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Light-regulated translocation of signaling proteins in *Drosophila* photoreceptors

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Abstract

Illumination of *Drosophila* photoreceptor cells induces multi-facet responses, which include generation of the photoreceptor potential, screening pigment migration and translocation of signaling proteins which is the focus of recent extensive research. Translocation of three signaling molecules is covered in this review: (1) Light-dependent translocation of arrestin from the cytosol to the signaling membrane, the rhabdomere, determines the lifetime of activated rhodopsin. Arrestin translocates in PIP_3 and NINAC myosin III dependent manner, and specific mutations which disrupt the interaction between arrestin and PIP_3 or NINAC also impair the light-dependant translocation of arrestin and the termination of the response to light. (2) Activation of *Drosophila* visual G protein, DGq, causes a massive and reversible, translocation of the α subunit from the signaling membrane to the cytosol, accompanied by activity-dependent architectural changes. Analysis of the translocation and the recovery kinetics of DGq_α in wild-type flies and specific visual mutants indicated that DGq_α is necessary but not sufficient for the architectural changes. (3) The TRP-like (TRPL) but not TRP channels translocate in a light-dependent manner between the rhabdomere and the cell body. As a physiological consequence of this light-dependent modulation of the TRP/TRPL ratio, the photoreceptors of dark-adapted flies operate at a wider dynamic range, which allows the photoreceptors enriched with TRPL to function better in darkness and dim background illumination. Altogether, signal-dependent movement of signaling proteins plays a major role in the maintenance and function of photoreceptor cells.

Keywords

Photoreceptors; Translocation; TRPL; $G_q\alpha$; Arrestin; Adaptation

1. Introduction

Vertebrate and invertebrate photoreceptors are highly polarized cells comprised of the cell body and the signaling compartment (Hardie and Raghu, 2001). The signaling compartment is designated rhabdorere in invertebrates, and outer segment in vertebrates. The major proteins that are essential for phototransduction reside in the signaling compartment. Absorption of photons by the photopigment rhodopsin in fly photoreceptors activates $G_q\alpha$ protein (Scott et al., 1995), which in turn activates phospholipase C_β (PLC_β) (Devary et al., 1987) and lead, in a still unclear way, to activation of the light sensitive channels TRP (Hardie and Minke, 1992) and TRPL (Phillips et al., 1992). Inactivation of the photopigment is achieved by the binding of arrestin2 (Byk et al., 1993) to metarhodopsin, which prevents further association between metarhodopsin and the G_q-protein.

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Photoreceptor cells adjust their sensitivity to light (Fain et al., 2001;Minke and Hardie, 2000) to fit the changing ambient illumination and thus maintain their working range within a physiologically relevant range of light intensities, by a mechanism known as light adaptation. Short-term adaptation rapidly reduces the amplitude of the electrical response to light. Ca^{2+} is a key regulator of this process, in which the amplitude of the response to light recovers within less than a minute. Long-term adaptation of invertebrate photoreceptors, which takes place in a time-scale of many minutes to hours, is governed mainly by translocations of signaling proteins into and out of the signaling membrane.

In this review we cover a striking phenomenon found in *Drosophila* photoreceptors and in mammalian signaling pathways. This phenomenon is manifested in a light-regulated translocation of several critical signaling proteins from the rhabdomere, to the cell body, and vice versa. These signal-regulated translocations play critical roles in the physiology of *Drosophila* photoreceptor cells and mammalian cells and tissues.

2. Light adaptation through phosphoinositide and NINAC myosin III dependant translocation of arrestin

Absorption of photons converts rhodopsin to its dark stable and physiologically active photoproduct, metarhodopsin. Binding of arrestin2 (Arr2) to the phosphorylated metarhodopsin prevents the activation of $G_q\alpha$ by metarhodopsin and thus determines the lifetime of functional metarhodopsin. The dissociation of Arr2 from rhodopsin requires photoconversion of metarhodopsin back to rhodopsin, followed by dephosphorylation of rhodospsin by the Ca^{2+} dependent protein phosphatase RDGC (retinat degeneration C) (Byk et al., 1993;Dolph et al., 1993;Steele et al., 1992;Yamada et al., 1990). Arr2 is phosphorylated by calcium/calmodulin-dependent kinase II (CamKII) (Matsumoto et al., 1994;Yamada et al., 1990), which is activated by Ca^{2+} influx through the light activated TRP and TRPL channels (Alloway and Dolph, 1999;Byk et al., 1993;Kiselev et al., 2000;Peretz et al., 1994). Mutations which cause stable association between Arr2 and metarhodopsin result in clathrin-dependent endocytosis of the metarhodopsin–Arr2 complexes into the cell body that eventually lead to degeneration of the photoreceptor cell (Alloway and Dolph, 1999;Alloway et al., 2000;Kiselev et al., 2000;Orem and Dolph, 2002).

Recent immunolocalization studies by Lee et al. (2003) have shown that light-dependant translocation of Arr2 to and from the rhabdomere is impaired in *cds* (Wu et al., 1995) and *rdgB* (Vihtelic et al., 1993,1991) mutants, in which PI biogenesis and trafficking are disrupted, respectively. They, furthermore, showed that in *PTEN; dAkt* and *PTEN* overexpression (Huang et al., 1999;Stocker et al., 2002) mutants, in which metabolism of PIP_3 is defective, Arr2 translocation was also impaired. Using a nitro-cellulose phospholipids binding assay they have shown that the Arr2 C-terminal, but not N-terminal, binds directly to several PIs in a pattern similar to CRAC proteins that bind preferentially PIP_3. Since the amino acid residues that contribute to the binding of PI in β-arrestin (Gaidarov et al., 1999) are not conserved in Arr2, a structural homology modeling was used to identify candidate residues participating in the interaction between Arr2 and PIP_3. The arrestin/PIP_3 binding model suggests that Lysine 228, 231 and 257 create a basic amino acid pocket and form hydrogen bonds with the three phosphates in PIP_3.

To determine the physiological role of PI binding, Montell and colleagues have generated transgenic flies that express the full length Arr2 with substitutions of all three lysine residues with glutamine (*3K/Q*), and with arginine (*3K/R*) as a control. Both mutants showed no defect in association or dissociation of Arr2 from metarhodopsin, but in the $arr2^{3K/Q}$ alone both the light-dependent movement of Arr2 to the rhabdomere, and the clathrin-dependant endocytosis were delayed. Moreover, blocking phototransduction by the PLC mutant *norpA* in the

norpA;arr2$^{3K/Q}$ double mutant suppressed light-dependent retinal degeneration typical for *norpA* mutants (Alloway and Dolph, 1999;Alloway et al., 2000;Ostroy, 1978).

To investigate the molecular basis of the PI-dependent translocation of Arr2 into the rhabdomere, Montell and colleagues used the *ninaC* (neither inactivation nor after-potential C) mutants (Lee and Montell, 2004). The NINAC protein consists of a myosin III head domain linked at the N-terminal to a protein kinase catalytic domain. The *ninaC* gene (Montell and Rubin, 1988) encodes two isoforms: p132 which is a shorter protein localized to the cell body and a larger protein, p174, which is localized to the rhabdomere. The null mutant *ninaC*^{P235} lacks both proteins. Although the precise role of NINAC is not entirely clear absence of NINAC results in abnormal cytoskeleton in the signaling compartment. The light-induced translocation of Arr2 was impaired in *ninaC*^{P132} and in the null mutant *ninaC*^{P235}, but not in the *ninaC*^{P174}. Although Arr2 did not coimmunoprecipitate with NINAC, Arr2 did bind to calmodulin-agarose beads in wild type and in the two NINAC mutants *ninaC*^{P132} and *ninaC*^{P174} but not in *ninaC*^{P235} null mutant, indicating that Arr2 interacts with both isoforms of NINAC. In contrast, the binding of Arr2 to the calmodulin-agarose beads in the *arr2*$^{3K/R}$ mutant was dramatically decreased. Binding assays have shown that both NINAC isoforms bind to PIP_2 and PIP_3 beads. This interaction was not dependent on Arr2, as NINAC was also bound to PIP_3 and PIP_2 beads in *arr2*^{5} null mutant.

Electroretinogram (ERG) recordings, is a measure of the extracellular response to light of the entire eye in vivo. ERG measured from dark and light adapted wild-type flies revealed that illumination with white light, similar to that used to induce light-dependent translocation of Arr2 into the rhabdomere, increased significantly the speed of ERG responses termination. This phenomenon was interpreted as long-term adaptation arising from Arr2 translocation, since the binding of Arr2 to metarhodopsin is a rate limiting step in the termination of the photoresponse (Dolph et al., 1993;Ranganathan and Stevens, 1995) to intense white or blue lights and a severe reduction in the concentration of Arr2 throughout the cell result in slow response termination. In support of this view, the termination rate of the ERG in *arr2*^{5} null mutant was hardly affected by prior illumination. In order to examine the relationship between the adaptation phenomenon and the translocation of Arr2, the time required for maximal adaptation to light, as measured by the rate of response termination, was examined in mutant flies. Indeed, in the *arr2*$^{3K/Q}$ mutants slower ERG response termination was observed even after 10 min illumination that was sufficient for almost maximal adaptation in wild type and *arr2*$^{3K/R}$. Similar results were obtained in the *PTEN overexpression* mutants in which slow trafficking of Arr2 into the rhabdomere was found, thus supporting the conclusion that Arr2 interaction with PI is essential for this long-term adaptation. Adaptation was also defective in *ninaC*^{P132} and even more severely affected in the *ninaC*^{P235} null mutant but not in the *ninaC*^{P174} mutant.

Altogether the authors suggest that both Arr2 and NINAC^{P132} binds to PI-containing vesicles independently, and that myosin motor activity facilitates the movement of the vesicle together with Arr2 towards the rhabdomere, while a rise in the Arr2 concentration in the rhabdomere mediates the acceleration of ERG response termination and hence shorten long-term adaptation.

3. Light regulation of $G_q\alpha$ translocation and morphological changes in *Drosophila* photoreceptors

Many signal transduction cascades use the heterotrimeric G-proteins as a molecular transducers. One of the two major determinants of the localization of G-proteins to the membrane is the lipid modification of G-proteins. All α subunits of heterotrimeric G-proteins (with the exception of transducin) are reversibly modified by palmitoylation, i.e., the

attachment of palmitate to a cysteine residue near the N-terminus (reviewed in Chen and Manning (2001), Mumby (1997),Smotrys and Linder (2004),Wedegaertner (1998)). The second major determinant of G-protein localization is the anchoring of the Gβγ subunits to the membrane (Evanko et al., 2001,2000).

Although heterotrimeric G-proteins are subject of intensive research little is known about the regulation of G_{α} subunit localization within the natural endogenous environment of a specialized signaling cells. Recent studies by Kosloff et al. (2003) using live *Drosophila* flies, have indicated that light causes massive and reversible translocation of the visual $G_q\alpha$ to the cytosol. In these studies the *Drosophila* eye-specific $G_q\alpha$ ($DG_q\alpha$) translocates to the cytosol during illumination and subsequently returns to the membrane as an inactive $G_q\alpha$-GDP form, via binding to the βγ dimmer. The role of the β-subunit in $DG_q\alpha$ movement was studied in *Drosophila* photoreceptors by using the $G^{1}_{\beta e}$ mutant (Dolph et al., 1994), which expresses highly reduced amounts of the eye-specific $DG_q\beta$. Analysis of the heterozygot $G^{1}_{\beta e}$ revealed that the kinetics of the light-dependent translocation of $DG_q\alpha$, and in particular the subsequent recovery were markedly slowed down.

The movement of $G_q\alpha$ was associated with marked architectural changes in the signaling compartment, the rhabdomere (Fig. 1). Genetic dissection together with detailed kinetic analysis, were used to characterize the translocation cycle and to unravel how signaling molecules that interact with $G_q\alpha$ affect these processes. Epistatic analysis showed that $G_q\alpha$ is necessary but not sufficient to bring about the morphological changes in the signaling organelle. Furthermore, mutant analysis indicated that $G_q\beta$ is essential for targeting of $G_q\alpha$ to the membrane and suggested that $G_q\beta$ is also needed for efficient activation of $G_q\alpha$ by rhodopsin. These results support the ‘two signal model’ hypothesis (Resh, 1999;Wedegaertner, 1998) for membrane targeting in a living organism, which suggests that both binding to $G_{\beta\gamma}$ and lipid modification are required or Gα binding to the membrane. These studies, furthermore, characterize the regulation of both the activity-dependent $G_q\alpha$ localization and the cellular architectural changes in *Drosophila* photoreceptors.

4. Light-regulated translocation of the TRPL channel

To achieve a very high gain, characteristic of the electrical response to light, the photoreceptor cell maximizes the current produced by a single photon using both the TRP channel and a relatively high level of TRPL channels. To prevent saturation of the light response upon an increase in ambient light, the Ca^{2+} flow via the Ca^{2+}-selective TRP channel attenuates the non-selective cation channel, TRPL (Reuss et al., 1997), and also induces its translocation out of the signaling membranes (Bähner et al., 2002). This novel mechanism to fine-tune visual responses is also used by other cellular signaling mechanisms such as fine tuning of growth cone path finding due to the action of a growth factor on TRPC5 activation (Bezzerides et al., 2004).

The relatively high level of TRPL, observed in photoreceptor membranes of dark-raised flies, is decreased when the flies are transferred to light (Fig. 2). Furthermore, the amount of rhabdomeral TRPL of flies that were kept in light for 16 h was close to the detection limit of the Western blot analysis but increased to a high level within 1 h after transferring of the flies to darkness. The amount of rhabdomeral TRP and also that of other components of the INAD signaling complex (Shieh and Niemeyer, 1995;Tsunoda et al., 1997;Tsunoda and Zuker, 1999) PLCβ, ePKC, and INAD, was unaffected by light. This finding indicates that the changes in the rhabdomeral TRPL level are specific to this ion channel subunit.

Direct visualization of intracellular movements of TRPL in photoreceptors upon illumination was obtained by immunolabeling of cross-sections through *Drosophila* eyes. This study

revealed that unlike TRPL, TRP and INAD are confined to the rhabdomeres, independent of whether the flies are kept in darkness or in light prior to sectioning. Antibodies directed against TRPL specifically labeled the rhabdomere area of cross-sectioned eyes obtained from dark-raised flies (Fig. 2). In the eye-sections of light-raised flies the TRPL-specific immunofluorescence was distributed over the cell body of the photoreceptor cell and was not detected in the rhabdomeres (Fig. 2) in line with the Western blot analysis.

Since the translocation of TRPL depends on illumination, the question arises whether or not the response to light through activation of the TRP and TRPL channels is the trigger for TRPL movement to the cell body. Using immunocytochemistry, TRPL translocation from the rhabdomere to the cell body was tested in the nearly null PLC mutant, $norpA^{P24}$ (Bloomquist et al., 1988), the null mutant of TRP, trp^{P343} (Scott et al., 1997) and the null INAD mutant, $inaD^{1}$. Young $inaD^{1}$ flies contain rhabdomeral TRP, which is most likely detached from the signaling complex and therefore degrades with age and is largely missing in older $inaD^{1}$ flies (Tsunoda et al., 1997). Light-induced translocation of TRPL to the cell body was observed in young $inaD^{1}$ flies, but did not occur in the *trp* null mutant and in old $inaD^{1}$ flies. In $norpA^{P24}$ mutants, translocation of TRPL tagged with Green Fluorescent Protein (TRPL-eGFP) was blocked (Bähner et al., 2002 note added in proofs). These findings reveal that when TRP is missing, as in the *trp* mutant and in old $inaD^{1}$ flies, TRPL internalization is not observed (Bähner et al., 2002). Therefore, the presence of TRP seems to be required for TRPL internalization. If the lack of PLC blocks TRPL translocation as found in TRPL-eGFP fusion experiments, then the block of TRPL translocation (Hardie and Minke, 1992;Peretz et al., 1994), by the absence of either TRP or PLC, suggests that light-induced Ca^{2+} influx is the trigger for TRPL translocation.

The light-induced current (LIC) of wild-type flies is composed of two independent components arising from activation of the TRP and TRPL channels (Reuss et al., 1997). Patch clamp whole cell recordings in isolated ommatidia of *Drosophila* were used to examine two properties of the LIC which discriminate between the contribution of the TRP and TRPL channels to the LIC: the block by La^{3+} and the reversal potential (Reuss et al., 1997).

Application of La^{3+} in micromolar concentration is known to specifically block the TRP but not the TRPL channels (Hardie and Minke, 1992;Hochstrate, 1989;Suss Toby et al., 1991). In wild-type *Drosophila* application of La^{3+} specifically blocks the TRP channels leaving a residual response, which is mediated by the TRPL channels and is indistinguishable from the response measured in *trp* mutant photoreceptors. In light-raised wild-type flies, application of La^{3+} largely reduced the peak amplitude of the LIC in response to intense light and modified the waveform of the response to prolonged light displaying the typical *trp* phenotype. In dark-raised wild-type flies application of La^{3+} under identical experimental conditions, had a much smaller effect on the peak amplitude of the LIC in response to similar light intensity. The weak trp phenotype in dark-raised flies indicates a reduced effect for La^{3+} (Fig. 3). Quantitative analysis at dim lights shows that La^{3+} suppressed the LIC suggesting a relative contribution of TRPL to the LIC of ~9% and 38%, in light- and dark-raised flies, respectively. Roughly similar conclusions were derived from measurements of the change in the reversal potential of light and dark-raised flies, together suggesting that a significantly larger amount of functional TRPL channels is present in dark-raised flies relative to light-raised flies (Bähner et al., 2002).

Measurements of the sensitivity to light of dark- and light-raised flies in vivo revealed that wild-type flies kept in darkness are very sensitive to dim background lights and respond within a relatively wide dynamic range having relatively low sensitivity to small changes in stimulus intensity. Wild-type light-raised flies are less sensitive to dim background lights, have a smaller dynamic range, but their photoreceptors are more sensitive to small changes in light intensity

within their dynamic range. The fact that *trpl* mutants, when kept in either light or darkness, behave similar to light-raised wild-type flies, strongly suggests that translocation of TRPL underlies the fine tuning of long-term adaptation (Bähner et al., 2002).

Translocation of TRP channels of both *Drosophila* and mammalian cells (Bähner et al., 2002;Bezzerides et al., 2004;Boels et al., 2001;Kanzaki et al., 1999) emerges as a novel and important regulatory mechanism with wide implications to a variety of signaling mechanisms.

5. Concluding remarks

Signal-dependent movement of proteins has recently emerged as an important mechanisms to fine tune the function of several processes including neuronal path finding and long-term adaptation of both vertebrate and invertebrate photoreceptors. While the molecular mechanism underlying translocation of soluble proteins such as DGq, transducin and arrestin has been partially elucidated, the molecular mechanism underlying translocation of channel proteins is largely unknown and imposes a challenge for future studies.

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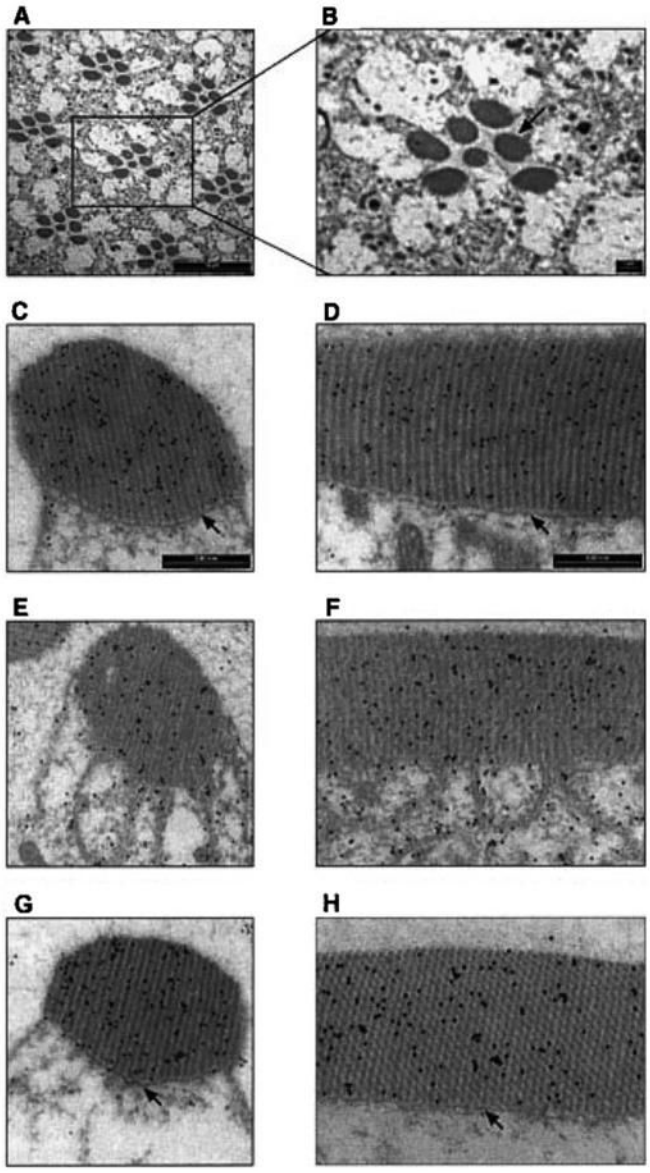

Fig 1.

Light-dependent translocation of $DG_q\alpha$ and rhabdomeral architectural changes shown by immunogold labeling. Panels A and B show a low magnification cross-section of the *Drosophila*'s compound eye for orientation. The seven dark oval structures of each visual unit (ommatidium) are the rhabdomeres, the signaling compartment of the photoreceptor cell composed of tightly packed microvilli. Panels C, E, G show cross-sections of a single rhabdomere while panels D, F, H show a longitudinal section along the axis of the rhabdomere. In these panels, $DG_q\alpha$ is localized by immunogold labeling, seen as dark dots. Panels C and D are from dark-adapted flies. Panels E and F are from flies illuminated for 60 min with blue light. Panels G and H are from flies that had been illuminated for 60 min and subsequently kept for 2 h in the dark. Illumination (panels E and F) caused remarkable architectural changes in the cell. The boundary between the rhabdomere and the rest of the cell disappears due to disruption of the cytoskeletal Rhabdomeral Terminal Web (RTW—marked with arrows in panels C and D). As a result, the rhabdomere collapses and membrane tendrils resembling a house painter's brush are seen intruding into the cytosol. These architectural changes are

completely reversible, as after 2 h in the dark (panels G and H) the rhabdomeres are indistinguishable from those of dark-adapted flies (panels C and D). Similar observations were found in six different flies. (From Kosloff et al., 2003.)

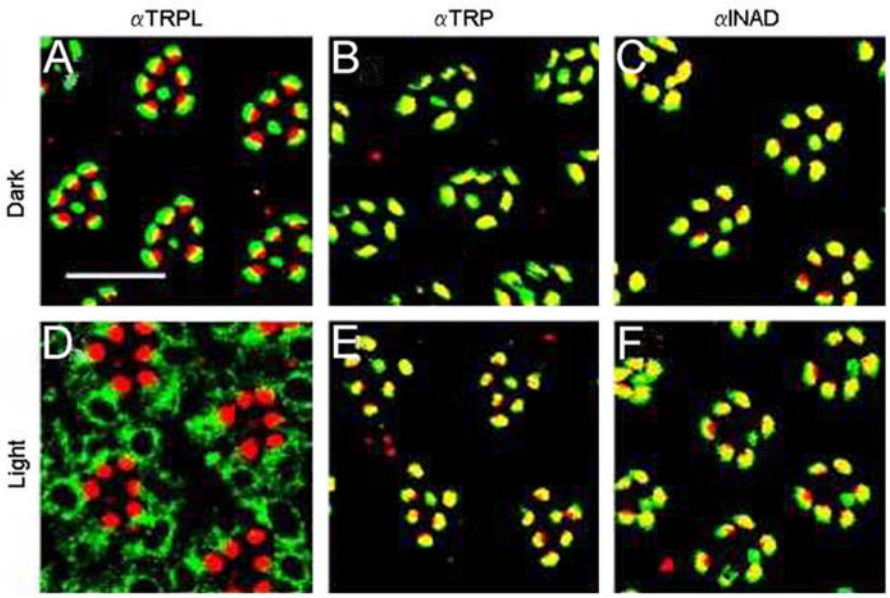

Fig 2.

Light-dependent translocation of TRPL molecules in the photoreceptor cells of *Drosophila* compound eyes. Cross-sections through wild-type *Drosophila* (A–F) eyes of light- and dark-raised flies were double labeled with rhodamin-coupled wheat germ agglutinin, which specifically labels rhabdomeral photoreceptor membranes (red fluorescence), and antibodies against TRPL, TRP, and INAD, as indicated. The overlay of both markers appears yellow. Scale bars in (A), 10 μm. (Modified from Bähner et al., 2002.)

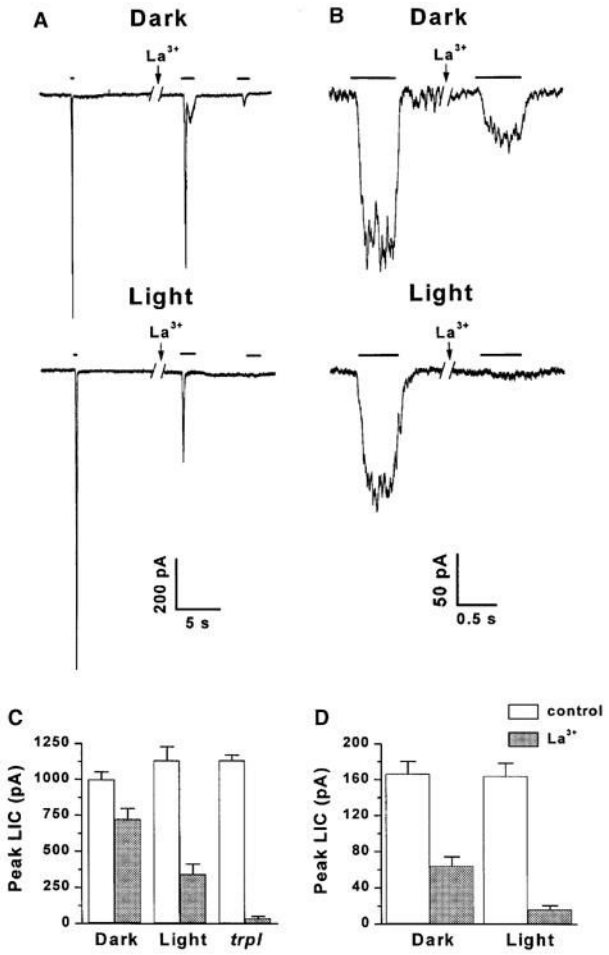

Fig 3.

Differential block of the LIC by La^{3+} in light- and dark-raised wild-type flies and in the *trpl* mutant. (A) Responses to relatively intense lights of wild-type *Drosophila* cells of flies raised in light or darkness (as indicated) during whole cell recordings at a holding potential of −60 mV. Cells were stimulated by constant white light, of durations indicated by bars, attenuated by 3.6 or 2.5 log units for dark- and light-raised flies, respectively. The left LIC are control responses. Application of La^{3+} (10 μM) to the bath (as indicated by arrow) led to a reduction in peak amplitude and suppression of the response to a second light pulse, typical for the trp mutant. The break in the traces indicates a time of 2 min. (B) Responses to relatively dim lights (orange lights, attenuated by 2.8–3.3 log units) in a paradigm similar to that of Fig. 3A, except that 0.5 mM external Ca^{2+} was used and the La^{3+} concentration was 20 μM. The effective intensity of the orange light was ~100 fold dimmer than the white light used in A. (C) Histogram plotting the peak amplitudes of the LIC of Fig. 3A, in response to the constant light before (control) and after application of La^{3+} in dark- and light-raised wild-type flies and in the *trpl* mutant (as indicated). The error bars are SEM calculated from 6–8 cells for each group. (D) Histogram plotting the peak amplitudes of the LIC of Fig. 3B in response to the dim test light before (control) and after application of La^{3+} in dark- and light-raised flies. The error bars are SEM calculated from 5 cells for each group. (From Bähner et al., 2002.)