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Report

Dependence on the Lazaro Phosphatidic Acid Phosphatase for the Maximum Light Response

Young Kwon[1] and Craig Montell[1,*]
[1] Department of Biological Chemistry and Department of Neuroscience
The Johns Hopkins University School of Medicine
Baltimore, Maryland 21205

Summary

The *Drosophila* phototransduction cascade serves as a paradigm for characterizing the regulation of sensory signaling and TRP channels in vivo [1, 2]. Activation of these channels requires phospholipase C (PLC) and may depend on subsequent production of diacylglycerol (DAG) and downstream metabolites [3, 4]. DAG could potentially be produced through a second pathway involving the combined activities of a phospholipase D (PLD) [5] and a phosphatidic acid (PA) phosphatase (PAP). However, a role for a PAP in the regulation of TRP channels has not been described. Here, we report the identification of a PAP, referred to as Lazaro (Laza). Mutations in *laza* caused a reduction in the light response and faster termination kinetics. Loss of *laza* suppressed the severity of the phenotype caused by mutation of the DAG kinase, RDGA [6, 7], indicating that Laza functions in opposition to RDGA. We also showed that the retinal degeneration resulting from overexpression of the PLD [5] was suppressed by elimination of Laza. These data demonstrate a requirement for a PLD/PAP-dependent pathway for achieving the maximal light response. The genetic interactions with both *rdgA* and *Pld* indicate that Laza functions in the convergence of both PLC- and PLD-coupled signaling in vivo.

Results and Discussion

To identify new proteins that are involved in phosphoinositide (PI) signaling and participate in *Drosophila* phototransduction, we focused on a gene (*CG11440*), which we previously identified in a microarray screen for genes expressed predominately in the adult eye [8]. *CG11440* mRNA is expressed in the adult eye at a concentration 15-fold higher than in the rest of the head and encodes a 334 amino acid protein that is >35% identical to known lipid phosphate phosphatases (reviewed in [9]). As with other lipid phosphate phosphatases (LPPs), *CG11440* is characterized by multiple transmembrane segments and three conserved domains that form the catalytic domain. An LPP would be predicted to catalyze the reverse reaction promoted by RDGA (Figure 1A).

To address a potential function for the *CG11440* (referred to as *lazaro*, *laza*; see figure legends for an explanation of the gene name) in phototransduction, we obtained a P element insertion line, GE27043 (GenExel), which was inserted in the 5′ untranslated region (*laza^{GE}*; Figure 1B). We created a null mutation in *laza* by genetically mobilizing the P element, because excisions of P elements are frequently associated with deletions of the flanking genomic DNA. We screened pools of genomic DNA by PCR and obtained two lines (*laza^{1}* and *laza^{2}*) that deleted the first ATG and additional portions of the coding region (Figure 1B). In addition, we retained one revertant line in which the P element excised precisely (*laza^{RE}*).

We raised antibodies against the C-terminal region of Laza and identified a protein of 42 kDa (predicted molecular weight of 37 kDa) that was not detected in any of the *laza* mutants (*laza^{GE}*, *laza^{1}*, *laza^{2}*) or in flies containing *laza^{GE}* in *trans* with a deficiency chromosome, *Df(3L)ED230*, that removed *laza* (Figures 2A and 2B). The 42 kDa protein was restored in *laza^{RE}* as well as in *laza^{1}* flies in which we introduced a wild-type *CG11440* genomic transgene (Figure 2B). These data indicated that expression of the Laza protein was disrupted in *laza^{GE}*, *laza^{1}*, and *laza^{2}*. Protein levels of other phototransduction proteins were unchanged in each of the lines studied (Figure 2C).

To evaluate whether the Laza protein was expressed in the retina, we used a biochemical approach because the antibodies were ineffective for immunostaining. We detected Laza exclusively in extracts from wild-type heads but not bodies (Figure 2D). Moreover, the Laza protein was absent in head extracts prepared from a mutant lacking eyes (*sine oculis*, *so*) and was greatly reduced in the heads of a mutant (*glass*), which were missing photoreceptor cells specifically [10] (Figure 2E). Thus, Laza was expressed primarily in photoreceptor cells.

We evaluated the requirement for *laza* for phototransduction by performing electroretinogram (ERG) recordings, which assay the summed responses of all retinal cells to light. Exposure of wild-type flies to light results in a corneal negative receptor potential, which decays to baseline after cessation of the light stimulus (Figure 3A; 10.3 ± 0.7 mV, n = 17). The amplitude of the light response was reduced nearly 40% in *laza^{GE}* flies (6.5 ± 0.4 mV, n = 29) and 3-fold in *laza^{1}* (3.7 ± 0.4 mV, n = 19) and *laza^{2}* flies (2.8 ± 0.3 mV, n = 23) (Figures 3B–3D and 3H). These reductions in ERG amplitudes were not due to background mutations because they were observed in *laza^{GE}/Df* flies (Figures 3E and 3H; 5.9 ± 0.3 mV, n = 25) and the *laza^{GE}* phenotype reverted to wild-type upon precise excision of the P element (Figures 3F and 3H; 10.1 ± 0.2 mV, n = 19). Furthermore, the wild-type *laza^{+}* transgene rescued the reduction in the ERG amplitude (Figures 3G–3H; 10.9 ± 0.3 mV, n = 12). Although it was difficult to formally exclude that the *laza* mutation had no effects on the overall physiology of the photoreceptor cells, the reduced ERG amplitude in *laza* flies did not appear to be due to retinal degeneration because 7-day-old adults did not display retinal degeneration (Figure S1 in the Supplemental Data

*Correspondence: cmontell@jhmi.edu

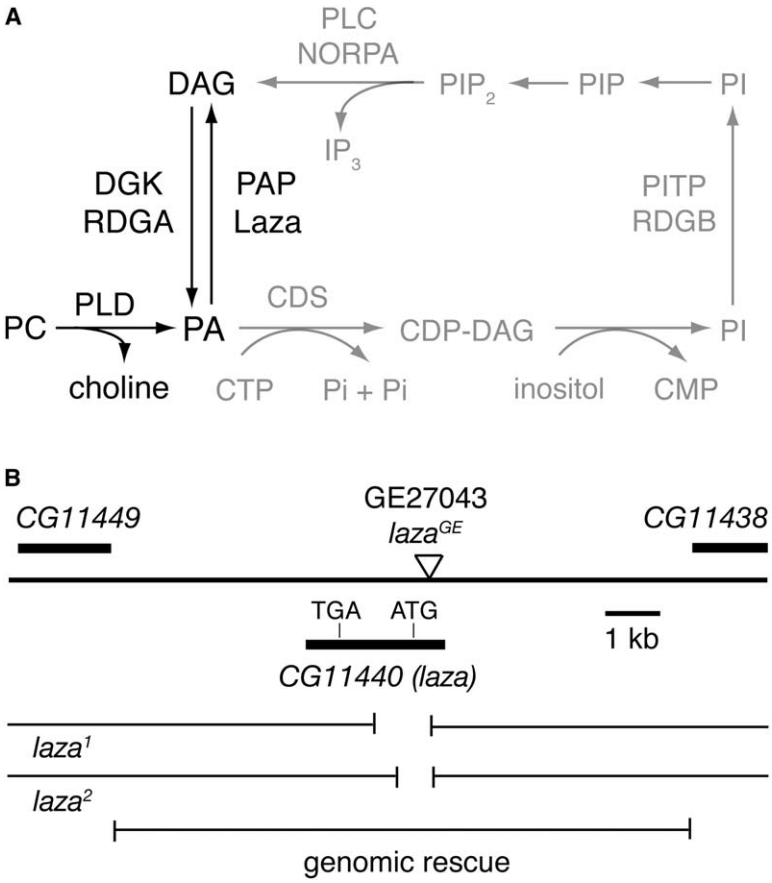

Figure 1. Phosphoinositide Signaling and the *laza* Genomic Region

(A) PI cycle. Activation of PLC (NORPA), which occurs following light stimulus, leads to hydrolysis of PIP_2 and generation of DAG and IP_3. The PIP_2 is regenerated through a multistep cycle initiated with the phosphorylation of DAG by the DAG kinase (RDGA) to produce PA. The proposed site of action of Laza is indicated, and it is in opposition to RDGA. PA is also produced from PC through the action of PLD. The following abbreviations are used: CDS, CDP-diacylglycerol synthase; DAG, diacylglycerol; DGK, DAG kinase; IP_3, inositol 1,4,5-trisphosphate; Laza, Lazaro; NORPA, No Receptor Potential A; PA, phosphatidic acid; PAP, phosphatidic acid phosphatase; PC, phosphatidylcholine; PI, phosphatidylinositol; PITP, PI-transfer protein; PIP, phosphatidylinositol-phosphate; PIP_2, phosphatidylinositol 4,5-bisphosphate; PLC, phospholipase C; PLD, phospholipase D; RDGA, Retinal Degeneration A; and RDGB, Retinal Degeneration B.

(B) Schematic representation of the *laza* mutations. A P element insertion (GE27043) was used to generate the deletions in $laza^1$ and $laza^2$ through imprecise excision of the transposable element. The 1.1 and 0.6 kb deletions in $laza^1$ (−317 to +1381) and $laza^2$ (−309 to +913), respectively, are indicated.

available online) and the ERGs were performed on flies <7 days old. As is the consequence of most mutations that affect phototransduction, in older flies (≥15 days old) the *laza* mutation resulted in retinal degeneration, which was light-dependent (data not shown). Nevertheless, the decreased ERG responsiveness in the *laza* flies was not age dependent (Figure 3I).

To assess whether the decreased amplitude in *laza* was more likely a reflection of a lower sensitivity to light or a decreased maximal obtainable amplitude, we measured the ERG responses at three different intensities. Over the 130-fold intensity range tested, both wild-type and *laza* flies showed a linear intensity-responsive relationship when the amplitudes were plotted against the logs of the light intensities (Figure 3J). However, in the *laza* mutant, the plot is shifted down. If there was a reduction strictly in the sensitivity of the light response, the lines generated from the wild-type and *laza* intensity-responsive relationships should have been parallel. This was not the case, because the amplitude of the *laza* response increased less sharply than in wild-type (Figure 3J). These data are most consistent with a defect in the maximum light response in the mutant; however, the data do not allow us to assess whether or not there is a defect in the sensitivity to light. If *laza* operates in a pathway downstream of PLD (Figure 1A), then there would be expected to be overlaps in the *pld* and *laza* mutant phenotypes. Interestingly, in the *pld* null mutant, there is also a defect in the maximum achievable ERG amplitude [5].

Mutations in *rdgA*, which encodes a diacylglycerol (DAG) kinase [11], prolong termination kinetics [4]. To test whether elimination of *laza* might accelerate deactivation kinetics, consistent with the predicted biochemical function (Figure 1A), we assayed the time required for an 80% return to the baseline after cessation of the photoresponse. We found that the termination of the photoresponse was approximately 4-fold faster in the $laza^1$ (0.29 ± 0.02 s, n = 19) and $laza^2$ (0.25 ± 0.03 s, n = 18) mutant flies than in wild-type (1.24 ± 0.11 s, n = 20) (Figures 4A–4C and 4G). Introduction of the genomic rescue transgene in the $laza^1$ mutant background restored the deactivation kinetics to that of wild-type (Figures 4D and 4G; 1.29 ± 0.11 s, n = 16).

The apparent increase in the speed of termination of the photoresponse in the *laza* mutants may have been due to the smaller ERG amplitude. Therefore, we exposed wild-type flies to a lower light intensity so that the amplitudes of the wild-type and *laza* ERGs were comparable (Figures 4B, 4C, and 4E). Under these conditions, the time required for 80% termination of the photoresponse was 2-fold shorter in the $laza^1$ (0.29 ± 0.02 s, n = 19) and $laza^2$ (0.25 ± 0.03 s, n = 18) mutant flies than in wild-type (0.53 ± 0.04 s, n = 20) (Figures 4B, 4C, 4E, and 4H). Thus, the more rapid termination in *laza* flies was the reverse of that observed in *rdgA* flies.

If *laza* functions in the regulation of the photoresponse by catalyzing the PI-cycle reaction that is the reciprocal of that catalyzed by RDGA (Figure 1A), then it is plausible that introduction of *laza* into an *rdgA* background would