-dependent manner (18) and it is, in addition, dynamically controlled by extracellular pH (19). In the case of TRPC channels, epidermal growth factor induces rapid insertion of TRPC4 and TRPC5 into the plasma membrane (20). Despite these numerous examples, the mechanisms and the molecular determinants of vertebrate TRP channels translocation are only beginning to be understood.

Another prominent example for ion channel translocation is the Drosophila TRPL channel. Together with TRP, TRPL is expressed in the photoreceptor cells of the compound eye where it becomes activated by a G-protein coupled, phospho-
lipase C-mediated visual transduction cascade (for reviews see Refs. 10, 21, 22). In dark-raised flies TRPL is located, together with other components of the phototransduction cascade, in the microvillar photoreceptor membrane, which forms the rhabdomere along one side of the photoreceptor cell. Upon illumination, TRPL translocates from the rhabdomere into a storage compartment in the cell body at a time scale of hours (23). This translocation is a two-stage process in which TRPL is first transported to the base of the rhabdomeral membrane and to the adjacent stalk membrane followed by internalization into the cell body. This is performed by a transport pathway utilizing vesicular structures that also contain internalized rhodopsin (24, 25). Subsequent dark adaptation of the flies results in redistribution of the stored TRPL channels back to the rhabdomeric membrane. The light-triggered internalization of TRPL depends on the activation of the phototransduction cascade and requires the presence of the second light-activated ion channel, TRP (24, 26). In contrast to TRPL, TRP is located in the rhabdomere irrespective of the light conditions. Besides their translocation behavior, TRP and TRPL also differ in their electrophysiological properties. Studies have revealed that TRP is a highly Ca\(^{2+}\)-selective ion channel with a reversal potential of about +13 mV at 1.5 mM external Ca\(^{2+}\) while TRPL is a non-selective cation channel with a reversal potential of about −4 mV (27, 28). TRP and TRPL also differ in their single channel conductance, which was estimated from noise analysis to be about 4 pS for TRP and 35 pS for TRPL in physiological Ringer’s solution. These channels also differ in their susceptibility to the channel blocker La\(^{3+}\) that blocks TRP but not TRPL at micromolar concentrations. Therefore, the light-dependent translocation of TRPL alters the properties of the light-response of the photoreceptor cells (23).

In the current study, we addressed the question: which segments of the TRPL protein are responsible for the observed light-triggered internalization of the ion channel. To this end, we generated chimeric eGFP-tagged ion channels composed of segments from TRP and TRPL. We found that the light-dependent translocation of TRPL requires both the N- and C-terminal segments, indicating that this process is not mediated by a simple single internalization motif. To further establish the role of Ca\(^{2+}\) entry into photoreceptor cells for initiating TRPL translocation, we used mutants that affect cellular Ca\(^{2+}\) concentrations. We found that TRPL translocation was inhibited in these mutants, indicating that sustained Ca\(^{2+}\) influx is required for TRPL translocation. Electrophysiological characterization of the chimeric channels expressed on trpl\(^{302}\); trp\(^{P543}\) double null background revealed functional channels with pore properties of either TRP or TRPL that were solely determined by the transmembrane region. All chimeras revealed a decline of the light response toward baseline during prolonged intense light, characteristic of the trp\(^{P543}\) mutant phenotype, thus limiting Ca\(^{2+}\)-influx required for the normal translocation process.

**EXPERIMENTAL PROCEDURES**

**Fly Stocks**—The following strains of *Drosophila melanogaster* were used: w\(^{118}\) Oregon R (here referred to as wild type), cn\(^{1}\), bw\(^{1}\); trp\(^{P543}\) (29), w\(^{+}\); cn\(^{1}\), bw\(^{1}\); trp\(^{P543}\); trp\(^{P432}\) (30), w\(^{+}\); trp\(^{P543}\) (31), yw; P[Rh1 > TRPL-eGFP, y\(^{+}\)] (26), w\(^{+}\); sn{\(^{nco}\)}CyO; trp\(^{14}\) (32), yw; P[Rh1 > Calx, w\(^{+}\)]CyO; P[Rh1 > TRPL-eGFP, y\(^{+}\)] (33), w\(^{+}\); P[Rh1 > TRPL-eGFP, y\(^{+}\)] (26). The transgenic flies expressing chimeras 1–4, which were generated in this study, were crossed with trpl\(^{302}\) or a trpl\(^{302}\); trp\(^{P543}\) double mutant to obtain the genotypes indicated in the figure legends using standard *Drosophila* genetics. Flies were raised at 25 °C on standard corn meal food. For all experiments adult flies were used at an age of 1–2 days after eclosion. For whole cell and ERG recordings newly eclosed flies were used. At least 12 h before eclosion, fly vials were wrapped in aluminum foil and transferred into a light sealed box. For determining light-dependent ion channel translocation flies were kept in the dark, or were illuminated with orange light (acrylic glass cut off filter transmitting light >560 nm, ~200 Lux) for 16 h. Dark-raised flies were dissected under dim red light (Schott RG 630, cold light source KL1500, Schott, Germany), whereas light-raised flies were dissected under white light.

**Generation of Chimeric Constructs**—For generating the DNA constructs used to express chimeric eGFP-tagged proteins, we first combined the coding sequences for TRP and eGFP. The stop codon and the 3′-untranslated region of a trp cDNA clone were removed by substituting the sequence 3′ of a Sacl restriction site with a PCR fragment containing Sacl and Apal cloning sites. After partial digestion with EcoRI and Apal the modified trp cDNA was subcloned into a p-Bluescript vector containing the coding sequence for eGFP (originally obtained from the vector pEGFP-1, BD Biosciences, Germany). This resulted in a fusion construct in which eGFP was attached at the 3′-end of trp. The fused coding sequences of TRP and eGFP were then cloned into pENTR\(^{TM}\)1A (a vector containing attL sites for site-specific recombination; Invitrogen, Germany) with NotI and KpnI. For generating chimera 1, a conserved DraIII site present in the middle of the TRP-domain, downstream of the sixth transmembrane region was employed to exchange the C-terminal region of TRP with that of TRPL. Chimera 1 thus encodes amino acids 1–675 of TRP, amino acids 681–1124 of TRPL and eGFP. For exchanging the N-terminal region of TRP in chimera 2 a Stul site located immediately before the first transmembrane domain of TRP was used. Here, the DNA coding for the first 328 amino acids of TRP was substituted with a PCR product coding for the first 336 amino acids of TRPL. Chimera 3 was constructed by cloning the region encoding the N terminus of TRPL from chimera 2 into chimera 1. This clone encodes amino acids 1–336 of TRPL, 328–675 of TRP, 681–1224 of TRPL and eGFP. For generating chimera 4 the transmembrane regions of TRP were substituted by a PCR fragment of the TRPL transmembrane regions containing Stul and DraIII cloning sites. Chimera 4 containing the N- and C-terminal parts of TRP and only the transmembrane regions of TRPL thus encodes amino acids 1–328 of TRP, 336–681 of TRPL, 675–1275 of TRP, and eGFP. All constructs were verified by DNA sequencing.

The constructs that were cloned into the pENTR\(^{TM}\)1A vector, were transferred into a modified YC4 vector (a gift from S. Britt, University of Colorado) by site specific recombination of att sites using the Gateway\(^{®}\) technology from Invitrogen (Germany). The modified YC4 vector contained the *Drosophila*...
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*Rh1*-promoter (base pairs −833 to +67), attrR-sites and the last 0.6 kb of the 3′-untranslated region of *Rh1*. P-element-mediated transformation of *Drosophila* was carried out as described (34). The host strain used was *Drosophila* yellow white (yw).

**SDS-PAGE and Western Blot Analyses**—For analyzing the expression of ion channel subunits in the heads of transgenic flies, proteins were extracted with 1× SDS-PAGE extraction buffer (4% SDS, 4 mM phenylmethylsulfonyl fluoride in 65 mM Tris–HCl, pH 6.8) for 15 min at room temperature. The extracts were centrifuged with 16,000 × *g* at 22 °C for 10 min. Supernatants of four heads were separated by SDS-PAGE according to Laemmli (35), using 8% polyacrylamide gels (Bio-Rad). For immunoblotting, proteins were electrophoretically transferred to PVDF membranes (Bio-Rad) and processed as has been described previously (26). The antibodies used for Western blot analyses were α-DmTRP (Mab83F6-c; Developmental Studies Hybridoma Bank of the University of Iowa) and a guinea pig antibody against amino acids 1083 to 1097 of TRPL. The ECL Plus Western blotting analysis system (GE Healthcare, Germany) was used for signal detection.

**ERG Measurements and Light Stimulation**—Electroretinogram (ERG) recordings were applied to intact flies as described previously (36). Orange light (OG 590 Schott edge filter) from a 75-Watt Xenon high-pressure lamp (PTI, LPS 220, operating at 60 watts) was delivered to the compound eye via a light guide. The maximal luminous intensity at the eye surface was about 3.0 logarithmic intensity units above the intensity for a half-maximal response of the major photoreceptors (R1–R6). For whole-cell patch clamp, a Xenon high-pressure lamp (LEJ, ebx 75 isolated, operating at 75 W) was used, and the light stimuli were delivered to the ommatidia by means of epi-illumination via an objective lens. Absolute calibration of the effective number of photons in the stimuli was achieved by counting quantum bumps in dark adapted wild-type photoreceptors under control conditions with dim light.

**Whole Cell Recordings**—Dissociated ommatidia were prepared from newly eclosed dark-adapted adult flies (<1 h post eclosion). Whole-cell voltage-clamp recordings were performed as described previously (37). In short, recordings were made at 21 °C using patch pipettes of 8–12 MΩ pulled from fiber-filled borosilicate glass capillaries. Series resistance was below 25 MΩ and was carefully compensated (>80%) for currents >100 pA. Signals were amplified using Axopatch 1D (Molecular Devices, Sunnyvale, CA) patch-clamp amplifier, and the currents were sampled at 5 kHz and filtered at 2 kHz using the 8-pole low pass Bessel filter. Data were captured using Digidata 1440A interface and recorded using Clampex 10.2 (Molecular Devices). All recordings were filtered below 100 Hz and analyzed using pClamp 10.2 software (Molecular Devices).

The bath solution contained (in mM) 120 NaCl, 5 KCl, 4 MgSO_4_, 1.5 CaCl_2_, 10 N-Tris-(hydroxymethyl)-methyl-2-amino-ethanesulfonic acid (TES), 25 l-proline, 5 l-alanine. The recording pipette solution contained (in mM) 140 K-glucuronate, 2 MgSO_4_, 10 TES buffer, 4 MgATP, 0.4 NaGTP, and 1 nicotinamide adenine dinucleotide (NAD). For reversal potential measurements, the pipette solution contained (in mM) 140 CsCl, 15 tetaethylammonium chloride (TEA), 2 MgSO_4_, 10 TES buffer, 4 MgATP, 0.4 NaGTP, and 1 NAD. All solutions were adjusted to pH 7.15.

**Fluorescence Microscopy**—The subcellular localization of eGFP-tagged ion channels was analyzed by fluorescence microscopy of intact eyes using water immersion as described by Meyer *et al.* (26). Relative quantification of eGFP-tagged protein amounts in the rhabdomeres was calculated using the formula *r* = (Ir-Ib)/[(Ir-Ib) + (Ic-Ib)], where Ir, Ib, and Ic are the fluorescence intensities in rhabdomeres R1–6, in rhabdome R7 (background), and in the cell body as described before (26). The data were normalized to the values obtained for dark-raised flies expressing TRPL-eGFP that were set to 100%. Ommatidia from three to five different flies were examined for each sample.

**Immunocytochemistry of Fly Heads**—Immunocytochemistry was carried out as described before (38, 39). For labeling of the rhabdomeres an AF546-coupled phalloidin (Invitrogen) was used. The eGFP-tagged chimeras were visualized on cryosections by their GFP fluorescence. The sections were examined with the AxioImager.Z1m microscope (objective: EC Plan-Neofluar 40x/1.3 Oil, Zeiss) with ApoTome module (Zeiss) and documented with the AxioCam MRm (Zeiss).

**RESULTS**

*Generation and Expression of eGFP-tagged Chimeric TRP and TRPL Channels in Drosophila Photoreceptor Cells*—*Drosophila* TRP and TRPL display an overall amino acid identity of about 39% (40). The transmembrane part, composed of six putative transmembrane helices, short loops connecting the helices, and the pore forming loop between helices five and six shows 51.3% amino acid identity between TRP and TRPL. The same conservation (51.3% identity) is found in the N-terminal region, which contains three or four predicted ankyrin-like repeats and a predicted coiled-coil domain. TRP and TRPL are least conserved in their C-terminal region displaying only 19.9% identity between the two channels.

To study which regions of TRP and TRPL determine the biophysical properties and the localization of these ion channels, we generated chimeric constructs. In these constructs, the C-terminal, the N-terminal, both regions together, or the transmembrane regions of TRP were replaced by the corresponding regions of TRPL. These constructs are referred to as chimera 1 (which is a TRP channel with a C terminus of TRPL, TRP1–675 + TRPL681–1124 + eGFP), chimera 2 (which is a TRP channel with the N terminus of TRPL, TRP1–328 + TRPL336–675 + eGFP), chimera 3 (which is a TRP channel with both C- and N termini of TRPL, TRPL1–336 + TRP328–1275 + eGFP), and chimera 4 (in which the transmembrane regions of TRP were replaced with those of TRPL, (TRP1–328 + TRPL336–675 + TRPL681–1275 + eGFP)) (Fig. 1A). To allow identification of the chimeric proteins using fluorescence microscopy, the constructs were tagged with eGFP at the end of the C terminus. Besides the chimeric constructs, we also used the already reported TRPL-eGFP (26) as a control. All constructs were expressed in the peripheral photoreceptor cells of transgenic *Drosophila* under the control of the rhodopsin 1 (Rh1) promoter.
The expression of these constructs on wild-type background was verified by observing the eGFP fluorescence in the eyes (data not shown) and by Western blot analysis with antibodies against the C-terminal regions of TRPL (Fig. 1B, upper panel) or TRP (Fig. 1B, lower panel). The expression of chimeras 1 and 3 was detected by anti-TRPL antibodies at a molecular weight of about 170 kDa (upper panel). Chimera 2 and chimera 4 have molecular weights of about 170 kDa (lower panel) and they reacted with anti-TRP antibodies. The protein bands detected below the chimera proteins on the Western blot show the native TRPL and TRP.

**Chimeric TRP/TRPL Constructs Form Functional Ion Channels**—To examine whether the chimeric constructs form functional light-activated ion channels in photoreceptor cells, we crossed the transgenes into a functional light-activated ion channels in photoreceptor cells, showing a decline of the light response toward baseline during prolonged intense light (Fig. 2, upper middle trace). The ERG waveform of all chimeras was roughly similar to that of the mutant (trpP343), expressing only the TRPL channel, showing sustained response to prolonged light (Fig. 2, lower middle trace).

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Regardless of the C- or N-terminal domains identity, we measured the reversal potential of the light induced current (Fig. 2, upper panel). Fig. 2, B and C shows the reversal potential of the TRPL channel as measured in the trpP343 mutant (Fig. 2, B and C). We thus conclude that the chimeric channels form functional light-activated channels.

Both N- and C-terminal Regions but Not the Transmembrane Regions of TRPL Are Necessary for TRPL Translocation—A specific feature of the TRPL channel is the change of its subcellular localization from the rhabdomere to the cell body upon light exposure. Because the TRP channel but not the TRPL channel translocates upon illumination, it is interesting to determine which segments of these channels underlie the difference in their translocation behavior. Using the chimeric TRP/TRPL channels we tested which segments of TRPL are essential for light-induced TRPL translocation. The light-dependent subcellular localization of TRPL-eGFP and chimeric channels expressed on wild type background was analyzed by water immersion microscopy of intact eyes and by detecting the eGFP fluorescence on cross sections through the eyes (Fig. 3). As shown previously (26), TRPL-eGFP reveals light-induced translocation from the rhabdomere to the cell body. Accordingly, TRPL-eGFP was localized in the rhabdomeres in flies kept in the dark for 16 h, whereas it was localized in the cell body after raising the flies in orange light for 16 h. Chimera 1, 2, and 4 did not show light-induced translocation like TRPL-eGFP (Fig. 3, B, C, and E). Chimera 1 was localized predominantly in the cell body irrespective of the light condition. However, chimera 2 and 4 were distributed between the rhabdomere and cell body (Fig. 3F). Chimera 3 having both N and C termini of TRPL displayed a similar light-dependent localization as TRPL (Fig. 3D). Thus, motifs present both at the N- and C-terminal regions are necessary for proper channel localization and translocation.

To estimate the relative amount of chimera 3 in the rhabdomeres, we quantified the relative amount of eGFP-fluorescence in the rhabdomeres of flies expressing chimera 3 in comparison to flies expressing TRPL-eGFP (Fig. 4). Chimera 3 showed a higher level of the chimera protein in the rhabdomeres in the dark (~75%) than in the light (~30%), indicating that it is translocated to the cell body upon illumination. This translocation was reversible since subsequent dark-adaptation resulted in redistribution of the chimera protein to the rhabdomeres (~70%). It should be noted, however, that the relative amount of chimera 3 present in the rhabdomeres in the dark was smaller than that of TRPL-eGFP (Fig. 4B), suggesting that this protein is not transported to the rhabdomeres as efficiently as the TRPL-eGFP channel.

To test whether endogenous TRPL is required for the localization of chimera 3, we studied the subcellular distribution of chimera 3 in the trpP343 mutant (expressing only TRP) as compared with chimera 1, 2, and 4 channels on the same mutant.
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FIGURE 2. Chimeric proteins form functional light activated channels. A, ERG responses to prolonged (10 s) intense orange light pulse of the trpl002 and trpP343 mutant flies as well as chimeras 1–4 on double null trpl002;trpP343 mutant background. B, responses to a 20 ms light flash were measured at membrane potentials stepped in 3 mV intervals as indicated, using whole cell patch clamp recordings. These traces were used to determine the reversal potential ($E_{\text{rev}}$) of the native and chimeric channels. C, histogram plotting the $E_{\text{rev}}$ of the native and chimeric channels. Note that the chimeric channels with transmembrane domains of TRP showed $E_{\text{rev}}$ identical to the $E_{\text{rev}}$ of the trpl002 mutant fly, while, the chimera channel with transmembrane domains of TRPL showed $E_{\text{rev}}$ identical ($p > 0.05$) to that of the trpP343 mutant fly (mean ± S.E., $n = 5$).

background (Fig. 5, A–D, left columns). Fig. 5 shows that in the absence of TRPL the localization of chimera 3 is similar to that of chimera 3 on wild-type background, except that in dark-adapted flies more labeling was observed outside the rhabdomeres (Fig. 5C, left column) and the relative amount of chimera 3 in the rhabdomere was reduced to 60% compared with 75% on wild-type background (Fig. 5A, B, D, left columns). Notably, chimera 4 and, to a lesser extent, chimera 2 showed a significantly higher localization in rhabdomeres of light-adapted flies on trpl002;trpP343 mutant background compared with dark-adapted flies. It is not clear why chimeras 2 and 4 display this “reverse” translocation in this specific genetic background. One possible explanation would be that chimeras 2 and 4 become efficiently transported to the rhabdomere in the light only when TRP and TRPL are absent, possibly because they compete with the chimeras for the same transport machinery.
In summary, we conclude that both the N-terminal and C-terminal regions of TRPL are necessary for the specific light-dependent translocation that is typical of the TRPL ion channel. Chimera 3 Partially Autotranslocates upon Illumination in the Absence of Both the TRP and TRPL Channels—The presence of the TRP channel has been shown to be required for light-dependent TRPL channel translocation. This is presumably because it allows for sufficient and sustained Ca\(^{2+}\)-influx which triggers the translocation (26). Chimera 3 has channel permeability properties similar to that of the TRP channel (i.e., with high permeability to Ca\(^{2+}\), Fig. 2, B and C). We therefore examined whether this chimera can translocate on its own (autotranslocate) from the rhabdomere to the cell body in the absence of both the TRP and TRPL channels. Strikingly, on trpl302; trpP343 double null background, chimera 3 localization was significantly reduced in the rhabdomere during prolonged illumination (Fig. 5C, right columns, and F) unlike the case of TRPL-eGFP, which requires the presence of the TRP channels to translocate upon illumination (23, 26). To investigate why chimera 3 but not the TRPL channels are able to autotranslocate, we examined the electrophysiological phenotype of this chimera during prolonged lights in more detail. Our previous studies suggested that sustained Ca\(^{2+}\)-influx is required for TRPL translocation (26). In the trp mutant the expressed TRPL does not translocate during illumination, presumably because the LIC and the ensuing Ca\(^{2+}\)-influx is transient. The ERG recordings (Fig. 2A) already suggested that the response to sustained light of chimera 3 is transient, but it declined slower to baseline relative to the trp mutant indicating a weaker phenotype. To further investigate this phenomenon, we performed ERG measurements in response to two intense light pulses separated by a dark interval. The trp phenotype is manifested by two characteristics: a decline of the light response to baseline during prolonged intense light pulses and response inactiva-
tion. Chimera 3 on double null mutant background revealed that the light response to intense prolonged light is inactivated and declined to baseline during illumination (Fig. 6A, bottom trace). However, quantification of the degree of response inactivation and the time constant with which the response declined to baseline revealed a weaker trp phenotype (Fig. 6B and C). Accordingly, Fig. 6B shows a comparison between chimera 3, wild type and the trp mutant with respect to the degree of response inactivation. The trp mutant showed a complete response inactivation after both 5-s and 10-s dark intervals. Wild-type flies revealed the weakest inactivation: showing fast response recovery of 80.2% and 88.6% after 5-s and 10-s dark intervals, respectively. Chimera 3 on trpl302; trpP343 background revealed an intermediate degree of inactivation between the trp mutant and wild-type flies: showing 16.6 and 35.9% recovery of the response after 5-s and 10-s dark intervals, respectively (Fig. 6B). Quantification of the response decay time constant (τ) also revealed a weaker phenotype. While the response decay time constant of the trpP343 mutant was 1.96 s that of chimera 3 on trpl302; trpP343 background was 3.69 s (i.e. 53% difference, Fig. 6C). Thus, the relatively slow decline of the response of chimera 3 on double null trpl302; trpP343 background and the faster recovery should allow for a considerable Ca\(^{2+}\)-influx, which is necessary for the translocation process. To further investigate this phenomenon, we compared the LIC of chimera 3 (on double null trpl302; trpP343) in response to a long light pulse, to the responses of trpP343 and wild-type flies. Fig. 6D shows a relatively large (<200 pA) inward current at the end of 5 s light pulse measured in chimera 3 on double null trpl302; trpP343 background, while the LIC of trpP343 mutant already declined to baseline under similar conditions (Fig. 6D, right and middle traces). Accordingly, the LIC of chimera 3 in response to a long intense light pulse revealed a response inactivation phenotype weaker than that of the trp mutant but stronger than that of wild type, suggesting a considerable Ca\(^{2+}\)-influx during 5 s illumination in chimera 3. Nevertheless, the slow response inactivation allowed for only limited translocation (Fig. 5C).

Light-dependent Translocation of TRPL-eGFP Requires Sustained Ca\(^{2+}\)-influx through Functional TRP Channels—We have shown previously and in the present study that the translocation of TRPL and TRPL-eGFP from the rhabdomere to the cell body is inhibited in the trpP343-null mutant (23, 26). It is possible that TRP is required for TRPL translocation as a structural component of the phototransduction cascade in addition to its function as an ion channel. In order to investigate this possibility, we analyzed TRPL-eGFP translocation in another trp mutant, trp14 (32). This trp allele produces a TRP channel that is anchored to the INAD signaling complex and hence present as a structural component of the rhabdomeres but its ion channel function is disrupted such that light-activated Ca\(^{2+}\)-influx is highly reduced. Similar to the trpP343 null mutant, TRPL-eGFP translocation is inhibited in trp14 (Fig. 7, A and B), indicating that the function of TRP as an ion channel is required for the internalization of TRPL-eGFP. To further substantiate a requirement of internal Ca\(^{2+}\) for the internalization...
of TRPL-eGFP, we analyzed translocation of TRPL-eGFP in Drosophila photoreceptors that overexpressed the retinal Na\(^{+}\)/Ca\(^{2+}\)-exchanger CalX (33). After light-induced Ca\(^{2+}\)-influx CalX removes Ca\(^{2+}\) from the photoreceptor cells. Overexpression of CalX under the control of the rhodopsin1 promoter (ninaE-calx) was reported to result in a lower cytosolic Ca\(^{2+}\) concentration (33). Transgenic flies that contained one allel of ninaE-calx showed almost normal translocation of TRPL-eGFP (Fig. 7C). The introduction of two alleles of ninaE-calx, however, resulted in complete inhibition of TRPL-eGFP translocation (Fig. 7D). These findings indicate that a reduction of the cytosolic Ca\(^{2+}\) concentration by overexpression of Na\(^{+}\)/Ca\(^{2+}\)-exchanger CalX can interfere with TRPL-eGFP translocation.

**DISCUSSION**

In the present work we have studied the light-triggered translocation of Drosophila TRPL using chimeric ion channels composed of segments of TRP and TRPL as well as mutants that affect Ca\(^{2+}\) levels in photoreceptor cells. The electrophysiological characterization of photoreceptor cells that express the chimeras on a trpl302; trpP343 background revealed that all the chimeras formed functional light activated channels.

However, only chimera 3 formed a channel that displayed a similar light-dependent subcellular translocation as native TRPL. This finding indicates that both the N- and C-terminal regions of TRPL but not its transmembrane regions are essential for proper localization and light-triggered translocation of the TRPL channels. Compared with TRPL-eGFP, the portion of chimera 3 present in the rhabdomeres of dark-adapted flies was reduced (Fig. 4B). This finding may indicate that there is a quantitative difference in the efficiency of delivering TRPL-eGFP and chimera 3 to the rhabdomere. We also observed that in dark-adapted flies the rhabdomeres contained reduced amounts of chimera 3 on trpl302; trpP343 mutant background than on wild-type background (Fig. 5E). This finding may be explained by assuming that chimera 3 interacts with native TRPL (which is missing in trpl302) and that this interaction facilitates the delivery of chimera 3 to the rhabdomere.

Chimera 3 contains the transmembrane region of TRP and hence forms a pore with the properties of the TRP pore that is characterized by a high Ca\(^{2+}\) permeability as revealed by the identical \(E_{rev}\) of chimera 3 and the trpl302 mutant (Fig. 2C). We have previously shown that the presence of TRP is required to trigger the translocation of TRPL (23, 26). An in situ experiment with isolated retinas revealed that the presence of extra-

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**FIGURE 6.** Chimera 3 on trpl302; trpP343 mutant background shows a weaker inactivation phenotype compared with the trpP343 mutant fly. A, ERG responses to two intense 10 s long orange light pulses separated by a 5 s dark interval. B, histogram plotting the relative recovery from inactivation of wild type (WT), trpP343 and chimera 3 on trpl302; trpP343 background separated by a dark interval of 5 s and 10 s (Mean ± S.E., n = 5). C, histogram plotting the decay time constant (\(\tau\)) of the first light response of trpP343 and chimera 3 on trpl302; trpP343 mutant background (mean ± S.E., n = 5, **: \(p < 0.01\)). The decay time constant (\(\tau\)) was calculated by fitting an exponential function to the response decay waveform between 90–10% of the maximal response. D, whole cell recordings of the LIC in response to a 5 s intense light pulse of 10\(^{5}\) effective photons. Note that the LIC of wild type and chimera 3 on trpl302; trpP343 mutant background did not decline to baseline during the light pulse, while the light response of trpP343 declined to baseline during light.
Subcellular Translocation of Drosophila TRPL

FIGURE 7. Translocation of TRPL-eGFP requires Ca\(^{2+}\)-influx through TRP-channels. Water immersion microscopy of dark- (16 h, left panels) and light-adapted (16 h orange light, right panels) flies expressing TRPL-eGFP in various genetic backgrounds affecting Ca\(^{2+}\)-homeostasis of photoreceptor cells. A and B, localization of TRPL-eGFP in trp\(^{P343}\) and trp\(^{14}\) mutants, respectively. C, TRPL-eGFP localization in flies expressing one copy of the Na\(^+\)/Ca\(^{2+}\)-exchanger CalX under the control of the ninaE-promoter (yw; ninaE-calX/CyO; trp-eGFP). D, TRPL-eGFP localization in flies expressing two copies of the Na\(^+\)/Ca\(^{2+}\)-exchanger CalX under the control of the ninaE-promoter (yw; ninaE-calX; trp-eGFP), trp\(^{14}\) and CalX mutants are red-eyed flies. A reliable quantitative evaluation of red-eyed flies is not possible because the red screening pigments affect the detection of eGFP fluorescence in the cell body. E, TRPL-eGFP localization in red-eyed wild-type flies were used for comparison. Scale bar Aa, 10 μm.

cellular Ca\(^{2+}\) is necessary to initiate TRPL translocation (26). In the present study we provide further evidence that a sustained Ca\(^{2+}\)-influx through TRP-channels triggers robust TRPL translocation. We show that the trp\(^{14}\) allele that produces a nonfunctional TRP channel (32) as well as overexpression of the Na\(^+\)/Ca\(^{2+}\)-exchanger CalX in photoreceptor cells result in failure of TRP translocation. Because chimera 3 contains the TRP-pore with a high Ca\(^{2+}\) permeability we examined the possibility that this chimera could induce its own translocation (auto-translocation) in the absence of both the TRP and TRPL channels. Therefore, we studied the localization of chimera 3 on trpl\(^{302}\); trp\(^{P343}\) double null background. Unlike the native TRPL, the amount of chimera 3 in the rhabdomeres was reduced upon illumination. However, the autotranslocation of chimera 3 on trpl\(^{302}\); trp\(^{P343}\) mutant background was rather limited. Fig. 1B shows that chimera 3 expressed a relatively large amount of the chimera channel protein. We also examined transgenic flies expressing one copy of the chimera 3 transgene (data not shown). These flies showed translocation of chimera 3 on wild-type background but failed to autotranslocate on trpl\(^{302}\); trp\(^{P343}\) background. The latter observation was probably due to the smaller amount of the chimeric protein present, which presumably resulted in less Ca\(^{2+}\)-influx compared with flies that have two copies of the chimeric transgene. However, even at high expression level the observed limited translocation (Fig. 5F) may be attributed to the fact that chimera 3 revealed response inactivation albeit at a slower rate than observed in the trp\(^{P343}\) mutant. Thus, the presence of chimera 3 on trpl\(^{302}\); trp\(^{P343}\) background allows for limited amount of Ca\(^{2+}\)-influx that triggers a limited translocation of the chimeric channel. Moreover, when the chimera 3 channel translocates, its level in the rhabdomere is reduced thus further reducing the Ca\(^{2+}\) flux.

The requirement of cytosolic domains for subcellular channel translocation has been studied also for aquaporins and for vertebrate TRP channels. In Madin-Darby canine kidney cells, aquaporin 2 (AQ2) is localized in intracellular storage vesicles and translocates to the apical plasma membrane after stimulation with forskolin (42). Using chimeras in which N- and C-terminal regions were switched between APQ1 and APQ2, van Balkom et al. showed that both tails are essential for trafficking of APQ2 to intracellular vesicles and its translocation to the apical plasma membrane (43). The requirement of the C-terminal tail of APQ2 may be explained by the presence of a protein kinase A-dependent phosphorylation site (Ser-256) which is essential for the apical membrane expression of APQ2 (44, 45). Deletion studies of N- and C-terminal regions of the TRPM8 channel revealed that the N-terminal region of this channel is essential for plasma membrane localization (46). Deletions in the C-terminal domain of TRPM8 had no effect on plasma membrane localization although these deletions rendered the channel non-functional (46). For the TRPV4 channel, on the other hand, deletion studies established a role of the C-terminal region in its plasma membrane localization. The C-terminal region of TRPV4 is probably needed for the oligomerization of this channel into a tetramer that may be a prerequisite for transport to the plasma membrane (47). In neither case was it possible to identify a single sequence motif that determines the subcellular localization of TRP channels. Our present study on the Drosophila TRPL channel also reveals that both the N- and the C-terminal region and hence at least two sequence motifs located in these cytosolic regions are essential for determining the dynamic localization of this TRP channel.

Acknowledgments—We thank Craig Montell for providing Drosophila mutants. The monoclonal antibody MAAb38F6 was obtained from the Developmental Studies Hybridoma Bank (University of Iowa). We are also grateful to Alexander Cerny and Olaf Voolstra for helpful comments on the manuscript.

REFERENCES

1. Lai, H. C., and Jan, L. Y. (2006) Nat. Rev. Neurosci. 7, 548–562
2. Sheng, M., and Lee, S. H. (2001) Cell 105, 825–828
3. Rumpel, S., LeDoux, J., Zador, A., and Malinow, R. (2005) Science 308, 83–88
4. Park, M., Penick, E. C., Edwards, J. G., Kauer, J. A., and Ehlers, M. D. (2004) Science 305, 1972–1975
5. Cerny, A. C., and Huber, A. (2011) Adv. Exp. Med. Biol. 704, 545–572
6. Toro, C. A., Arias, L. A., and Brauchi, S. (2011) Curr. Pharm. Biotechnol. 12, 12–23
7. Montell, C., Birnbaumer, L., and Flockerzi, V. (2002) Cell 108, 595–598
8. Clapham, D. E. (2003) Nature 426, 517–524
9. Minke, B., and Cook, B. (2002) Physiol. Rev. 82, 429–472
10. Minke, B., and Parnas, M. (2006) Annu. Rev. Physiol. 68, 649–684
11. Montell, C. (2005) Sci. STKE. 2005, re3
12. Minke, B. (2006) Cell Calcium 40, 261–275
13. Ramsey, I. S., Delling, M., and Clapham, D. E. (2006) Annu. Rev. Physiol. 68, 619–647
14. Kanzaki, M., Zhang, Y. Q., Mashima, H., Li, L., Shibata, H., and Kojima, I.
(1999) Nat. Cell Biol. 1, 165–170
15. Van Buren, J. J., Bhat, S., Rotello, R., Pauza, M. E., and Premkumar, L. S. (2005) Mol. Pain. 1, 17
16. Zhang, X., Huang, J., and McNaughton, P. A. (2005) EMBO J. 24, 4211–4223
17. Boels, K., Glassmeier, G., Herrmann, D., Riedel, I. B., Hampe, W., Kojima, I., Schwarz, J. R., and Schaller, H. C. (2001) J. Cell Sci. 114, 3599–3606
18. Gkika, D., Topala, C. N., Chang, Q., Picard, N., Thébault, S., Houlliér, P., Hoenderop, J. G., and Bindels, R. J. (2006) EMBO J. 25, 4211–4223
19. Boels, K., Glassmeier, G., Herrmann, D., Riedel, I. B., Hampe, W., Kojima, I., Schwarz, J. R., and Schaller, H. C. (2001) J. Cell Sci. 114, 3599–3606
20. Gkika, D., Topala, C. N., Chang, Q., Picard, N., Thébault, S., Houlliér, P., Hoenderop, J. G., and Bindels, R. J. (2006) EMBO J. 25, 4211–4223
21. Hardie, R. C., and Raghu, P. (2001) Nature 413, 186–193
22. Huber, A. (2004) in Invertebrate phototransduction: Multimolecular Signaling Complexes and the Role of TRP and TRPL Channels, Transduction Channels in Sensory Cells (Friggs, S., and Bradley, J., eds), WILEY-VCH, Weinheim
23. Bähner, M., Frechter, S., Da Silva, N., Minke, B., Paulsen, R., and Huber, A. (2002) Neuron 34, 83–93
24. Cronin, M. A., Lieu, M. H., and Tsunoda, S. (2006) J. Cell Sci. 119, 2935–2944
25. Oberegelsbacher, C., Schneidler, C., Voolstra, O., Cerny, A., and Huber, A. (2011) Eur. J. Cell Biol. 90, 620–630
26. Meyer, N. E., Joel-Almagor, T., Frechter, S., Minke, B., and Huber, A. (2006) J. Cell Sci. 119, 2592–2603
27. Reuss, H., Mojet, M. H., Chyb, S., and Hardie, R. C. (1997) Neuron 19, 1249–1259
28. Hardie, R. C., and Minke, B. (1992) Neuron 8, 643–651
29. Niemeyer, B. A., Suzuki, E., Scott, K., Jalink, K., and Zuker, C. S. (1996) Cell 85, 651–659
30. Yang, Z., Emerson, M., Su, H. S., and Sehgal, A. (1998) Neuron 21, 215–223
31. Pak, W. L. (1979) Study of photoreceptor function using Drosophila mutants. In Breaksfield, X., editor. Neurogenetics: Genetic approaches to the nervous system, ELSEVIER, Amsterdam
32. Wang, T., Yucheng, L., and Montell, C. (2005) J. Cell Biol. 171, 685–694
33. Wang, T., Xu, H., Oberwinkler, J., Gu, Y., Hardie, R. C., and Montell, C. (2005) Neuron 45, 367–378
34. O’Toole, J. E. (1992) Vis. Neurosci. 8, 385–390
35. Laemmli, U. K. (1970) Nature 227, 682–685
36. Peretz, A., Sandler, C., Kirschfeld, K., Hardie, R. C., and Minke, B. (1994) J. Gen. Physiol. 104, 1057–1077
37. Peretz, A., Suss-Toby, E., Rom-Glas, A., Arnon, A., Payne, R., and Minke, B. (1994) Neuron 12, 1257–1267
38. Chorna-Ornan, I., Tzarfaty, V., Ankri-Eliahoo, G., Joel-Almagor, T., Meyer, N. E., Huber, A., Payne, R., and Minke, B. (2005) J. Cell Biol. 171, 143–152
39. Meyer, N. E., Oberegelsbacher, C., Dür, T. D., Schafer, A., and Huber, A. (2008) Fly 2, 384–394
40. Phillips, A. M., Bull, A., and Kelly, L. E. (1992) Neuron 8, 631–642
41. Scott, K., Sun, Y., Beckingham, K., and Zuker, C. S. (1997) Cell 91, 375–383
42. Deen, P. J. P., Mulders, S. M., Errington, R. J., van Baal, B. J., and van Os, C. H. (1997) Am. Soc. Nephrol. 8, 1493–1501
43. van Balkom, B. W., Graat, M. P., van Raak, M., Hofmann, E., van der Sluijs, P., and Deen, P. M. (2004) Am. J. Physiol. Cell Physiol. 286, C372–C379
44. Fushimi, K., Sasaki, S., and Marumo, F. (1997) J. Biol. Chem. 272, 14800–14804
45. Katsura, T., Gustafson, C. E., Ausillo, D. A., and Brown, D. (1997) Am. J. Physiol. 272, F817–F822
46. Phelps, C. B., and Gaudet, R. (2007) J. Biol. Chem. 282, 36474–36480
47. Becker, D., Müller, M., Leuner, K., and Jendrach, M. (2008) Mol. Membr. Biol. 25, 139–151