Enzymatic activity and gene expression of diacylglycerol kinase isozymes in developing retina of rats

Sakura SATO^{1,2}, Yasukazu HOZUMI¹, Sachiko SAINO-SAITO¹, Hidetoshi YAMASHITA², and Kaoru GOTO^{1,*}

¹ Department of Anatomy and Cell Biology, ² Department of Ophthalmology, Yamagata University School of Medicine, Yamagata 990-9585, Japan

(Received 20 July 2011; and accepted 10 August 2011)

ABSTRACT

Photoreceptors contain highly specialized structures for phototransduction, which is mediated by rhodopsins and heterotrimeric G-proteins. The signal is transmitted through the cGMP cascade, which controls cGMP-gated cation channels in mammals, while in flies it is operated by phosphoinositide (PI) cascade through a second messenger diacylglycerol (DG), which engenders the opening of Ca^{2+} channels. Recent studies suggest that PI-related signaling cascade is also involved in the phototransduction in mammalian retina. This study examined whether one PI-related enzyme, diacylglycerol kinase (DGK), which is regarded as a regulator of the DG signal through its metabolism, is expressed in mammalian retina. Enzymatic assay, Northern blot and RT-PCR analyses, and *in situ* hybridization histochemistry were performed to assess the expression profile of DGK isozymes and their cellular localization. In rat retina DGK ϵ , DGK ζ , and DGK ι are the dominant species with distinct patterns of expression. At the cellular level, DGK ϵ is the only one detected intensely in the photoreceptor layer, although DGK ι and DGK ζ are observed in bipolar and ganglion cell layers. These results suggest that each DGK isozyme plays a different role in the signal transduction in distinct cell types and that DGK ϵ is a candidate involved in the photoreceptor PI signaling machinery.

Signal transduction in retinal cells is initiated by the absorption of light by photopigments in the outer segment of the photoreceptor cells. Phototransduction in mammalian retinal photoreceptor cells involves a cyclic guanosine monophosphate (cGMP) signaling cascade that controls cGMP-gated cation channels, through which photoreceptors release glutamate continuously in the dark or attenuate its release when stimulated by light (1, 7). The photosignal is conveyed via bipolar cells to ganglion cells, the axons of which extend to the lateral geniculate nucleus through the optic nerve and tract.

In contrast, phototransduction in flies is operated by phosphoinositide (PI) signaling (14, 39). Triggering of the cell surface receptors, such as G-protein-coupled receptors, initiates the PI cycle by activating phospholipase C (PLC), resulting in the hydrolysis of phosphatidylinositol 4,5-bisphosphate (PIP_2) into two second messengers: diacylglycerol (DG) and inositol 1,4,5-trisphosphate (IP_3) (27). In fact, DG serves as a second messenger that modulates the activity of DG-sensitive molecules, whereas IP_3 mobilizes Ca^{2+} from the endoplasmic reticulum (23). It has been revealed that while the signal cascades in photoreceptor cells of vertebrates and flies are both initiated by activation of related rhodopsins and engagement of heterotrimeric G-proteins, there exist notable differences: Light stimulation of the rhodopsins in rods and cones of mammals increases the activity of a cGMP phosphodiesterase, resulting in closure of the cGMP-gated channels, while the cas-

Address correspondence to: Kaoru Goto, M.D., Ph.D.
Department of Anatomy and Cell Biology, Yamagata University School of Medicine
Iida-Nishi 2-2-2, Yamagata 990-9585, Japan
Tel: +81-23-628-5207, Fax: +81-23-628-5210
E-mail: kgoto@med.id.yamagata-u.ac.jp

cade in fly photoreceptors is operated through activation of PLC β and opening light-sensitive channels (transient receptor potential (TRP) and TRP-like (TRPL)) (14, 37, 39).

The importance of PI signaling in the fly retina is suggested by findings related to various *Drosophila* mutants of the PI cascade components (14, 26, 37). A defect in *Drosophila*-PLC enzyme results in a mutant called *norpA*, the photoreceptors of which fail to give any electrical response to light (3, 16, 24). Furthermore, *Drosophila* retinal degeneration A (*rdgA*) mutant, which lacks eye-specific DGK activity, has photoreceptor cells that differentiate normally but degenerate rapidly after eclosion (16, 18, 22). Analyses of other *Drosophila* retinal degeneration mutants, *cds* and retinal degeneration B (*rdgB*), have also revealed that their degeneration is closely related to PI synthesis and its supply through subrhabdomeric cisternae (SRC) to the rhabdomere. CDP-DG synthase (CDS), encoded by *cds* gene, catalyzes phosphatidic acid (PA) by converting it to CDP-DG. It plays a role in photoreceptor function and maintenance by regulating the amount of PI in the photoreceptive membrane (38). The PI-transfer protein encoded by the *rdgB* gene is localized to the SRC membranes and adjacent photoreceptive membranes, suggesting that the *rdgB* protein controls the PI concentration in the photoreceptive membrane by transporting PI from the SRC to the rhabdomere (31).

In this regard, it has been suggested that PI signaling pathway exists in the rod outer segment (ROS) of vertebrates (8, 25). The existence of a PIP₂-specific PLC in vertebrate retinas and ROS is demonstrated by enzyme characterization and immunocytochemical localization. Furthermore, recent studies have revealed a small subset of retinal ganglion cells, which are intrinsically photosensitive (ipRGCs) and which function in photoentrainment of circadian rhythm and in light-induced pupillary constriction (2). Moreover, the phototransduction cascade in the ipRGCs shares striking similarities to that in fly photoreceptors, suggesting that the phototransduction cascades in fly photoreceptors and mammalian ipRGCs originate from common machinery.

Based on this evidence, it can be readily inferred that PI signaling is involved in signal transduction in mammalian retina, although the detailed functional aspects remain undetermined. In this regard, we and others have isolated and characterized several DGK isozymes that play important roles in cellular PI signal transduction (13, 19, 28, 34). It is particularly interesting that most of the isozymes show

abundant expression in distinct patterns in the brain. We have described a unique expression pattern of each isozyme, suggesting the importance of this enzyme family in neuronal function (13). Nevertheless, little is known about the nature and the morphological aspects of DGKs in the retina, which is also of neuroectodermal origin. Therefore, we aimed in this study at examining the enzymatic activity and the expression pattern of mRNA using Northern blot and *in situ* hybridization analyses in the developing retina of rat to define how the different DGK isozymes are expressed.

MATERIALS AND METHODS

Animals. Developing and seven-week-old male Wistar rats (CLEA Japan Inc., Tokyo, Japan) were examined throughout this study. The day of birth was designated as P0. The rats were housed under freely ranging circumstances in our animal facilities under normal lighting conditions (lights on 08 : 00–20 : 00) and were allowed *ad libitum* access to normal rodent chow and water. The eyes were dissected under light conditions (12 : 00–15 : 00). All animals were treated according to the statement for the 'Principles of laboratory animal care' (NIH publication no. 85–23, revised 1985). Guidelines for the care and use of laboratory animals of the Yamagata University School of Medicine were also followed.

Measurement of DGK enzymatic activity. Whole eyeballs were used for the DGK assay. Samples were lysed in a buffer containing 50 mM Tris-HCl (pH 7.4), 0.25 M sucrose, 1 mM EDTA, 4 mM EGTA (pH 8.0), 1 mM DTT, 1 mM PMSF, and protease inhibitor cocktail (Roche Products, Indianapolis, IN) and they were sonicated. After removing cell debris by low-speed centrifugation (550 $\times$ g for 10 min), total lysate was used to measure the DGK activity by the octylglucoside mixed micelle assay as described (17). Briefly, an aliquot of 10 μ L of the supernatant was added to a reaction mixture (50 μ L total) containing either 1 mM 1,2-dioleoylglycerol-*sn*-glycerol (18 : 1/18 : 1 DG; Sigma, St. Louis, MO) or 1-stearoyl-2-arachidonoyl-*sn*-glycerol (18 : 0/20 : 4 DG; Sigma), 6 mM L- α -phosphatidyl-L-serine, 50 mM MOPS, 1 mM DTT, 100 mM NaCl, 20 mM NaF, 1 mM EGTA, 50 mM octylglucoside, and 10 mM MgCl₂ in the presence of 1 mM [γ -³²P]ATP (ICN Biomedicals Inc., Costa Mesa, CA). The reaction was initiated by adding the sample. Then the mixture was incubated for 5 min at 30°C. Subsequently, [³²P]phosphatidic acid generat-

ed by endogenous DGK was separated using thin-layer chromatography and was quantified using liquid scintillation. To assess significance, Kruskal-Wallis test was performed.

Northern blotting and RT-PCR. To prevent cross-hybridization among isozymes in Northern blot analysis, each probe was derived from a specific region of the cloned rat DGK α (9), DGK β (10), DGK γ (11), DGK ϵ (21), DGK ζ (12), and DGK ι (17). The probe sequence region for each isozyme was described previously (29, 35). Total RNAs were extracted from whole eyeball, retina, and brain (positive control) of adult rat using acid guanidinium thiocyanate/phenol/chloroform extraction (TRIzol; GIBCO BRL, Bethesda, MD). Each of the total RNA samples (20 μ g/lane) was denatured with formamide and size-separated using formalin/agarose gel electrophoresis. The RNAs were transferred and fixed to a nylon membrane (Hybond-N; Amersham Pharmacia Biotech Inc., Buckinghamshire, UK) and hybridized with the [α - 32 P]dATP-labeled cDNA probe for each DGK isozyme. For RT-PCR analysis, total RNA in developing retina was reverse transcribed, followed by PCR using specific primers as described previously (20).

In situ hybridization histochemistry. Eyeballs of adult seven-week-old male rats were cut into 20- μ m-thick sections. Then the sections were mounted onto silane-coated glass slides. After fixation in 4% paraformaldehyde in 0.1 M PB (pH 7.4) for 10 min at 4°C they were treated with 0.1 M triethanolamine containing 0.25% acetic anhydride for 10 min, dehydrated in a graded ethanol series (70–100%), and air-dried as described (29, 35). Prehybridization was performed in a buffer containing 50% deionized formamide, 10% dextran sulfate, 300 mM NaCl, 8 mM Tris-HCl (pH 7.4), 0.8 mM EDTA, 1 $\times$ Denhardt's solution, and 200 μ g/mL tRNA. The sections were subsequently hybridized in the same buffer supplemented with [α - 33 P]dUTP-labeled cRNA probe overnight at 55°C in a moist chamber. After hybridization, sections were washed in a buffer containing 50% formamide, 300 mM NaCl, 0.1% sarcosyl, 10 mM 2-mercaptoethanol at 65°C for 60 min, treated with ribonuclease-A (30 μ g/mL) at 37°C for 30 min, washed in 2 $\times$ SSC/0.1% sarcosyl/10 mM 2-mercaptoethanol at 65°C for 60 min, dehydrated through an ethanol/300 mM ammonium acetate series (70–100%), and exposed to Hyperfilm β Max (Amersham Pharmacia Biotech Inc., Buckinghamshire, UK) for 7 days. Some sections were pro-

cessed for liquid emulsion autoradiography using NTB-2 emulsion (Eastman Kodak Co., Rochester, NY), developed after 2 weeks, counterstained with hematoxylin, and examined using both bright-field and dark-field microscopy.

RESULTS

DGK activity in the developing eye

We first examined the total enzymatic activity of DGK in the eye. Reportedly DGK isozymes show different enzymological properties, such as substrate specificity. Of DGKs, DGK ϵ specifically phosphorylates arachidonoyl-containing DG (1-stearoyl-2-arachidonoyl-*sn*-glycerol), whereas the other isozymes show no substrate specificity (32). Therefore an octylglucoside mixed-micelle assay was used to evaluate substrate specificity toward dioleoyl-*sn*-glycerol (18 : 1/18 : 1 DG) and 1-stearoyl-2-arachidonoyl-*sn*-glycerol (18 : 0/20 : 4 DG). As shown in Fig. 1, DGK activity was detected clearly in the eye of all developing stages examined. The overall activity in the eye was roughly estimated as 1/20 of that in the brain (20) where DGKs are expressed abundantly (13). In the postnatal period, the activity was transiently elevated in early postnatal days (P3-P7) about two-fold compared with that at P0. Thereafter, the activity decreased gradually to the level of about half of that at P0. The activities toward 18 : 1/18 : 1 DG and 18 : 0/20 : 4 DG during the postnatal period were almost comparable. Collectively, the results suggest that in the eye the total DGK activity is highest in early postnatal days and that it does not exhibit predominant substrate specificity as a whole.

Expression profile of DGK isozymes

To date, six DGK isozymes have been cloned from rat tissues. We performed Northern blot analysis to examine the expression profile of the isozymes in the retina at the mRNA level. We used the whole eyeball, including the cornea, lens, sclera, ciliary and vitreous bodies, and the retinal layers extracted from the eyeball. As portrayed in Fig. 2, all isozymes were detected in the brain at various intensities. However, in adult retina, hybridization signals were detected intensely for DGK ι and DGK ϵ , moderately for DGK ζ , and weakly for DGK α and DGK β . DGK γ mRNA was not detected in the retina or eyeball. The signal intensities for the detectable DGK isozymes were generally higher in the extracted retinal layer than in the whole eyeball, showing that DGKs are expressed abundantly in neural retina, except for DGK α , the expression of which was

Fig. 1 DGK activities in postnatal developing eye. Homogenates of the whole eyeballs from postnatal day (P) 0, 1, 3, 5, 7, 14, 21, and 49 were assayed for DGK activity toward a single DG species in the octylglucoside mixed-micelle assay as described in Materials and Methods. DGK activities toward 1,2-dioleoyl-*sn*-glycerol (18:1/18:1 DG) (**A**) and 1-stearoyl-2-arachidonoyl-*sn*-glycerol (18:0/20:4 DG) (**B**) in the presence of 0.1 mM Ca^{2+} . The results are expressed in nmol PA formed/min per mg of protein. Each value is the mean of triplicate assays $\pm$ SD. Similar results were obtained in three independent experiments. Kruskal-Wallis test, * $P < 0.05$; ** $P < 0.01$.

lower in the extracted retina than that in the whole eyeball. These data show that DGK ϵ , DGK ζ , and DGK ι are the major species in the retina.

During the postnatal period, the retina becomes exposed to light, although the eyelids do not open until postnatal day 14 in rats. The enzymatic activity was shown to be highest in early postnatal days (Fig. 1). Therefore, we roughly estimated temporal expression patterns of mRNA for major DGK isozymes in the developing retina by RT-PCR (Fig. 3). Expression signals for DGK ϵ and DGK ζ increased

during the first week after birth and reached the plateau level after P14, whereas those for DGK ι increased transiently in early postnatal days and decreased after P14.

Cellular expression of DGK isozymes

To investigate the cellular expression of DGK isozymes, we examined mRNA localization using *in situ* hybridization histochemistry. In adult retina, the expression signals were detected clearly for DGK ϵ , DGK ζ , and DGK ι (Fig. 4). The mRNA signals for DGK ζ were detected intensely in the inner nuclear layer (INL) and ganglion cell layer (GCL) (Fig. 4N). The signals for DGK ι were predominant in the INL and moderate in the GCL (Fig. 4O). Both signals were below detection levels in the inner segment (IS) and outer nuclear layer (ONL), where photoreceptor cells are located. In contrast to the expression patterns observed for DGK ζ and DGK ι , the signals for DGK ϵ were detected intensely in the IS, moderately in the ONL and faintly in the GCL (Fig. 4M), suggesting the expression of this isozyme in photoreceptor cells.

In developing retina, the signals for all of those DGKs were first detected weakly in the inner blast cell layer at P0. Thereafter, the signals for DGK ζ and DGK ι increased gradually in the INL and GCL, whereas those for DGK ϵ were confined to the IS and ONL. Adult expression patterns were observed for all of those DGKs at P14 when the layers were differentiated distinctly. Control experiments using labeled sense strand RNA probes gave no detectable signals in the sections, confirming the specificity of the hybridization signals in this study (data not shown).

DISCUSSION

This study revealed for the first time the expression profile of DGKs in mammalian retina, *i.e.* the enzymatic activity and characteristic mRNA expression patterns of DGK isozymes. First, we show clearly that DGK ϵ mRNA is detected intensely in the photoreceptor layer (IS and ONL), whereas those for DGK ζ and DGK ι are below detection levels in the same region. This finding suggests that DGK ϵ is the only isozyme that is intensely expressed in photoreceptor cells. Combined with a previous study, showing the localization of PLC in the outer segment of mammalian retinal photoreceptor cells (25), this study further supports the existence of PI signaling machinery in photoreceptor cells, such as the production and attenuation of the second messenger DG.

Fig. 2 Northern blot analysis of the DGK isozymes in the retina. Total RNA (20 μ g per lane) from the retina (R), whole eyeball (E), and brain (B, as a positive control) was analyzed. After electrophoresis and transfer of the samples, the filter was hybridized to each of 32 P-labeled cDNA probes for rat DGK α , β , γ , ϵ , ζ , and ι . GAPDH probe was used as a control on the same filter.

Fig. 3 RT-PCR analysis of DGK ϵ , DGK ζ , and DGK ι in developing retina. In RT-PCR analysis of developing retinas from postnatal day (P) 0, 3, 7, 14, and 21, bands for DGK isozymes were amplified using specific primer for each isozyne and electrophoresed. β -actin primer was also used as a control. PCR was performed for 35 cycles. A similar tendency was obtained in PCR for 30 and 32 cycles.

As noted in the Introduction, mammalian phototransduction system is mainly operated in cGMP-gated channels, which remain open in the dark and quickly close in response to the light, leading to hyperpolarization (1, 7). Then, what is the functional role of DGK ϵ in photoreceptor cells? Clues to answer that question might be suggested by two

points: one is a specialized structure of these cells and the other is a characteristic of DGK ϵ . The outer segment is a site of the initial step of photoreception in photoreceptor cells. It comprises hundreds of stacked rhodopsin-laden membranous disks enveloped by the rod's plasma membrane. The old disks in the distal part of the segment are engulfed by pigment epithelial cells to be replaced by new ones, which are added to the proximal part. It is worth noting that the outer segment membrane is characteristically enriched in polyunsaturated fatty acids, such as arachidonic and docosahexaenoic acids (30). Although the reason of this enrichment remains enigmatic, it is speculated that phospholipids with polyunsaturated fatty acids stabilize the membrane structure of the outer segment. Considering that DGK ϵ catalyzes arachidonoyl-DG specifically, this isozyne is probably engaged in the PI cycle distinctly (36) because PI has a characteristic fatty acid composition, 1-stearoyl-2-arachidonoyl species (15). Taken together, one hypothesis might be that DGK ϵ participates in the turnover of the disk membrane in the outer segment.

As for DGK ζ and DGK ι , *rdgA* gene product in *Drosophila*, designated as DGK2, is identified as an eye-specific DGK characterized by ankyrin repeats (22). Molecular cloning of mammalian DGKs made it possible to compare the structural features, which reveals that class IV DGK—DGK ζ (5, 12) and DGK ι (6, 17)—share a similar structural motif: an-

Fig. 4 *In situ* hybridization analysis of mRNA localization for DGK ϵ (A, D, G, J, M), DGK ζ (B, E, H, K, N), and DGK ι (C, F, I, L, O) in adult and developing retinas. Sets of dark- and bright-field images for the individual isozymes are shown. Note the distinct mRNA expression patterns of the isozymes in the retina. In the adult retina, the mRNA signals for DGK ϵ are intensely detected in the IS, moderately in the ONL and faintly in the GCL (M). On the other hand, the signals for DGK ζ are detected intensely in the INL and GCL (N) while the signals for DGK ι are predominant in the INL and moderate in the GCL (O), both of which signals are below detection levels in the IS and ONL. Note in developing retina that the signals for all of those DGKs are first detected weakly in the inner blast cell layer at P0 (A, B, C). Thereafter, the signals for DGK ζ and DGK ι gradually increase in the INL and GCL, while those for DGK ϵ in the IS and ONL. Adult expression pattern is observed for all of those DGKs at P14 when the layers are distinctly differentiated. I, inner blast cell layer; O, outer blast cell layer; GCL, ganglion cell layer; IPL, inner plexiform layer; INL, inner nuclear layer; OPL, outer plexiform layer; ONL, outer nuclear layer; IS, inner segment; RPE, retinal pigment epithelium. Bars 20 μ m.

kyrin repeats. Recent studies show that a small subset of retinal ganglion cells, ipRGCs, function in photoentrainment of circadian rhythm and in light-induced pupillary constriction (2). Gene expression of DGK ζ and DGK ι in the GCL as well as INL, as revealed in the present study, may suggest that these isozymes are involved in the phototransduction cascade in the ipRGCs, which share striking similarities to the cascade in fly photoreceptors.

In terms of the enzymatic activity, the overall DGK activity in the eye is revealed to be highest in early postnatal days and decrease thereafter. This result might suggest that DGK activity plays a particular role for structural and functional development of retinal layers. However, the developmental change in the total enzymatic activity is not correlated with that in the mRNA expression levels of DGKs. This apparent discrepancy remains unclear, although it might be that the relative amount of DGK protein is decreased at the adult stage (P49) or that distinct regulatory mechanisms of the enzymatic activity exist in developing stages. Further study is needed to elucidate this point.

Retinal degeneration in various *Drosophila* mutants of the PI signaling components (37) suggests a possible involvement of mammalian DGKs in retinal diseases. Considering the tissue expression pattern, DGK ι is regarded as the most likely candidate for a human orthologue of *rdgA* (6). However, no apparent disease-associated mutations for human DGK ι have been identified in inherited retinopathy (autosomal dominant retinitis pigmentosa, adRP) (4). However, a search for disease gene linkage reveals that human DGK ϵ gene maps within the adRP disease gene interval, although no mutation in the coding sequence of human DGK ϵ from two patients is identified at the moment (33). Considering that photoreceptor cells are primary sites of retinal degeneration, it is reasonable that the defect of DGK ϵ might cause dysregulation of the DG metabolism, *i.e.*, accumulation of DG and/or deficiency of PA, in these cells. Further studies are necessary to dissect the functional role of DGK ϵ and the causative link to retinopathy at the protein level using specific antibodies.

Acknowledgements

This work was supported by Grant-in-Aid from The Ministry of Education, Culture, Sports, Science and Technology (MEXT) of Japan (K.G.).

REFERENCES

1. Arshavsky VY, Lamb TD and Pugh EN, Jr. (2002) G proteins and phototransduction. *Annu Rev Physiol* **64**, 153–187.
2. Berson DM (2007) Phototransduction in ganglion-cell photoreceptors. *Pflugers Arch* **454**, 849–855.
3. Bloomquist BT, Shortridge RD, Schneuwly S, Perdew M, Montell C, Steller H, Rubin G and Pak WL (1988) Isolation of a putative phospholipase C gene of *Drosophila*, *norpA*, and its role in phototransduction. *Cell* **54**, 723–733.
4. Bowne SJ, Sullivan LS, Ding L, Traer E, Prescott SM, Birch DG, Kennan A, Humphries P and Daiger SP (2000) Evaluation of human diacylglycerol kinase ι , DGKI, a homolog of *Drosophila* *rdgA*, in inherited retinopathy mapping to 7q. *Mol Vis* **6**, 6–9.
5. Bunting M, Tang W, Zimmerman GA, McIntyre TM and Prescott SM (1996) Molecular cloning and characterization of a novel human diacylglycerol kinase ζ . *J Biol Chem* **271**, 10230–10236.
6. Ding L, Traer E, McIntyre TM, Zimmerman GA and Prescott SM (1998) The cloning and characterization of a novel human diacylglycerol kinase, DGK ι . *J Biol Chem* **273**, 32746–32752.
7. Dowling JE (1987) *The Retina: An Approachable Part of the Brain*. Belknap Press of Harvard University Press, Cambridge, Mass.
8. Gehm BD and McConnell DG (1990) Phosphoinositide synthesis in bovine rod outer segments. *Biochemistry* **29**, 5442–5446.
9. Goto K, Watanabe M, Kondo H, Yuasa H, Sakane F and Kanoh H (1992) Gene cloning, sequence, expression and in situ localization of 80 kDa diacylglycerol kinase specific to oligodendrocyte of rat brain. *Brain Res Mol Brain Res* **16**, 75–87.
10. Goto K and Kondo H (1993) Molecular cloning and expression of a 90-kDa diacylglycerol kinase that predominantly localizes in neurons. *Proc Natl Acad Sci USA* **90**, 7598–7602.
11. Goto K, Funayama M and Kondo H (1994) Cloning and expression of a cytoskeleton-associated diacylglycerol kinase that is dominantly expressed in cerebellum. *Proc Natl Acad Sci USA* **91**, 13042–13046.
12. Goto K and Kondo H (1996) A 104-kDa diacylglycerol kinase containing ankyrin-like repeats localizes in the cell nucleus. *Proc Natl Acad Sci USA* **93**, 11196–11201.
13. Goto K, Hozumi Y, Nakano T, Saino-Saito S and Kondo H (2007) Cell biology and pathophysiology of the diacylglycerol kinase family: morphological aspects in tissues and organs. *Int Rev Cytol* **264**, 25–63.
14. Hardie RC (2003) Regulation of TRP channels via lipid second messengers. *Annu Rev Physiol* **65**, 735–759.
15. Holub BJ and Kuksis A (1978) Metabolism of molecular species of diacylglycerophospholipids. *Adv Lipid Res* **16**, 1–125.
16. Hotta Y and Benzer S (1970) Genetic dissection of the *Drosophila* nervous system by means of mosaics. *Proc Natl Acad Sci USA* **67**, 1156–1163.
17. Ito T, Hozumi Y, Sakane F, Saino-Saito S, Kanoh H, Aoyagi M, Kondo H and Goto K (2004) Cloning and characterization of diacylglycerol kinase ι splice variants in rat brain. *J Biol Chem* **279**, 23317–23326.
18. Johnson MA, Frayer KL and Stark WS (1982) Characteristics of *Rdga*—mutants with retinal degeneration in *Drosophila*. *J Insect Physiol* **28**, 233–242.
19. Kanoh H, Yamada K and Sakane F (1990) Diacylglycerol ki-

- nase: a key modulator of signal transduction? *Trends Biochem Sci* **15**, 47–50.
20. Katagiri Y, Ito T, Saino-Saito S, Hozumi Y, Suwabe A, Otake K, Sata M, Kondo H, Sakane F, Kanoh H, Kubota I and Goto K (2005) Expression and localization of diacylglycerol kinase isozymes and enzymatic features in rat lung. *Am J Physiol Lung Cell Mol Physiol* **288**, L1171–L1178.
 21. Kohyama-Koganeya A, Watanabe M and Hotta Y (1997) Molecular cloning of a diacylglycerol kinase isozyme predominantly expressed in rat retina. *FEBS Lett* **409**, 258–264.
 22. Masai I, Okazaki A, Hosoya T and Hotta Y (1993) *Drosophila* retinal degeneration A gene encodes an eye-specific diacylglycerol kinase with cysteine-rich zinc-finger motifs and ankyrin repeats. *Proc Natl Acad Sci USA* **90**, 11157–11161.
 23. Nishizuka Y (1992) Intracellular signaling by hydrolysis of phospholipids and activation of protein kinase C. *Science* **258**, 607–614.
 24. Pak WL, Grossfield J and Arnold KS (1970) Mutants of the visual pathway of *Drosophila melanogaster*. *Nature* **227**, 518–520.
 25. Peng YW, Rhee SG, Yu WP, Ho YK, Schoen T, Chader GJ and Yau KW (1997) Identification of components of a phosphoinositide signaling pathway in retinal rod outer segments. *Proc Natl Acad Sci USA* **94**, 1995–2000.
 26. Raghu P, Colley NJ, Webel R, James T, Hasan G, Danin M, Selinger Z and Hardie RC (2000) Normal phototransduction in *Drosophila* photoreceptors lacking an InsP(3) receptor gene. *Mol Cell Neurosci* **15**, 429–445.
 27. Rhee SG, Suh PG, Ryu SH and Lee SY (1989) Studies of inositol phospholipid-specific phospholipase C. *Science* **244**, 546–550.
 28. Sakane F, Imai S, Kai M, Yasuda S and Kanoh H (2007) Diacylglycerol kinases: why so many of them? *Biochim Biophys Acta* **1771**, 793–806.
 29. Sasaki H, Hozumi Y, Hasegawa H, Ito T, Takagi M, Ogino T, Watanabe M and Goto K (2006) Gene expression and localization of diacylglycerol kinase isozymes in the rat spinal cord and dorsal root ganglia. *Cell Tissue Res* **326**, 35–42.
 30. Simopoulos AP and Bazán NG (2009) *Omega-3 Fatty Acids, the Brain, and Retina*. Karger, Basel; New York.
 31. Suzuki E and Hirosawa K (1994) Immunolocalization of a *Drosophila* phosphatidylinositol transfer protein (*rdgB*) in normal and *rdgA* mutant photoreceptor cells with special reference to the subrhabdomic cisternae. *J Electron Microsc (Tokyo)* **43**, 183–189.
 32. Tang W, Bunting M, Zimmerman GA, McIntyre TM and Prescott SM (1996) Molecular cloning of a novel human diacylglycerol kinase highly selective for arachidonate-containing substrates. *J Biol Chem* **271**, 10237–10241.
 33. Tang W, Bardien S, Bhattacharya SS and Prescott SM (1999) Characterization of the human diacylglycerol kinase ϵ gene and its assessment as a candidate for inherited retinitis pigmentosa. *Gene* **239**, 185–192.
 34. Topham MK and Epand RM (2009) Mammalian diacylglycerol kinases: molecular interactions and biological functions of selected isoforms. *Biochim Biophys Acta* **1790**, 416–424.
 35. Toya M, Hozumi Y, Ito T, Takeda M, Sakane F, Kanoh H, Saito H, Hiroi M, Kurachi H, Kondo H and Goto K (2005) Gene expression, cellular localization, and enzymatic activity of diacylglycerol kinase isozymes in rat ovary and placenta. *Cell Tissue Res* **320**, 525–533.
 36. Walsh JP, Suen R, Lemaitre RN and Glomset JA (1994) Arachidonoyl-diacylglycerol kinase from bovine testis. Purification and properties. *J Biol Chem* **269**, 21155–21164.
 37. Wang T and Montell C (2007) Phototransduction and retinal degeneration in *Drosophila*. *Pflugers Arch* **454**, 821–847.
 38. Wu L, Niemeyer B, Colley N, Socolich M and Zuker CS (1995) Regulation of PLC-mediated signalling *in vivo* by CDP-diacylglycerol synthase. *Nature* **373**, 216–222.
 39. Zuker CS (1996) The biology of vision of *Drosophila*. *Proc Natl Acad Sci USA* **93**, 571–576.