The latency of the light response is modulated by the phosphorylation state of Drosophila TRP at a specific site

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Abstract

Drosophila photoreceptors respond to oscillating light of high frequency (~100 Hz), while increasing the oscillating light intensity raises the maximally detected frequency. Recently, we reported that dephosphorylation of the light-activated TRP ion channel at S936 is a fast, graded, light-, and Ca2+-dependent process. We further found that this process affects the detection limit of high frequency oscillating light. Accordingly, transgenic Drosophila, which do not undergo phosphorylation at the S936-TRP site (trpS936A), revealed a short time-interval before following the high stimulus frequency (oscillation-lock response) in both dark- and light-adapted flies. In contrast, the trpS936D transgenic flies, which mimic constant phosphorylation, showed a long-time interval to oscillation-lock response in both dark- and light-adapted flies. Here we extend these findings by showing that dark-adapted trpS936A flies reveal light-induced current (LIC) with short latency relative to trpWT or trpS936D flies, indicating that the channels are a limiting factor of response kinetics. The results indicate that properties of the light-activated channels together with the dynamic light-dependent process of TRP phosphorylation at the S936 site determine response kinetics.

Keywords

light induced current; transgenic Drosophila; TRP channel; TRP dephosphorylation

Results

To investigate the physiological roles of light-dependent pS936 dephosphorylation in the most direct manner, in our previous study, we generated transgenic flies expressing TRP channels in which Ser 936 was exchanged to Ala (trpS936A) or to Asp (trpS936D), permanently preventing or mimicking phosphorylation of this site, respectively. In addition, we generated a transgenic fly expressing an unmodified TRP channel (trpWT) as control. All transgenes were expressed under control of the rh1 (rhodopsin 1) promoter and crossed onto a trpP343 (trp null mutant) background. Here we extended our study by analysis of the above 3 transgenic strains on a double null trp1302;trpP343 background (see Methods). Accordingly, in our...
previous study, the native light-activated TRP channel was eliminated in the transgenic flies expressing either mutant or wild type TRP, while in the present study both the native light-activated TRP and TRPL channels were eliminated.

To rule out possible effects of the transgenic expression of TRP per se, we performed several control experiments. In principle, an abnormal light response may be associated with impaired function of TRP channels due to effects on channel gating, large reduction in channel protein levels, failure to interact with the INAD scaffold protein, failure to assemble and form homotetramers, or mislocalization of the TRP channel outside the signaling compartment, the rhabdomere. Accordingly, in the previous study, we performed the above controls for the transgenic flies on a single null trp$^{P343}$ background. In the present study, we repeated the necessary controls on transgenes expressed on double null trp$^{P302}, trp^{P343}$ background. We examined whether trp$^{S936A}$ and trp$^{S936D}$ mutant channels are expressed at levels comparable to wild type TRP and whether they induce cross effects on the expression of other phototransduction proteins, using Western blot analyses. Indeed, protein levels of TRP$^{S936A}$, TRP$^{S936D}$, and TRP$^{WT}$ in the transgenic flies (86.9 ± 20.4%, 96.1 ± 11.6% and 106.7 ± 27.2%, respectively) were comparable to that of wild type TRP (designated “wild type” in Fig. 1A). Furthermore, other signaling proteins of the phototransduction cascade were present at comparable amounts to their wild type levels (Fig. 1B). Examination of the rhododermic localization of TRP$^{WT}$, TRP$^{S936A}$ and TRP$^{S936D}$ proteins, using immunocytochemistry, revealed proper localization of the channels to the rhabdomere, similar to that of wild type TRP (Fig. 1C). However, both TRP$^{WT}$ and TRP$^{S936A}$ showed increased signal at the cell body compared with TRP$^{S936D}$ and wild type TRP. Together, the control studies indicated that the trp$^{S936A}$, trp$^{S936D}$, and trp$^{WT}$ transgenes expressed on double null trpl;trp background were suitable for examining the effect of permanent dephosphorylation or having a phosphomimetic mutation at the S936-TRP site, on the physiological response to light.

A crucial experimental finding of the previous study was the marked difference in the time interval to frequency-locked response at high frequencies (70 Hz) between light-adapted trp$^{S936A}$ and trp$^{S936D}$ transgenic flies, indicating that the time interval to frequency-locked response depends on the phosphorylation state of S936-TRP. In the present study, we present an additional independent experimental finding showing a marked physiological difference between dark-adapted trp$^{S936A}$ and trp$^{WT}$ or trp$^{S936D}$ transgenic flies. The above transgenic flies were also useful to identify an additional factor of the light response kinetics, which is reflected in the latency of the light response. Transduction stages affecting the latency of the single photon response (quantum bumps) were previously investigated by Scott and Zuker and by Minke and colleagues. These studies revealed that mutations of the Gqα protein had a relatively small effect on the latency of the light response, reflecting the small contribution of rhodopsin-G-protein coupling to the latency. In contrast, mutations in phospholipase C (PLC) dramatically increased the response latency. However, the effects of manipulating the light-activated channels on the response latency were not investigated. This issue was investigated in the present study.

When expressed on a single null trp$^{P343}$ background, the response latency of trp$^{S936A}$ fly was significantly shorter relative to trp$^{WT}$ at dim (3 × 10$^3$ EP/s) light intensity, while similar latencies of the two fly strains were found for medium and intense light stimulation, with a tendency of shorter mean latency of the trp$^{S936A}$ flies (Fig. 2A, B). This tendency was also observed when measuring the latency distribution of the quantum bumps of the above two fly strains, in response to repeated dim light pulses of constant intensity and duration. Since the quantum bump latency is a highly variable parameter, even when measured in the same cell under identical stimulus intensity (see Fig. 2C), we plotted the measured bump latency distribution and fitted the data to a log-normal distribution for the two fly strains (Fig. 2D). Consistent with measurements of the latency of the macroscopic light-induced current (LIC), the bump latency distribution of trp$^{S936A}$ was left shifted compared with trp$^{WT}$ (mode of trp$^{S936A}$ = 65.56 ms, mode of trp$^{WT}$ = 83.9 ms, Fig. 2D).

In the previous study, we measured the effect of S936-TRP phosphorylation in vivo using transgenic flies on a trp$^{P343}$ single null background. These flies lack the native TRP but express normal levels of the TRPL channel. The reason for choosing a single null trp$^{P343}$ background over the double null trp$^{P302}, trp^{P343}$ background for in-vivo analysis of the frequency response was because of an additional mutation of the...


trpl302 null mutant fly. Accordingly, the trpl302 mutant fly, which eliminates the native TRPL channel,9 harbors an additional mutation in the inebriated gene, which, upon illumination, induces membrane potential oscillations at the synaptic terminal and thus alters the frequency response to oscillating light23 and prevents accurate analysis. However, in the present study, the isolated ommatidia preparation used for whole cell recordings lack the synaptic terminal.10-12 Therefore, transgenic flies on double null trpl302;trpP343 background could be used. In the experiments of Fig. 2E and F, we measured the latency of the macroscopic LIC of transgenic flies expressing trpS936A, trpS936D, or trpWT on trpl302;trpP343 double null background. Importantly, the trpS936A strain revealed significantly shorter response latencies at all light

Figure 1. Biochemical and immunocytochemical analysis of transgenic flies that express TRP channels with altered S936-TRP phosphorylation on double null trpl302;trpP343 background. (A) TRP and Tubulin expression levels were quantified using Western blot analyses of trpS936A, trpS936D, and trpWT transgenes on a trpl302;trpP343 double null mutant background as well as trpP343 and WT flies as controls. For quantification of TRP expression level, the integrated density values of the TRP bands were divided by the integrated density values of the Tubulin bands. These values were normalized to the wild type value which was set to 100%. The graph was obtained from four independent experiments. Error bars show S.E.M. (B) Quantification of the expression levels of major signaling proteins in various fly strains as indicated using Western blot analyses. Molecular mass markers are indicated to the left of the blots. The experiment was performed once (see method). (C) Immunocytochemical analysis of TRP localization in photoreceptors. Cross-sections through the eyes of wild type flies and flies expressing the transgenes trpWT, trpS936A, and trpS936D on trpl302;trpP343 double null background were probed with an α-TRP (green) and with phalloidin (red), which labels the rhabdomeres. Overlay of both colors in the merged panels appears yellow. Scale bar, 10 μm.
intensities tested, relative to \( trp^{S936D} \) or \( trp^{WT} \), while the response latencies of \( trp^{S936D} \) and \( trp^{WT} \) strains were similar (Fig. 2E and F).

Interestingly, under dark adaptation, activation of the TRP channel revealed latencies longer than the latencies of the TRP-like (TRPL) channel (measured in WT flies, Fig. 3A, B). The light response of the TRP channel can be measured in isolation in the \( trpl^{302} \) mutant\(^9\) expressing only the TRP channel (see Western blot analysis at Fig. 3D). Furthermore, when the cell membrane was clamped to voltages close to the reversal potential of the LIC (~8 mV), a biphasic positive-negative LIC appeared when recording from WT flies (Fig. 3C and see\(^11\),\(^14\),\(^15\)) which is absent when recording from \( trpl^{302} \) mutant flies. The positive current fits the activation of the TRPL channel (reversal potential ~0 mV), while the negative current arises from activation of the TRP channels (reversal potential ~16 mV). It is thus evident that the positive TRPL-dependent current appears faster than the negative TRP-dependent current. Strikingly, the mutant S936A-TRP channel (in the \( trp^{S936A} \) fly) revealed even shorter response latencies at various light intensities than the TRPL channel. Since we used transgenic flies

Figure 2. (For figure legend, see page 682.)
in this study, a question arises as to the effect of transgenic expression on the latency of the light response. A comparison of the latency of WT and transgenic fly expressing unmodified TRP (trpWT) on trpP343 background (Fig. 3B and Fig. 2B, respectively) showed a significant shortening of the latency of the transgenic fly (two-way ANOVA). Similarly, a comparison of the latency of trpS936A mutant flies and transgenic fly expressing unmodified TRP (trpWT) on double null trpP343 background (Fig. 3B and Fig. 2F, respectively) showed a significant shortening of the latency of the transgenic fly (two-way ANOVA). However, Bonferroni’s multiple comparison test revealed no statistical significant difference of the latencies at all measured light intensities. Most importantly, the trpS936A transgenic fly revealed the shortest latency, indicating that the effect of transgenic expression has a minor effect on the latency.

In conclusion, the ability to modulate the latency of the light response by affecting the phosphorylation state of the TRP channel indicates that properties of the light-activated channels together with the dynamic light-dependent process of TRP phosphorylation at the S936 site determine response kinetics.

Materials and methods

Fly strains - Transgenic flies on single trp null background (yw;[rh1-trpS936A]trpP343, yw;[rh1-trpS936D]); trpP343, and yw;p[rh1-trpWT, trpP343]). Transgenic flies on double null trpl;trp background (yw;p[rh1-trpS936A], trpP343, yw;p[rh1-trpS936D], trpl302;trpP343 and yw;[rh1-trpWT, trpl302;trpP343].

Light stimulation - A xenon high-pressure lamp (PTI, LPS 220, operating at 50W, Lawrenceville, NJ) was used and the light stimuli were delivered to the ommatidia by means of epo-illumination via an objective lens (in situ). The intensity of the orange light (Schott OG 590 edge filter) at the specimen, without intervening neutral density filters, was 13 mW/cm². The light intensity was attenuated by using neutral density filters in log scale. Absolute calibration of the effective number of photons in the stimuli was achieved by counting quantum bumps in dark-adapted WT photoreceptors under control conditions with dim light.

Solutions - For Drosophila isolated ommatidia, the extracellular solution contained the following (in mM): NaCl, 120 KCl, 1.5 CaCl2, 4 MgCl2, 10 TES, 25 L-proline, 5 L-alanine. For measuring light-induced bumps and macroscopic response, we used an intracellular pipette solution containing (in mM): 140 K-glucanote, 10 TES, 2 MgCl2, 4 MgATP, 0.4 NaGTP and 1 nicotinamide adenine dinucleotide (NAD). For reversal potential measurements, the pipette solution contained (in mM) 140 CsCl, 15 tetraethylammonium chloride (TEA), 2 MgSO4, 10 TES, 4 MgATP, 0.4 NaGTP, and 1 NAD. All solutions were adjusted to pH 7.15.

Figure 2. (see previous page) Reduced latencies measured in trpS936A transgenic flies expressed on single null trpP343 and double null trpl;trpP343 background. (A) Whole cell patch clamp recordings comparing the latency of the LICs in trpP343 and trpWT transgenic flies expressed on single trpP343 null background. Representative latencies at medium light intensities. (B) A histogram summarizing the data of the latencies measured from the LICs in response to increasing light intensities obtained from different flies of the trpP343 and trpWT. A two-way ANOVA was conducted that examined the effect of transgenic fly strains and light intensity on response latency. The effect of light strain on the latency was statistically significant (P = 0.0005). The effect of light intensity on the latency was statistically significant (P < 0.0001) and no statistically significant interaction was found (P = 0.4921). Bonferroni’s multiple comparison test revealed statistical significance at a light intensity of 3 × 10⁵ EP/s between the trpP343 and trpWT transgenic flies on trpP343 background (P = 0.0338). Error bars are SEM, n = 5–7. (C) Representative single photon responses (quantum bumps) recorded from trpWT transgenic flies (black) and trpS936A (red). The quantum bumps were elicited by repeated stimulation with a constant dim orange light pulse (gray bar) and the individual traces are superimposed to show the variability in latencies and amplitudes. (D) Histograms plotting quantum bumps latency distribution of the trpP343 (red) and trpWT (black) transgenic flies, using the protocol of C. The smooth curve is a fit of a log-normal function of the bump latency distribution. The histogram shows shorter bump latency distributions of the trpP343 relative to trpWT transgenic flies. (E) Whole cell patch clamp recordings comparing the latency of LIC of trpS936A, trpP343, and trpWT transgenic flies expressed on double null trpl;trpP343 background. Superposition of representative LIC traces of trpP343 and trpWT (gray and black traces, respectively) and of trpP343 and trpWT (red and black traces, respectively). Note that trpP343 and trpWT show similar latencies, while trpP343 show shorter latency compared with trpWT. (F) A histogram summarizing the data of the latency measured from the LICs in response to increasing light intensities obtained from different trpP343, trpP343, and trpWT transgenic flies on double null trpP343 background. A two-way ANOVA was conducted that examined the effect of transgenic fly strains and light intensity on response latency. The effect of light strain on the latency was statistically significant (P < 0.0001). The effect of light intensity on the latency was statistically significant (P < 0.0001) and no statistically significant interaction was found (P = 0.9121). Tukey’s multiple comparison test revealed statistical significance between the trpP343 and trpP343 transgenic flies at light intensities of 3 × 10⁴ and between the trpP343 and trpWT transgenic flies at light intensities 3 × 10⁴ and 3 × 10⁵ EP/s on trpl;trpP343 double null background. The error bars are SEM, n = 3–6.
Antibodies - For Western blot analyses, the following antibodies were used: polyclonal α-RH1 antibody, monoclonal α-TRP antibody (MAb83F6, Developmental Studies Hybridoma Bank), polyclonal α-TRP antibody, monoclonal α-β-tubulin antibody (E7, Developmental Studies Hybridoma Bank), polyclonal α-PLC antibody, monoclonal α-TRPL antibody, monoclonal α-Gqα antibody and polyclonal α-Moesin antibody. To generate polyclonal α-INAD and α-PKC antibodies, fragments spanning the last

Figure 3. A comparison of latencies of macroscopic light-induced current measured from the trpl302 mutant and wild type fly. (A) Representative LIC showing a longer latency of the trpl302 mutant (expressing only the TRP channel) relative to the wild type fly. (B) A histogram summarizing the data of the latencies measured from LICs in response to increasing light intensities obtained from the trpl302 mutant and wild type flies. A two-way ANOVA was conducted that examined the effect of fly strain and light intensity on response latency. The effect of the fly strain on the latency was statistically significant (P = 0.0006). The effect of light intensity on the latency was statistically significant (P < 0.0001) and no statistically significant interaction was found (P = 0.8727). Bonferroni’s multiple comparison test revealed statistical significance at a light intensity of 3 × 10^4 EP/s between the trpl302 mutant and wild type flies (P = 0.038). Error bars are SEM, n = 5–7 for WT and n = 10 for the trpl302 mutant. (C) The reversal potential (E_{rev}) is biphasic (positive-negative) in wild type flies (black), but monophasic in the trpl302 mutant (red). Families of superimposed LICs in response to a 20 ms orange light pulse measured in dark-adapted wild type (left) and trpl302 mutant (right) flies. The current traces were recorded during a series of voltage steps of 3 mV in the voltage ranges around E_{rev} of the LIC, as indicated. (D) Western blot analysis showing that wild type flies express both the TRP and TRPL channels at similar levels to the expression levels of TRP in the trpl302 mutant and of TRPL in the trpP343 mutant.
300 amino acids or amino acids 232–369, respectively, were heterologously expressed in bacteria with a His tag. The polypeptides were cleaned up using Ni-NTA agarose (Qiagen) according to the manufacturer’s protocol. Immunization of rabbits was done by Pineda Antibody Service. Antisera were cleaned up using a HiTrap protein A HP column (GE Healthcare Life Sciences) according to the manufacturer’s protocol.

Western blot analyses - Western blotting was performed as described previously by using polyvinylidene difluoride membranes (Bio-Rad). Chemiluminescent signals were recorded using an XRS documentation system (Bio-Rad). Quantification of Western blot bands was accomplished using the Image Lab 4.0 software (Bio-Rad). Bands were selected manually with background subtraction (disk size, 10 mm) enabled. The volume (Int) value that reflects band area and intensity was used. For the combination of transgenes in the trpl;trp double mutant background as shown in Fig. 1B, we performed the Western blot analysis once. Some of the transgenic flies in double null background were tested for expression of photoreceptor proteins in other contexts and we have never observed an obvious deviation from wild type in the expression level of the phototransduction proteins. In addition, the results were similar to those obtained with the transgenes in the trp single mutant background. The assessment of photoreceptor protein amounts in Drosophila mutants is often done qualitatively rather than quantitatively (for example, see).  

Immunocytochemistry - Immunocytochemistry of fly eyes was carried out as described previously.  

α-TRP (MAB83F6, Developmental Studies Hybridoma Bank) was used as primary antibody. Secondary antibody was α-mouse Cy5 (Dianova). AlexaFlor 546-coupled phalloidin (Life Technologies) was used to label the actin cytoskeleton of the rhabdomeres.

Whole-Cell Recordings - Dissociated Drosophila ommatidia were prepared as described in from newly eclosed flies (<1 h post eclosion) and transferred to a recording chamber on an inverted Olympus microscope. Whole-cell recordings were performed at 21°C using patch pipettes of 8–12 MΩ resistance, pulled from fiber-filled borosilicate glass capillaries. Signals were amplified using an Axopatch-1D (Molecular Devices) patch-clamp amplifier. Currents were filtered at 2 kHz using the 8-pole low pass Bessel filter of the patch-clamp amplifier and sampled at 5 kHz using the A/D converter Digidata 1440A (Molecular Devices). Data acquisition and analysis were performed using the pClamp 10.2.0.14 software (Molecular Devices). Series resistance values were below 25 MΩ and were routinely compensated to above 80% when recording macroscopic responses greater than 100 pA, but not when recording bumps. Membrane potential was clamped to −70 mV.

Bump analysis - Quantum-bumps were elicited by repeated stimulation with dim orange illumination (Schott OG 590 edge filter). Bumps were detected offline using the event detection threshold search function of pClamp 10.2.0.14 software (Molecular devices). The following parameters were used; trigger: −3 pA, re-arm: −2 pA, pretrigger: 1 ms, post-trigger: 1 ms and minimum allowed duration: 10 ms.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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References

[1] Wu CF, Wong F. Frequency characteristics in the visual system of Drosophila: Genetic dissection of electroretinogram components. J Gen Physiol. 1977;69:705-24. doi:10.1085/jgp.69.6.705. PMID:894240
[2] Voolstra O, Rhodes-Mordov E, Katz B, Bartels JP, Obereggsbacher C, Schotthöfer SK, Yasin B, Tzadok H, Huber A, Minke B. The phosphorylation state of the Drosophila TRP channel modulates the frequency response to oscillating light in Vivo. J Neurosci. 2017;37:4213-24. doi:10.1523/JNEUROSCI.3670-16.2017. PMID:28314815
[3] Wang T, Jiao Y, Montell C. Dissecting independent channel and scaffolding roles of the Drosophila transient receptor potential channel. J Cell Biol. 2005;171:685-94; doi:10.1083/jcb.200508030. PMID:16301334
[4] Tsunoda S, Sierralta J, Sun Y, Bodner R, Suzuki E, Becker A, Socolich M, Zuki C. A multivalent PDZ-domain protein assembles signalling complexes in a G-protein-coupled cascade. Nature 1997;388:243-9. doi:10.1038/40805. PMID:9230432
[5] Katz B, Oberacker T, Richter D, Tzadok H, Peters M, Minke B, Huber A. The Drosophila TRP and TRPL are assembled as homomultimeric channels in vivo. J Cell Sci. 2013;126:3121-33. doi:10.1242/jcs.123505. PMID:23687378
[6] Scott K, Zuker CS. Assembly of the Drosophila phototransduction cascade into a signalling complex shapes elementary responses. Nature. 1998;395:805-8. doi:10.1038/27448. PMID:9796815

[7] Cook B, Bar YM, Cohen-Ben AH, Goldstein RE, Paroush Z, Selinger Z, Minke B. Phospholipase C and termination of G-protein-mediated signalling in vivo. Nat Cell Biol. 2000;2:296-301. doi:10.1038/35010571. PMID:10806481

[8] Henderson SR, Reuss H, Hardie RC. Single photon responses in Drosophila photoreceptors and their regulation by Ca$^{2+}$. J Physiol. 2000;524(Pt 1):179-94. doi:10.1111/j.1469-7793.2000.00179.x. PMID:10747191

[9] Niemeyer BA, Suzuki E, Scott K, Jalink K, Zuker CS. The Drosophila light-activated conductance is composed of the two channels TRP and TRPL. Cell. 1996;85:651-9. doi:10.1016/S0092-8674(00)81232-5. PMID:8646774

[10] Hardie RC. Whole-cell recordings of the light induced current in dissociated Drosophila photoreceptors: Evidence for feedback by calcium permeating the light-sensitive channels. Proc R Soc Lond B. 1991;245:203-10. doi:10.1098/rspb.1991.0110

[11] Hardie RC, Minke B. The trp gene is essential for a light-activated Ca$^{2+}$ channel in Drosophila photoreceptors. Neuron. 1992;8:643-51. doi:10.1016/0896-6273(92)90086-S. PMID:1314617

[12] Katz B, Gutchorov R, Rhodes-Mordov E, Hardie RC, Minke B. Electrophysiological method for whole-cell voltage clamp recordings from Drosophila photoreceptors. J Vis Exp. 2017;13

[13] Scott K, Sun Y, Beckingham K, Zuker CS. Calmodulin regulation of Drosophila light-activated channels and receptor function mediates termination of the light response in vivo. Cell. 1997;91:375-83. doi:10.1016/S0092-8674(00)80421-3. PMID:9363946

[14] Reuss H, Mojet MH, Chyb S, Hardie RC. In vivo analysis of the Drosophila light-sensitive channels, TRP and TRPL. Neuron. 1997;19:1249-59. doi:10.1016/S0896-6273(00)80416-X. PMID:9427248

[15] Bähner M, Frechter S, Da Silva N, Minke B, Paulsen R, Huber A. Light-regulated subcellular translocation of Drosophila TRPL channels induces long-term adaptation and modifies the light-induced current. Neuron. 2002;34:83-93. doi:10.1016/S0896-6273(02)00630-X. PMID:11931743

[16] Bentrop J, Schwab K, Pak WL, Paulsen R. Site-directed mutagenesis of highly conserved amino acids in the first cytoplasmic loop of Drosophila Rh1 opsin blocks rhodopsin synthesis in the nascent state. EMBO J. 1997;16:1600-9. doi:10.1093/emboj/16.7.1600. PMID:9130705

[17] Bähner M, Sander P, Paulsen R, Huber A. The visual G protein of fly photoreceptors interacts with the PDZ domain assembled INAD signaling complex via direct binding of activated Galpha(q) to phospholipase c beta. J Biol Chem. 2000;275:2901-4. doi:10.1074/jbc.275.4.2901. PMID:10644758

[18] Huber A, Belusic G, Da-Silva N, Bähner M, Gerdon G, Draslar K, Paulsen R. The Calliphora rpa mutant lacks the PDZ domain-assembled INAD signalling complex. Eur J Neurosci. 2000;12:3909-18. doi:10.1046/j.1460-9568.2000.00276.x. PMID:11069586

[19] Xue T, Do MT, Riccio A, Jiang Z, Hsieh J, Wang HC, Merbs SL, Welsbie DS, Yoshioka T, Weissgerber P, et al. Melanopsin signalling in mammalian iris and retina. Nature. 2011;479:67-73. doi:10.1038/nature10567. PMID:22051675

[20] Elia N, Frechter S, Gedi Y, Minke B, Selinger Z. Excess of Gbeta over Galpha in vivo prevents dark, spontaneous activity of Drosophila photoreceptors. J Cell Biol. 2005;171:517-26. doi:10.1083/jcb.200506082. PMID:16260498

[21] Wang T, Wang X, Xie Q, Montell C. The SOCS box protein STOPS is required for phototransduction through its effects on phospholipase C. Neuron. 2008;57:56-68. doi:10.1016/j.neuron.2007.11.020. PMID:18184564

[22] Voolstra O, Beck K, Oberegelsbacher C, Pfannstiel J, Huber A. Light-dependent phosphorylation of the drosophila transient receptor potential ion channel. J Biol Chem. 2010;285:14275-84. doi:10.1074/jbc.M110.102053. PMID:20215118

[23] Strauch L, Cerny A, Belusic G, Huber A. Oscillations in the ERG of the Drosophila trp$^{82}$ mutant are caused by an additional mutation in the inebriated gene. Dros Inf Serv. 2016;99:8-11.