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Diacylglycerol Kinases as Sources of Phosphatidic Acid

Jinjin Cai^{a,b}, Hanan Abramovici^{d,e}, Stephen H. Gee^{d,e}, and Matthew K. Topham^{a,b,c,*}

^aHuntsman Cancer Institute, University of Utah, 2000 Circle of Hope, Salt Lake City, Utah 84112, U.S.A

^bDepartment of Oncological Sciences, University of Utah, 2000 Circle of Hope, Salt Lake City, Utah 84112, U.S.A

^cDepartment of Internal Medicine, University of Utah, 2000 Circle of Hope, Salt Lake City, Utah 84112, U.S.A

^dDepartment of Cellular and Molecular Medicine, University of Ottawa, 451 Smyth Road, Ottawa, Ontario, K1H 8M5, Canada

^eCenter for Neuromuscular Disease, University of Ottawa, 451 Smyth Road, Ottawa, Ontario, K1H 8M5, Canada

Summary

There are ten mammalian diacylglycerol kinases (DGKs) whose primary role is to terminate diacylglycerol (DAG) signaling. However, it is becoming increasingly apparent that DGKs also influence signaling events through their product, phosphatidic acid (PA). They do so in some cases by associating with proteins and then modifying their activity by generating PA. In other cases, DGKs broadly regulate signaling events by virtue of their ability to provide PA for the synthesis of phosphatidylinositols (PtdIns).

Keywords

diacylglycerol kinase; diacylglycerol; phosphatidic acid; phosphatidylinositol; lipid signaling

1. Introduction

DAG is generated by the hydrolysis of phosphatidylinositol-4,5-bisphosphate (PtdIns(4,5)P₂) by PtdIns-specific phospholipase C (PLC) enzymes [1]. Remaining in the membrane, it binds proteins with cysteine-rich, C1 domains, and activates several of these proteins including protein kinase C (PKC) isoforms [2] and Ras guanyl nucleotide-releasing proteins (RasGRPs) [3]. In other cases, DAG recruits but does not activate proteins such as protein kinase D, the Munc13 proteins, and the chimaerins [3]. Additionally, DAG appears to activate some transient receptor potential channels that do not harbor C1 domains [4]. Its effects on numerous and diverse targets underscores the importance of DAG signaling and indicates that DAG modulates a broad array of biological events. It is critical then that intracellular DAG levels be tightly regulated and it is now widely agreed that under most circumstances, conversion of DAG to PA by the DGKs is the major route to terminate DAG signaling. But PA, itself, has a

*Corresponding Author: Matthew K. Topham; tel. 801-585-0304; FAX: 801-585-0101; E-mail: matt.topham@hci.utah.edu.

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broad array of signaling properties that are very distinct from those of DAG. Their ability to generate PA suggests that DGKs might also influence biological events by generating this lipid. In fact, there are now several examples indicating that DGKs modulate signaling events not only by metabolizing DAG to terminate its effects, but also by producing PA.

DGKs have been identified in unicellular organisms such as bacteria, but these DGKs are structurally different from those identified in higher eukaryotes such as *Drosophila melanogaster* [5-7], *Arabidopsis thaliana* [8,9], *Caenorhabditis elegans* [10,11], and mammals [12]. For example, bacterial DGK, unlike higher eukaryote DGKs, is a small, integral membrane protein that phosphorylates other lipids in addition to DAG [13]. And a recently identified yeast DGK is similar to cytidyltransferases and uses CTP as a phosphate donor rather than the ATP used by DGKs in higher eukaryotes [14,15]. Finally, a multisubstrate lipid kinase (MuLK) that also phosphorylates DAG was recently identified [16,17]. Unlike mammalian DGKs, MuLK phosphorylates non-DAG lipids and its structure is not very homologous to that of DGKs. This review will focus on DGKs in higher eukaryotes, with a specific focus on their role in generating PA that influences biological events.

2. Common structural features of the mammalian DGK enzymes

DGK activity was first identified about forty years ago [18], and an 80kDa DGK enzyme was purified to homogeneity from pig brain cytosol in 1983 [19]. Based on the partial sequence from the 80kDa enzyme, Sakane and colleagues isolated a full-length, porcine DGK cDNA in 1990 [20]. But based on antibody studies, additional DGK enzymes appeared to exist [21], prompting an exhaustive search for other DGK isoforms. Nine additional isoforms have since been identified (Fig. 1), making this a rather large family of enzymes. Their diversity is even broader because DGKs β , γ , δ , ζ , ι , and η are alternatively spliced [22]. All mammalian DGKs have two common structural elements: a catalytic domain and at least two C1 domains. The functions and structural properties of these domains have been described in detail in recent reviews [22,23], so only the important features of these motifs are highlighted below.

2.1 The catalytic domain

DGK catalytic domains are composed of accessory and catalytic subunits. In most cases, these subunits are joined to create an uninterrupted catalytic domain. However, in DGKs δ , η and κ [24-26], these domains are separated by a long peptide sequence that does not have any apparent functional motif. Each catalytic subunit has an ATP binding site where mutation of a glycine in this motif to an aspartate or alanine renders the DGK kinase dead [27-29]. The DGK catalytic domains may also require other motifs for maximal activity because catalytic domains of DGKs ϵ , ζ , and θ have very little DGK activity when expressed as isolated subunits (M.K.T. unpublished observations and [30]). Moreover, the isolated catalytic domain of DGK α retained only about 1/3 the activity of this fully active mutant [25]. Thus, it appears that mammalian DGK catalytic domains, unlike bacterial DGK, require other motifs for maximal activity. These other motifs likely function in coordination with the catalytic domain or confer structural stability that allows full catalytic activity.

2.2 The C1 domains

All DGKs have at least two cysteine-rich regions homologous to the DAG-binding C1A and C1B motifs of PKCs [31]. The C1 domain closest to the catalytic domain has an extended region of fifteen amino acids not present in C1 domains from other proteins or in the other C1 domains of DGKs. This extended motif appears to contribute to DGK activity because mutations within this domain significantly reduced the kinase activity of the enzyme [30]. In theory, C1 domains bind DAG, perhaps localizing DGKs to where DAG accumulates. However, no DGK C1 domain has so far been conclusively shown to bind DAG, but some

bind phorbol esters [32-34], which are DAG analogues. Based on sequence alignments, Hurley and colleagues [31] proposed that most DGK C1 domains were sufficiently different from those in PKCs that they might not bind DAG. Instead of binding DAG, the C1 domains of some DGKs appear to act as protein-protein interaction sites. For example, the C1 domains of DGK ζ directly bind to Rac1 [35] and they also associate with β -arrestins [36]. Collectively, these data have led to the suggestion that the C1 domains in some DGKs might not bind DAG. However, it seems unlikely that a DAG kinase would have C1 domains without using them to bind DAG. The question of whether or not DGK C1 domains bind DAG will not be answered until the crystal structure of a DGK C1 domain has been solved.

2.3 The five DGK subfamilies

Based on other structural motifs, mammalian DGK isoforms are classified into five subtypes (Fig. 1). Type I DGKs [20,37,38] have calcium-binding EF hand motifs that make them more active in the presence of calcium [39]. Type II DGKs have pleckstrin homology (PH) domains at their amino termini [24-26]. This domain in DGK δ has been shown to bind weakly and non-selectively to PtdIns [40]. Some Type II DGKs also have sterile alpha motifs (SAM domains) at their carboxy termini that appear to act as localization cues [28] and/or cause homo- and hetero-oligomerization of DGKs δ and η [41,42]. DGK ϵ , the only type III enzyme, has an unusual specificity toward acyl chains of DAG, strongly preferring a specific fatty acid—arachidonate—in the *sn*-2 (middle) position of the glycerol backbone [43]. Its preference for arachidonoyl-DAG suggests that DGK ϵ may be a component of the biochemical pathway that accounts for the enrichment of PtdIns(4,5)P₂ with arachidonate [44]. This possibility is discussed in more detail below. Type IV DGKs [45,46] have domains similar to the phosphorylation site domain of the MARCKS protein. This motif in type IV DGKs appears to function as a localization cue. Type IV DGKs also have four ankyrin repeats and a carboxy terminal PDZ binding domain [47]. The type V enzyme, DGK θ , has three C1 domains and a putative PH domain with a Ras association (RA) domain embedded within it [48]. There is no evidence that the RA domain is functional [49] and the affinity of the PH domain for PtdIns has not been tested. Overall, the DGK family is structurally diverse, which indicates that these enzymes likely modulate numerous important biological events.

3. DGKs influence specific biological events depending on their binding partners

Each DGK isotype is expressed in numerous tissues, and usually multiple DGK isotypes are expressed in the same tissue and even within the same cell [50]. For example we detected all known DGK isoforms in mouse brain extracts [51] and have found expression of at least six DGK isoforms in mouse embryo fibroblasts (J.C. and M.K.T. unpublished observations). When multiple DGK isoforms are expressed in a cell type, they are usually from different subfamilies, suggesting that the subfamilies have distinct functional roles. Thus, specific pools of DAG could be uniquely regulated by directing DGK isoforms to appropriate cellular compartments in order to metabolize the DAG. Conversely, DGK isotypes could uniquely generate PA depending on their intracellular localization and modes of regulation. Consistent with this model, evidence indicates that DGKs achieve functional specificity by accessing specific pools of DAG and binding to a unique subset of DAG- or PA-activated proteins in order to regulate their activity (Fig. 2). This concept agrees with an emerging body of evidence indicating that specificity in signal transduction is often achieved by gathering together signaling proteins in common pathways along with their regulators [52].

The outcome of DGK function will thus depend on the binding partners of each DGK isoform, and the effects that they exert can be quite different. A clear example of this concept has been demonstrated for the type IV DGKs, ζ and ι , which are structurally very similar but have

opposing effects on Ras signaling. DGK ζ was found to attenuate Ras signaling, both *in vitro* [53,54] and *in vivo* [55]. Its effects on Ras are due to the ability of DGK ζ to bind and inhibit RasGRP1 [53], a Ras activator that requires DAG for its function. By metabolizing DAG, DGK ζ inhibits the activity of RasGRP1. Its ability to regulate RasGRP1 was unique among the five other DGK isoforms that were tested; even an alternatively spliced form DGK ζ did not inhibit RasGRP1.

Given the structural similarity between DGK ζ and DGK ι , one would predict that they would have similar signaling outcomes. It was surprising then when we subsequently discovered that DGK ι had the opposite effects on Ras signaling: while DGK ζ deficiency enhanced Ras activity, DGK ι deficiency reduced it [56]. The effects of DGK ι on Ras signaling were caused by its inhibition of RasGRP3. In conditions of DGK ι deficiency, RasGRP3 activity was augmented, which led to activation Rap1 that then interfered with Ras signaling [56]. Collectively these observations indicate that DGKs achieve functional specificity based upon the company that they keep.

Additional examples of DGKs specifically binding to DAG target proteins to regulate their activity have been published [57,58], indicating that this is a common way to regulate DAG levels and the proteins that this lipid influences. Based on the structural diversity of the DGK family, it is likely that each DGK regulates a distinct set of DAG signaling proteins, a concept that is supported by mouse knockout studies showing that mice with targeted deletion of individual DGK isoforms have distinct phenotypes [51,55,56,59,60]. But, in addition to inhibiting the activity of proteins influenced by DAG, DGKs also appear to modulate proteins that are influenced by PA (Fig. 2, right panel). Several examples of DGKs modulating signaling events by producing PA are discussed in detail below.

4. PA signaling events mediated by DGKs

Most of the mechanisms described below involve DGKs ζ and α , suggesting that these might be the only DGK isoforms that modulate signaling events by producing PA. However, other DGK isoforms have not been similarly tested, so one cannot rule out the possibility that they might also function in this manner.

4.1 DGK ζ activates phosphatidylinositol-4-phosphate 5-kinase type I α

Almost seventeen years ago, phosphatidylinositol-4-phosphate (PtdIns4P) 5-kinase enzymes were shown to be potently activated by PA [61]. A subsequent study demonstrated that DGK activity co-immunoprecipitated with a complex that included a PtdIns4P 5-kinase [62], suggesting that DGKs might bind to PtdIns4P 5-kinases and then generate PA to modulate their activity. We investigated this possibility in more detail and found that DGK ζ co-localized and co-immunoprecipitated with PtdIns4P 5-kinase type I α , and we showed that expression of DGK ζ dramatically increased generation of PtdIns(4,5)P $_2$ in cells [63]. A kinase dead DGK ζ also co-immunoprecipitated with the PtdIns4P 5-kinase, but failed to enhance its activity. Together, these data strongly argue that localized PA generation, rather than a conformational change mediated by association of the PtdIns4P 5-kinase with DGK ζ , augmented PtdIns4P 5-kinase activity.

In a separate study, DGK ζ was shown to mediate DAG signaling downstream of the M1 muscarinic receptor (M1R), a seven-transmembrane receptor (7TMR) [36,64]. Its translocation to M1R required binding to β -arrestins—which are scaffolding proteins that bind 7TMRs. The binding site on DGK ζ for β -arrestins mapped to the C1 domains [36], and mutating either of the C1 domains abolished translocation of DGK ζ [64]. Blocking the interaction of β -arrestins with DGK ζ attenuated DAG metabolism, which led to the conclusion that the function of DGK ζ in this complex was to terminate DAG signaling that was initiated by M1R. Since β -

arrestins bind other 7TMRs, this mechanism is likely broadly applied to limit DAG signaling initiated by many different 7TMR agonists. Indeed, over-expressing DGK ζ enhanced decay of ERK phosphorylation following activation of the gonadotropin-releasing hormone receptor, another 7TMR [65].

It was subsequently shown that PtdIns4P 5-kinase type Ia also translocated to 7TMRs by binding to β -arrestins. Its function at the 7TMR was to promote internalization of the receptor [66]. Since DGK ζ also binds β -arrestins, this collection of observations raises the possibility that DGK ζ might function in this complex not only to metabolize DAG, but also to promote PtdIns4P 5-kinase activity by generating PA. This would provide a two step mechanism to shut down the receptor (Fig. 3). First, DGK ζ could metabolize DAG to reduce the impact of this signaling lipid, and then the PA that is produced could activate the PtdIns4P 5-kinase enzyme in order to promote receptor internalization. This hypothetical model has not been specifically tested, but it agrees with data showing that transgenic over-expression of DGK ζ in mouse myocardium protects the mice against cardiac hypertrophy initiated by excessive activation of a 7TMR [67].

4.2 DGK ζ modulates Rac1 activation to influence neurite outgrowth

As noted above, DGK activity was found to co-immunoprecipitate with a PtdIns4P 5-kinase [62]. In addition to these two proteins, the precipitate also contained Rac1, a member of the Rho family of GTPases that helps regulate changes in actin organization. Rho GTPases are molecular switches that oscillate between an inactive GDP-bound state and an active GTP-bound state. We followed up these studies and found that DGK ζ directly interacted with Rac1 and colocalized with it at sites of actin remodeling [68]. DGK ζ appeared to promote the activity of Rac1 because its over-expression in a neuronal cell line induced neurite outgrowth that was inhibited by a dominant-negative Rac1 [35]. As an upstream activator of Rac1, it is appealing to speculate that DGK ζ provides a component that is necessary for Rac1 activation. PA is a likely candidate because it is known to promote dissociation of Rac1 from its inhibitor Rho guanine dissociation inhibitor (RhoGDI) [69], a protein that was also identified in the DGK precipitates [62]. PA is also known to activate p21-activated kinase 1 (PAK1) [70], which phosphorylates RhoGDI, causing it to release Rac1 to enable its activation [71]. Thus, in this complex of proteins, there are at least two mechanisms by which DGK-derived PA might promote actin reorganization and cell motility. First, PA could activate PtdIns4P 5-kinase activity that would provide localized PtdIns(4,5)P₂ to promote actin polymerization [72]. Second, the PA could also activate Rac1 by causing its dissociation from RhoGDI. Supporting the second mechanism, Abramovici et al. recently found reduced PAK1 phosphorylation and attenuated dissociation of RhoGDI from Rac1 in DGK ζ -deficient fibroblasts [93]. These defects were rescued by exogenous PA and by expression of wild-type DGK ζ , but not by kinase dead DGK ζ . Additionally, they found that DGK ζ stably associated with both PAK1 and RhoGDI. Together, these data indicate that DGK ζ has a major role in regulating Rac1-mediated signaling. It should be noted that DGK ζ might not be the only isoform that can regulate Rac1, because there is also evidence that DGK α positively influences Rac1 following activation of tyrosine kinase receptors [73].

4.3 DGK ζ regulates mTOR activation

The serine/threonine kinase mammalian target of rapamycin (mTOR) is an important intermediate in several pathways that manage cellular responses to environmental stress. Its activity is regulated, in part, by PA, which appears to bind the same region of mTOR to which rapamycin binds. This observation has led to a hypothetical model in which rapamycin inhibits mTOR by competing with PA or displacing it from its binding site so that mTOR can't be activated by PA. There is strong evidence indicating the phospholipase D (PLD) isoforms are largely responsible for providing the pool of PA that activates mTOR [74]. But a recent report

indicates that DGK ζ might also activate mTOR under some circumstances. Avila-Flores and colleagues demonstrated that over-expression of DGK ζ led to enhanced, serum-induced phosphorylation of p70 S6 kinase (p70S6K)—a major downstream target of mTOR—and rendered the cells resistant to the effects of rapamycin. Conversely, RNAi-mediated knockdown of DGK ζ reduced phosphorylation of p70S6K. It appears that PA is important in this mechanism to activate mTOR, because DGK ζ could not promote activation of a mutant mTOR that had reduced ability to bind PA. And demonstrating that among the DGKs, this might be a specific property of DGK ζ , over-expression of DGK α did not promote p70S6K phosphorylation. Collectively, these data indicate that DGK ζ can activate mTOR, presumably through its ability to generate PA. The target of this PA, however, is not clear because another report showed in the same cell line that inhibiting PLD almost completely abolished serum-induced S6 kinase activity, indicating that PLD is largely responsible for activating mTOR [75]. It is possible then that instead of directly activating mTOR, DGK ζ activates PtdIns4P 5-kinases, which could provide PtdIns(4,5)P₂, an important activator of PLD enzymes [76]. Regardless of the mechanism, these data suggest that DGK ζ can potentially activate mTOR and that it does so by producing PA.

4.4 DGKs ζ and α modulate immune cell signaling

Gene knockout studies in mice have demonstrated that DGKs ζ and α have central roles in modulating immune cell function. Most data indicate that their primary function in immune cells is to metabolize DAG, and that in their absence DAG accumulates and causes aberrant immune cell function. For example, deficiency of either DGK ζ or DGK α in T cells causes hyper-reactive responses largely due to excess DAG that promotes Ras activation [55,60,77]. But there is also evidence that DGKs ζ and α might also provide PA that is critical for proper immune cell function. For example, compound mutant mice lacking both DGK ζ and DGK α have defects in T cell development that can be partially rescued by exogenous PA [78]. Additionally, generation of PA by DGK ζ also appears to be important for proper signaling from Toll-like receptors (TLRs) in macrophages [79]. TLRs are key mediators that promote the production of pro-inflammatory cytokines which are important for both innate and adaptive immune responses. In DGK ζ -deficient macrophages, production of two cytokines, TNF α and IL-12, was blunted. The reduced levels of cytokines suggested that DGK ζ might provide a component, such as PA, that is necessary for their production. Supporting this possibility, exogenous PA restored the levels of IL-12 in DGK ζ -deficient macrophages. The role of PA is not clear, but it might be necessary to inhibit PtdIns 3-kinases, which were excessively active in the DGK ζ deficient cells. Finally, a recent report suggested that PA derived from DGK α influenced neutrophil responses to anti-neutrophil cytoplasmic antibodies [80]. Collectively, these observations indicate that DGKs α and ζ regulate immune cell function not only by influencing DAG levels, but also by producing PA.

4.5 Plant DGKs affect stress responses by generating PA

DGK isoforms have also been identified in several plant species. For example, seven DGK genes (AtDGK1-7) has been identified in *Arabidopsis thaliana* [81] and in rice there are eight putative DGK isoforms [82]. There is very little data to indicate their specific roles, but based on current information indicating that the number of PA targets in plants vastly outnumbers DAG targets, it has been hypothesized that the primary role of DGKs in plants is to generate PA rather than to consume DAG [83]. In plants, PA is usually produced in response to stress, suggesting that DGKs might influence the stress response. Supporting this possibility, expression of plant DGKs is induced in response to stresses such as wounding, chemicals, and fungal infection [81,82], and over-expression of a rice DGK in tobacco plants enhanced the resistance of those plants to disease [82]. Although it is not clear exactly how these DGKs are protective in conditions of stress, numerous PA targets have been identified in plants [84] and these proteins are probably critical effectors in the stress response.

5. Role of DGKs in regenerating PtdIns

PtdIns(4,5)P₂ is enriched in unsaturated fatty acids [44], so there must be a mechanism that promotes this enrichment. PtdIns(4,5)P₂ is re-synthesized from DAG in a series of reactions known as the PtdIns cycle (Fig. 4) and the DGK reaction is the first step in this sequence. Evidence indicates that DGK ϵ , by virtue of its specificity for DAG that has unsaturated fatty acids, helps enrich PtdIns(4,5)P₂ with unsaturated fatty acids. But there is also evidence that other DGK isoforms might also contribute.

5.1 DGK ϵ in neural tissues

As noted above, DGK ϵ prefers to phosphorylate DAG with an arachidonate group in the *sn*-2 position [43]. PtdIns, including PtdIns(4,5)P₂, are enriched at the *sn*-2 position with unsaturated fatty acids—usually arachidonate [44]. Consequently, the DAG generated by PtdIns-specific PLC isoforms such as PLC β and PLC γ is enriched with this fatty acid. While it may seem that the fatty acid components of DAG would not significantly affect its signaling ability, some DAG targets, including PKCs, appear to be specifically activated by unsaturated DAG [85]. How the fatty acid components of DAG affect target proteins is unclear, but it is possible that the fatty acids in PtdIns(4,5)P₂ and/ or DAG might help enrich these lipids in membrane microdomains that may, in turn, recruit other necessary signaling components. *In vitro*, most DGKs don't distinguish between DAG species with fatty acid components. But DGK ϵ is an exception [43]. Its selectivity suggests that DGK ϵ may have a prominent role in the re-synthesis of PtdIns from DAG, because incorporating DGK ϵ into the cycle would maintain the enrichment of PtdIns with arachidonate.

To examine the biological function of DGK ϵ , we disrupted the gene encoding DGK ϵ in mice. Since proper PtdIns signaling is important for normal neuronal transmission, in a collaborative effort we studied seizure threshold in the mice. We found that DGK ϵ null mice had significantly shorter seizures following electroconvulsive shock and they recovered faster than wild type mice [59]. Examination of brain lipids showed reduced levels of arachidonate in both PtdIns (4,5)P₂ and DAG in the DGK ϵ -deficient mice. This lipid profile demonstrated a critical role for DGK ϵ in maintaining a proper balance of arachidonate-enriched PtdIns. Subsequent analysis of lipids in embryo fibroblasts from the DGK ϵ knockout mice also revealed reduced levels of arachidonate in PtdIns [86]. There were minimal changes in the lipid species of DAG in the embryo fibroblasts, probably because those experiments were conducted under basal conditions. Together these observations indicate that DGK ϵ is a component of the PtdIns cycle and that through its selectivity for arachidonoyl-DAG it helps maintain the fatty acid composition of PtdIns and consequently modulates DAG signaling events and seizure susceptibility.

But DGK ϵ does not appear to be expressed in some tissues [59], suggesting that there are other mechanisms to enrich PtdIns with unsaturated fatty acids. Since other DGKs do not seem to exhibit specificity for DAG that has unsaturated fatty acids, one way to maintain this enrichment of PtdIns with unsaturated fatty acids would be to couple PtdIns-specific phospholipase C enzymes with DGKs. Indeed, we found that DGK ζ co-immunoprecipitated with the PtdIns-specific PLCs β and γ (M.K.T., unpublished observations and [87]). Collectively, these observations suggest that by producing PA, DGK ϵ and possibly other DGKs, help maintain the proper fatty acid composition of PtdIns, and consequently DAG. They may do this either by having specificity for the fatty acid components of DAG or by associating with elements of the PtdIns cycle that have specificity.

5.2 A *Drosophila* DGK in the retina

A *Drosophila* eye-enriched DGK encoded by the *rdgA* locus that is similar to mammalian type IV DGKs also appears to be important for PtdIns re-synthesis. This DGK functions downstream from the 7TMR *Drosophila* photoreceptors. When a photon of light is absorbed by these receptors, PLC enzymes produce DAG, which is critical, because flies that harbor mutant PLC enzymes do not respond to light [88]. This DAG probably activates PKCs and other targets that promote activation of TRP channels, which leads to increased intracellular calcium [89]. The importance of the *Drosophila* DGK is indicated by *rdgA* mutant flies, which have constitutively active TRP channels and eventually develop retinal degeneration [90]. Presumably, the channels are constitutively active because of excess DAG, but no one has yet demonstrated high levels of DAG in *rdgA* mutant flies.

In addition to providing an avenue to metabolize DAG, the DGK reaction also seems to be important for regenerating PtdIns(4,5)P₂ from DAG. This re-synthesis cycle appears to be disrupted in *rdgA* mutant flies because there are dramatic reductions in the levels of PA [91] and PtdIns [92] in their retinæ. Once PA is produced by the DGK, a series of reactions occurs to regenerate PtdIns(4,5)P₂, and the first step in this sequence is the production of CDP-DAG by CDP-DAG synthase (Fig. 4). Supporting the possibility that impaired re-synthesis of PtdIns(4,5)P₂ contributes to retinal degeneration in *rdgA* mutant flies, mutation of the CDP-DAG synthase gene enhanced the retinal degeneration in *rdgA* mutant flies [92]. Presumably their phenotype is made worse because mutation of CDP-DAG synthase shuts off other avenues—such as the PLD reaction—to synthesize PtdIns(4,5)P₂. Moreover, mutating a PtdIns4P 5-kinase that catalyzes the final step of PtdIns(4,5)P₂ synthesis had the same effect as mutating CDP-DAG synthase in the *rdgA* mutant background [92]. Conversely, mutating *lazaro*, a lipid phosphate phosphohydrolase (LPP) that might function as a phosphatidic acid phosphatase (PAP) to reverse the DGK reaction, reduced the rate of retinal degeneration in *rdgA* mutant flies [92]. Presumably the *lazaro* mutation is protective because, by blocking the reverse reaction, it leads to enhanced synthesis of PtdIns(4,5)P₂. However, LPP and PAP enzymes are structurally distinct and it has not been conclusively demonstrated that the *lazaro* gene product can, indeed, dephosphorylate PA [92]. Nonetheless, this collection of data strongly suggests that DGK activity in the *Drosophila* retina is not only important to metabolize DAG, but is also critical as an initial step in regenerating PtdIns(4,5)P₂. While these data indicate that mammalian type IV DGKs—the orthologs of the *rdgA* gene product—might have important roles in visual signal transduction, there is no evidence that deleting type IV DGK genes in mice affects their vision (M.K.T. unpublished data).

6. Summary and conclusions

While DGKs are better known as enzymes that metabolize diacylglycerol, there are also several reports indicating that they can additionally function by providing PA. In some cases, this PA binds and activates proteins such as PtdIns4P 5-kinases to influence cell function, while in other cases the PA is a critical component for the re-synthesis of PtdIns. In either case, disrupting DGK function causes aberrant signaling. This concept in which DGKs provide PA to influence signaling events is relatively new and it is very likely that additional examples will be identified in the future.

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Abbreviations used

DGKs	diacylglycerol kinases
DAG	diacylglycerol
PA	phosphatidic acid
PtdIns	phosphatidylinositols
PtdIns(4,5)P₂	phosphatidylinositol-4,5-bisphosphate
PLC	phospholipase C
PKC	

	protein kinase C
RasGRPs	Ras guanyl nucleotide-releasing proteins
MuLK	multisubstrate lipid kinase
PH	pleckstrin homology
SAM	sterile alpha motif
RA	Ras association domain
7TMR	seven-transmembrane receptor
M1R	M1 muscarinic receptor
RhoGDI	Rho guanine dissociation inhibitor
PAK1	p21-activated kinase 1
mTOR	mammalian target of rapamycin
PLD	phospholipase D
p70S6K	p70 S6 kinase
TLR	Toll-like receptor
LPP	lipid phosphate phosphohydrolase
PAP	PA phosphatase
PtdIns4P	phosphatidylinositol-4-phosphate
PI5K	PtdIns4P 5-kinase

Fig. 1.

Structure of mammalian DGK isoforms. The ten members of the mammalian DGK family are grouped by sequence homology into five subtypes. Shown are protein motifs common to several DGKs. The significance of several of these motifs is discussed in the text. Additional motifs of uncertain significance are not shown.

Fig. 2.

Model of compartmentalized DGK function. Evidence indicates that DGKs bind and influence the activity of proteins regulated by either DAG or PA. In the left panel, a DGK binds and inhibits a DAG-activated protein. In the right panel, a DGK binds a protein and, by generating PA, increases its function.

Fig. 3.

Hypothetical model in which DGK ζ could negatively regulate 7TMR signaling. Upon activation of the 7TMR, β -arrestin is recruited to the receptor along with its bound DGK ζ . The DGK terminates DAG signaling by converting this lipid to PA. The PA, in turn, might serve to activate a PtdIns4P 5-kinase (PI5K), which generates PtdIns(4,5)P₂. This lipid promotes internalization of the 7TMR to limit access to its ligand.

Fig. 4.

A simplified diagram of the PtdIns cycle. Shown are lipids that are components of the PtdIns cycle and enzymes in this cycle that are relevant to the discussion in the text.