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Mammalian Diacylglycerol Kinases: Molecular Interactions and Biological Functions of Selected Isoforms

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Abstract

The mammalian diacylglycerol kinases (DGK) are a group of enzymes having important roles in regulating many biological processes. Both the product and the substrate of these enzymes, i.e. diacylglycerol and phosphatidic acid, are important lipid signaling molecules. Each DGK isoform appears to have a distinct biological function as a consequence of its location in the cell and/or the proteins with which it associates. This review discusses three of the more extensively studied forms of this enzyme, DGK α , DGK ϵ , and DGK ζ . DGK α has an important role in immune function and its activity is modulated by several mechanisms. DGK ϵ has several unique features among which is its specificity for arachidonyl-containing substrates, suggesting its importance in phosphatidylinositol cycling. DGK ζ is expressed in many tissues and also has several mechanisms to regulate its functions. It is localized in several subcellular organelles, including the nucleus. The current state of our understanding of the properties and functions of these proteins is reviewed.

Keywords

Diacylglycerol kinase; diacylglycerol; phosphatidylinositols; phosphatidic acid; lipid signaling

1. Introduction

Diacylglycerol (DAG) is generated by the hydrolysis of phosphatidylinositol-4,5-bisphosphate (PtnIns(4,5)P₂) by PI-specific phospholipase C (PLC) enzymes [1]. It activates several important proteins including protein kinase C (PKC) isoforms[2–4], RasGRP nucleotide exchange factors[5–11], and some transient receptor potential channels[12]. DAG also recruits several proteins to membrane compartments, including the chimaerins, protein kinase D, and the Munc13 proteins[13]. Its effects on numerous and diverse targets underscores the

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importance of DAG signalling and indicates that DAG modulates a broad array of biological events. It is critical then that intracellular DAG levels be tightly regulated. Under most circumstances, conversion of DAG to phosphatidic acid (PA) by the diacylglycerol kinases (DGKs) is the major route to terminate DAG signalling. PA, itself, has a set of signalling properties that are distinct from DAG [14], indicating that DGKs might modulate lipid signalling either by terminating DAG or by producing PA.

DGK isoforms have been identified in unicellular organisms such as bacteria and yeast, but these DGKs are structurally different from those identified in higher eukaryotes such as *Caenorhabditis elegans* [15,16], *Drosophila melanogaster* [17–19], *Arabidopsis thaliana* [20, 21], and mammals [22]. For example, bacterial DGK, unlike mammalian DGKs, is a small, integral membrane protein that phosphorylates other lipids in addition to DAG [23]. And the yeast DGK is similar to cytidyltransferases and uses CTP as a phosphate donor rather than the ATP used by DGKs in higher eukaryotes [24,25]. This review will focus on DGKs in higher eukaryotes, with a specific focus on three of the best characterized mammalian DGK enzymes.

2. Common structural features of the mammalian DGK enzymes

Ten mammalian DGKs have been identified and all of them 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 [14,26], 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 the type II DGKs δ , η and κ [27–29], 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 [30–32]. The DGK catalytic domains may also require other motifs for maximal activity because catalytic domains from DGKs ϵ , ζ , and θ have very little DGK activity when expressed as isolated subunits (M.K.T. unpublished observations and [33]). Moreover, the isolated catalytic domain of DGK α retained 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

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 [34]. 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 [33]. In theory, C1 domains bind DAG, perhaps localizing DGKs to where DAG accumulates. However, only the C1 domains of DGKs β and γ could bind phorbol esters [35–37], which are DAG analogues, while the C1 domains of DGKs δ , η , and θ did not bind. These results correlated with sequence alignments performed by Hurley and colleagues [34], who predicted that only the C1 domains from DGKs β and γ could bind DAG while other DGK C1 domains were sufficiently different from those in PKCs that they might not bind DAG. In fact, it appears that the C1 domains of some DGKs, like those in other proteins, can act as protein-protein interaction sites. For example, the C1 domains of DGK ζ bind directly to Rac1 [38] and they also associate with β -arrestins [39]. This suggests that the C1 domains in some DGKs might not bind DAG. It will be of interest to test the phorbol ester binding capacity of additional DGK

C1 domains and to solve their crystal structures so that we can understand the differences between the C1 domains of DGKs and those of other proteins that contain them.

3. The five DGK subfamilies

Based on shared structural motifs, mammalian DGK isoforms in higher eukaryotes are classified into five subtypes (Fig. 1). Type I DGKs [40–42] have calcium-binding EF hand motifs that make them more active in the presence of calcium [43]. Type II DGKs have pleckstrin homology (PH) domains at their amino termini [27–29]. This domain in DGK δ has been shown to bind weakly and nonselectively to phosphatidylinositols (PtnIns) [44]. Type II DGKs also have sterile alpha motifs (SAM domain) at their carboxy termini that might act as localization cues [31] and/or cause homo- and hetero-oligomerization of type II DGKs [45, 46]. 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 position [47]. Its preference for arachidonoyl-DAG suggests that DGK ϵ may be a component of the biochemical pathway that accounts for the enrichment of PtnIns with arachidonate [48]. This possibility is discussed in more detail below. Type IV DGKs [49,50] have domains similar to the phosphorylation site domain of the MARCKS protein. This motif functions as a localization cue and is discussed in more detail below. Type IV DGKs also have four ankyrin repeats and a carboxy terminal PDZ binding domain [51]. The type V enzyme, DGK θ , has three C1 domains and a putative PH domain that has a Ras association (RA) domain embedded within it [52]. The function of its RA domain is not clear, and although most RA domains bind to Ras, this domain in DGK θ does not appear to associate with Ras [53]. In summary, the mammalian DGK family is structurally diverse, which indicates that these enzymes likely modulate numerous important biological events.

3.1 Differences in biological function of DGKs depend on their binding partners

Each DGK isoform is expressed in numerous tissues, and multiple DGK isotypes have been identified in the same tissue and even within the same cell [54]. When multiple DGK isoforms are expressed in a cell type, they are usually from different subfamilies, suggesting that these subfamilies have distinct, tissue- or cell-specific roles. Thus, cells might be able to differentially regulate pools of DAG by directing DGK isoforms to appropriate subcellular compartments and then integrating their DGK activity within specific signalling complexes. Consistent with this model, evidence indicates that some DGKs achieve functional specificity by accessing specific pools of DAG, in part, by binding to DAG-activated proteins in order to regulate their activity (Fig. 2, left panel). This concept agrees with an emerging body of evidence indicating that specificity in signal transduction is often achieved by gathering signalling proteins in a common pathway with their regulators [55]. For example, endogenous DGK ζ binds to endogenous RasGRP1 in A172 cells. Presumably DGK ζ regulates RasGRP1 by metabolizing DAG, and consequently modulates the active state of Ras [56]. Indeed, over-expressing wild-type DGK ζ along with RasGRP1 in HEK293 cells inhibited its ability to activate Ras, while over-expressing kinase dead DGK ζ did have this effect [56]. In fact, over-expressing kinase dead DGK ζ in jurkat cells augmented and prolonged Ras activation caused by stimulation of the T cell receptor [56], and deleting the DGK ζ gene in mice had the same effect in their T cells [57]. This regulation was selective: over-expression of five other DGK isotypes or the alternatively spliced DGK ζ 2 did not inhibit RasGRP1 in HEK293 cells [56]. Collectively, these data suggest that DGK ζ binds and inhibits RasGRP1 in T cells, where this RasGRP is predominantly expressed. Conversely, deleting the gene encoding DGK ι , which is structurally similar to DGK ζ , had the opposite effects on Ras signalling in embryo fibroblasts derived from DGK ι knockout mice, and rendered the mice resistant to induced skin tumors [58]. Presumably the effects of deleting DGK ι were due to its ability to bind and regulate RasGRP3, but association of endogenous DGK ι and RasGRP3 has never been demonstrated due to lack of

appropriate reagents. These opposing signalling outcomes have led to the hypothesis that DGKs ζ and ι , and possibly other DGKs, regulate signalling events based on the company that they keep. Indeed, specific DGK isotypes or DGK activity has been demonstrated in endogenous immunoprecipitates of numerous signalling proteins such as PKC α , phospholipase C γ , β -arrestins, Rac1, and others [39,59–61]. In addition to inhibiting the activity of proteins activated by DAG, DGKs might also bind proteins that are activated by PA to enhance their activity [62,63] (Fig. 2, right panel). Indeed, endogenous DGK ζ and phosphatidylinositol 5-kinase type 1 α , an enzyme activated by PA, co-immunoprecipitated from rat brain extracts [62]. Based on their structural diversity, it is quite possible that each DGK will regulate a distinct set of DAG signalling events, a concept that is supported by mouse knockout studies showing that mice with targeted deletion of individual DGK isoforms have distinct phenotypes [57,58,64–66]. Unfortunately, space does not allow a review of each DGK isoform, instead we chose three of the best characterized mammalian enzymes—DGKs α , ϵ , and ζ —and discuss their unique features below.

4. DGK α modulates adaptive immune cell function

DGK α is more active in the presence of calcium and is the best characterized enzyme in the type I DGK subfamily. Knockout, knockdown, and inhibitor studies indicate that it has important roles in immune function, angiogenesis, proliferation, and cell migration [66,67].

4.1 The amino terminus of DGK α functions as a regulatory motif

DGK α is one of three members of the Type I DGKs. One hallmark of this DGK subfamily is the presence of two calcium-binding EF hand motifs. Similar motifs are found in numerous calcium-binding proteins such as calmodulin. When expressed in bacteria, the EF hand motifs of DGK α bind 2 moles of Ca²⁺/mole of protein fragment. The Ca²⁺ binding affinity of the DGK α EF hands was 10-fold weaker than for those of the two other Type I DGK isotypes [43]. The significance of this observation is not clear. The affinity of the isolated DGK α EF hands for Ca²⁺ is 9.9 μ M while the affinity of intact DGK α for Ca²⁺ is 0.3 μ M [68]. The large increase in affinity of the intact protein suggests that either the conformation of the EF hand domain is stabilized when it is part of the larger protein or that other motifs within the full-length protein participate in the binding of Ca²⁺.

In addition to being activated by Ca²⁺, DGK α is also activated when it binds to membranes. This process is regulated, in part, by the presence of Ca²⁺ [68], suggesting that by binding Ca²⁺, the EF hand motifs promote membrane translocation. In addition to the EF hand motifs, DGK α also contains an N-terminal recoverin homology domain that is related to the amino terminus of the recoverin family of neuronal calcium-sensors. Deletion of the recoverin homology domain of DGK α renders the enzyme incapable of being activated by Ca²⁺ [68], indicating that this domain functions in coordination with the EF hand motifs. Curiously, when the EF hand motifs are deleted along with the recoverin homology domain, the resulting truncated enzyme is constitutively active and no longer exhibits sensitivity to Ca²⁺ [68]. These findings suggest that in addition to promoting the binding of DGK α to membranes that contain phosphatidylserine [69], Ca²⁺ also triggers a conformational change in the protein that results in enhanced DGK activity.

4.2 Role of membrane physical properties

Although it has maximal activity in the presence of Ca²⁺, DGK α also exhibits a basal level of activity that is independent of calcium, provided that the activity is measured in the presence of certain lipids. For example, either phosphatidylethanolamine or cholesterol will support the calcium-independent activity of DGK α [70]. These lipids have in common small, poorly hydrated head groups that appear to provide an environment that allows DGK α to bind to

membranes and become activated in the absence of Ca^{2+} . In addition to these lipids, the lipid products of phosphoinositide 3-kinases, such as phosphatidylinositol(3,4)bisphosphate and phosphatidylinositol(3,4,5)trisphosphate, also activate DGK α and induce its relocation to the plasma membrane in intact lymphocytes [71]. Activation by phosphoinositide 3-kinase lipid products appears to be a unique property of DGK α among the type I DGK isoforms, because DGKs β and γ are not activated by these lipids.

4.3 Regulation of DGK α activity by phosphorylation

Another mechanism to activate DGK α is by the phosphorylation of Tyr³³⁵. This occurs during T-cell activation [72] or after exposing cells to hepatocyte growth factor[73], and it appears to be mediated by Src tyrosine kinases [67,73]. This phosphorylation both promotes membrane localization of the enzyme and augments its activity [67,72,74]. These changes, in turn, help modulate Rac activation and consequent remodeling of the actin cytoskeleton in migrating epithelial cells [67]. Thus, there are two mechanisms by which Src can activate DGK α . One is by direct phosphorylation of a Tyr residue in DGK α and the other is by Src-dependent activation of phosphatidylinositol-3-kinases that then produce anionic lipids that promote DGK α activity.

4.4 Biological functions of DGK α

Studies with DGK α mutants and the DGK α inhibitor, R59949 [75], demonstrated that DGK α enhances interleukin 2-induced transition of T cells from G1 to S phase of the cell cycle, consequently augmenting their rate of proliferation [76]. Activation of DGK α by interleukin 2 causes its translocation to a perinuclear or intranuclear region where it generates phosphatidic acid that activates as yet unidentified proteins. DGK α also promotes cell proliferation and migration in response to vascular endothelial cell growth factor [77] and hepatocyte growth factor [78], but these effects appear to be dependent on DGK α localized at or near the plasma membrane, and it is not clear whether these effects are due to metabolism of DAG, generation of PA, or both.

In addition to localizing within the nucleus and at the plasma membrane, DGK α also associates with the trans-Golgi network in T-cells [79]. The Golgi network is a membrane transport hub, and the formation of secretory vesicles there critically depends on the presence of DAG [79]. Thus, it was not surprising that DGK α was found to have an important role in this process: it inhibits the secretion of Fas ligand-bearing exosomes in T-cells through an apparent effect at the Golgi [79]. By helping regulate exosome trafficking, DGK α modulates activation-induced T cell death, a mechanism that is critical for maintaining immune tolerance.

Since DGK α is expressed in many of the cells of the immune system, deleting its gene in mice has tested its function there. The overall effect is to alter T cell tolerance or anergy, a state in which the cells ignore self antigens [66,80]. Normally, T cells respond to antigens through activation of the T cell receptor, which activates signalling pathways that promote proliferation, increase cytokine gene expression, and support survival. To avoid reacting to self antigens, some signalling pathways downstream of the T cell receptor are quiescent, including those that generate DAG [66]. This observation suggested that through its ability to regulate DAG levels DGK α might modulate T cell anergy. Supporting this possibility, DGK α expression is increased in anergic T cells while DGK α knockout mice were resistant to anergy. Its effects on anergy presumably occur because DGK α regulates T cell mediated DAG levels and in its absence, DAG accumulates, which leads to anergy resistance.

In T cells, DAG links the T cell receptor to Ras signaling through Ras guanyl releasing protein 1 (Ras GRP1), a Ras activator that depends on DAG for its function [5]. Ras activation is known to reverse anergy, leading to the possibility that DGK α modulates anergy by controlling

the levels of DAG and consequently the activity levels of RasGRP1 and as a result also of Ras. Aspects of this model were tested in the DGK α knockout mice and the data are consistent with this model. For example, there is increased Ras activity in DGK α deficient T cells [81] and over-expression of DGK α reduced Ras signalling [80]. Additionally, RasGRP1 was not effectively recruited to the plasma membrane in anergic cells—an event that likely requires DAG. So based on these data, it appears that DGK α has a central role in modulating T cell anergy through its ability to control DAG levels, which likely activates RasGRP1 and possibly other proteins. Collectively, these data suggest that modulating the activity of DGK α could be harnessed clinically to treat diseases such as cancer where anergy toward tumor antigens has detrimental effects.

5. DGK ϵ phosphorylates specific forms of DAG

With a mass of 64 kD, DGK ϵ is the smallest known mammalian DGK isoform. It is the only isoform that, outside of the catalytic C1 domains, does not have any known regulatory motifs. This observation raises the issue of how its activity is regulated. One hypothesis is that DGK ϵ is not regulated, but instead is constitutively active and has an important housekeeping role in maintaining the composition of PtdIns lipids in cell membranes. This possibility is consistent with its selectivity for specific lipid components of DAG that is discussed below. Conversely, it is also possible that there are mechanisms to regulate its activity that are not yet known.

5.1 Specificity for DAG substrates and evidence supporting a role for DGK ϵ in PtnIns(4,5)P₂ cycling

DGK ϵ exhibits specificity for DAG substrates containing an arachidonoyl chain in the *sn*-2 position [47], but the motif in DGK ϵ that confers this selectivity has not been discovered. Its selectivity for arachidonoyl-DAG is of particular interest because it suggests that DGK ϵ might have a role in maintaining the fatty acid composition of PtdIns. It is known that phosphatidylinositol-(4,5)-bisphosphate (PtnIns(4,5)P₂) is highly enriched with arachidonic acid [48] and this does not result from remodelling the acyl chain composition of this lipid [82]. Since a major pathway for the resynthesis of PtnIns(4,5)P₂ from DAG begins with the phosphorylation of DAG by DGKs, it is possible that by selectively phosphorylating arachidonoyl-DAG, DGK ϵ provides an avenue to enrich PtnIns(4,5)P₂ with arachidonate.

To study this possibility, we undertook a lipidomics analysis comparing the acyl chain compositions of lipids from mouse embryo fibroblasts (MEFs) obtained from mice with genetic deletion of DGK ϵ (DGK ϵ -KO) or from their wild-type (WT) siblings [64,83]. We grew the cells under basal conditions and then examined their levels of DAG. Surprisingly, we found no differences between WT and DGK ϵ -KO cells in either the amount or the acyl chain composition of DAG. In a separate analysis, there were also no differences in the levels of DAG or its acyl chain composition in brain extracts from resting WT and DGK ϵ -KO mice [64]. But in that analysis, there were significant reductions in the levels of total DAG and arachidonoyl-DAG when the mice were exposed to electroconvulsive shock [64]. The reduced levels of DAG upon stimulation were surprising because knocking out other DGK isoforms leads to excessive levels of DAG [65,83].

One possibility to explain the reduced levels of DAG in DGK ϵ -KO cells is that DGK ϵ promotes the re-synthesis of PtdIns from DAG, so that when DGK ϵ is absent, the levels of PtdIns fall, leading to reduced PtnIns(4,5)P₂ substrate that can be hydrolyzed to DAG by PtnIns(4,5)P₂-specific PLC enzymes. Supporting this possibility, we found significant reductions in arachidonate-containing lipids for several phospholipid classes in DGK ϵ -KO cells, and the changes in arachidonate content between WT and DGK ϵ -KO cell lines were greatest for the lipids involved in PtnIns cycling. These data were consistent with a second study using

DGK ϵ -KO mice [64]. Together, these observations indicate that DGK ϵ plays an important role in determining the final enrichment of PtdIns(4,5)P₂ with arachidonic acid. Supporting this possibility, we also observed an increase in arachidonoyl content between DAG and PA in WT cells. In addition, there was an even greater increase in arachidonoyl content between PA and phosphatidylinositol in the WT cells compared with the DGK ϵ -KO cells. This additional enrichment in a step that does not involve the catalytic activity of DGK ϵ suggests that DGK ϵ might have a non-catalytic role in this step of PtdIns synthesis. One possibility is that DGK ϵ facilitates the specific transfer of arachidonoyl-rich PA from the plasma membrane to the endoplasmic reticulum where it is converted to PtnIns. This would provide a function for the DGK ϵ found in the ER.

5.2 Inhibition by anionic lipids

We have shown that DGK ϵ is inhibited by a number of anionic phospholipids [84]. This inhibition had been previously shown with an arachidonoyl-specific DGK from bovine testes [85]. The anionic lipid, PtnIns(4,5)P₂, is a particularly potent inhibitor, suggesting that there may be some regulatory function of this inhibition in inositol cycling. It is possible that DGK ϵ becomes activated when PtnIns(4,5)P₂-specific phospholipase C catalyzes the hydrolysis of this polyanionic lipid, thus relieving the inhibition of DGK ϵ and allowing it to catalyze the phosphorylation of the arachidonoyl-rich DAG that is liberated by this process.

Walsh *et al.* [86] showed that the inhibition of arachidonoyl-specific DGK from bovine testes caused by 1-palmitoyl-2-oleoyl phosphatidic acid is identical to that caused by 1-stearoyl-2-arachidonoyl phosphatidic acid, in spite of the known affinity of this isoform of DGK for substrates containing an arachidonoyl group. However, we have recently shown that there are differences in inhibition by PA depending on the nature of the acyl chains in this lipid (M. Lung, unpublished results). Not only is the inhibition greater for PA with an arachidonoyl group in the *sn*-2 position, but the length of the saturated acyl chain on the *sn*-1 position also determines the extent of inhibition. Our results suggest that there may be a specific binding site on DGK ϵ for PA that has the highest affinity for the most abundant form of PA formed as an intermediate in inositol cycling, *i.e.* 1-stearoyl-2-arachidonoyl phosphatidic acid. The reason these differences were not observed previously may be because the arachidonoyl-specific DGK from bovine testes is not identical to DGK ϵ , as indicated by the difference in its mass.

5.3 The putative transmembrane segment

Most isoforms of DGK reside in the cytosol, but are capable of translocating to membranes to bind DAG. DGK ϵ , however, might have a unique mechanism to bind to membranes. Using a fusion protein of DGK ϵ and GFP it was found that DGK ϵ associated with the endoplasmic reticulum (ER) [87]. It also appears to be present in the plasma membrane [88]. Additionally, we observed that DGK ϵ contains a segment of about 20 residues that are predicted to form a transmembrane helix according to algorithms such as IMPALA, TM Finder and DAS that identify such motifs. Although it does not appear that this segment affects the selectivity for DAG, it is hypothesized to permanently anchor DGK ϵ to membranes. The motif comprises approximately residues 21–41 with the sequence LILWTLCSVLLPVFITFWCSL in the human and monkey enzymes. In rat and mouse DGK ϵ the segment is found at residues 19–39 and each of the rodent forms has two conservative amino acid replacements compared with the primate form. Hence this hydrophobic segment is highly conserved, suggesting its functional importance. There is also a stretch of hydrophobic amino acids between residues 16–36 in *Drosophila* and between residues 20 to 40 in *Arabidopsis thaliana* (DGK2), but with low sequence homology to the mammalian isoforms.

Although the segment from residues 20 to 40 in DGK ϵ is predicted to be a transmembrane helix, several observations do not coincide with this prediction. Modeling studies for the native DGK ϵ 18–42 fragment using the Boltzmann-Stochastic *in silico* method, PepLook detects a tendency to structural polymorphism. The 99 models of lower energy give two equally frequent conformations: a long helix and a U-bent helix (Fig. 3). Interestingly, the two representative models (The Prime and the second model) are the two most stable conformations of the 99 models supporting the conclusion that both conformations might coexist. Since both conformations have low self-stability, their relative ratio might depend upon the medium and factors such as the protein/lipid ratio and the nature of the lipid. A mechanism to shift the equilibrium between the two conformations could provide a mechanism for the regulation of the enzymatic properties of this protein. In addition to changes in the membrane environment, it is predicted that the conformational state of the protein can be shifted toward the transmembrane arrangement of the helix by a single amino acid mutation, changing the Pro³² residue to Ala (Fig. 4).

The PepLook predictions indicate that the hydrophobic segment of the wild type DGK ϵ enters and leaves the membrane on the same side, without accessing the opposing monolayer, i.e. it is a re-entrant helix. There are other protein segments that are predicted to be hydrophobic helices that form reentrant loops. To test the membrane topology of DGK ϵ we expressed an N-terminal FLAG-tag labeled form of both the wild type protein and the P32A mutant in cells to determine if the FLAG epitope is exposed to the cell exterior, as would occur if the hydrophobic segment were a transmembrane helix. In support of the modeling studies, we found that the FLAG epitope was exposed to antibody only on in cells whose membrane was permeabilized with detergent, while the P32A mutant had an exposed Flag tag even in intact cells [87]. It was also shown in this work that the lack of exposure of the FLAG tag in the cells expressing the wild type protein was not the consequence of the protein being targeted to intracellular membranes.

We have also assessed the role of the hydrophobic segment through N-terminal truncations [89]. In one mutation we removed the hydrophobic segment entirely by eliminating 40 residues from the N-terminus. In addition, we added a FLAG-tag to the amino terminus in order to quantify the level of expression of this construct that was compared with a full length, N-terminally FLAG-tagged construct. These proteins will be referred to as FLAG-DGK ϵ (Δ 40) and FLAG-DGK ϵ , respectively. The lack of influence of the FLAG epitope on the enzymatic properties of the protein were confirmed by comparing FLAG-DGK ϵ with a full length construct devoid of an epitope label as well as with a DGK ϵ construct having a C-terminal His-tag. It was found that there was a three-fold increase in k_{cat} and a two-fold increase in K_m when comparing FLAG-DGK ϵ (Δ 40) with FLAG-DGK ϵ [89]. We also made two other N-terminally truncated mutations, FLAG-DGK ϵ (Δ 13) and FLAG-DGK ϵ (Δ 58). The FLAG-DGK ϵ (Δ 13) exhibited no change in the Michaelis-Menten constants (Y. Shulga, unpublished observations). The fact that the FLAG-DGK ϵ (Δ 13) did not exhibit these changes in kinetic constants suggests that its membrane topology more closely resembles that of the wild type enzyme with a re-entrant helix. Thus, the bend in the hydrophobic segment that causes the hydrophobic segment to deviate from a transmembrane helix is not formed because of interactions between the amino-terminal region of the protein and segments on the carboxyl-terminal side of the hydrophobic segment. With regard to the FLAG-DGK ϵ (Δ 58) construct, analysis of the Michaelis-Menten kinetics showed that this construct had an approximately 2-fold reduction in both K_m and k_{cat} . It is interesting to contrast this with the results of the kinetic analysis of the P32A mutant [87]. The reduction of both K_m and k_{cat} in that case is about 4.5-fold. Thus the change in kinetic properties is greater for a mutant with a single amino acid replacement than it is for one with the entire N-terminal 58 residues removed. Many proteins are known that have only a single transmembrane helix. These have been categorized as bitopic proteins [90] because the polypeptide chain passes the membrane only once and portions of the protein are exposed on

both sides of the bilayer. A protein with a single re-entrant helix corresponds to the less common kind of membrane topology that is classified as a monotopic protein. Bitopic proteins are integral membrane proteins that can only be extracted from the membrane by completely destroying the membrane structure with detergent or with organic solvent. In contrast monotopic proteins that insert into membranes but do not traverse the membrane form a continuum between integral and peripheral membrane proteins. Those monotopic proteins that insert deeply into the membrane will be difficult to extract without disrupting the membrane structure, while those that insert more peripherally may be extracted with high salt or high pH. In the case of DGK ϵ only a fraction of the enzyme is extracted with high salt or high pH [89]. Part of the membrane affinity of the native enzyme is contributed by the hydrophobic segment since a much larger fraction of the FLAG-DGK ϵ (Δ 40) construct is extracted with high salt or high pH compared with the native enzyme [89]. This is opposite to what happens with the P32A mutant that is very poorly extracted from membranes with high salt or high pH [87].

5.4 Role of DGK ϵ in cardiac and brain function

Cardiac hypertrophy is a condition that can lead to development of heart failure. DGK ϵ is one of the DGK isoforms expressed in the heart and its levels of expression are reduced in cardiac hypertrophy [91]. To test the functional effects of DGK ϵ in the heart, a transgene encoding DGK ϵ was expressed in mice under control of a myosin heavy chain promoter [92]. Its over-expression protected the mice from changes caused by chronic pressure overload. Unlike controls, the transgenic animals were protected from cardiac hypertrophy as measured by increased heart weight after phenylephrine infusion or thoracic aorta constriction. Phenylephrine activates G protein-coupled receptors (GPCR) that then stimulate PtnIns(4,5)P₂-specific phospholipase C. The DAG produced by this reaction activates PKCs that then promote the development of cardiac hypertrophy [92]. Over-expression of DGK ϵ led to reduced levels of DAG in the myocardium and limited the activation of several PKC isoforms as measured by their association with cell membranes. Taken together, these data indicate that DGK ϵ might have an important role in modulating GPCR signaling in the myocardium.

The brain is another organ with high expression of DGK ϵ . Its mRNA is ubiquitously distributed in the gray matter suggesting that DGK ϵ fulfills a fundamental, rather than a specific function in the brain. The cellular distribution of DGK ϵ in brain is similar to that of glutamate metabotropic receptors (mGluRs), being localized in Purkinje cerebellar neurons, mitral cells of the olfactory bulb, hippocampal interneurons, and neurons of the thalamus and substantia nigra [64]. The mGluRs have an important role in seizures caused by electroconvulsive shock [64] and their activation results in the generation of DAG, which promotes seizures. During seizures the principle DAG species that accumulates is the arachidonoyl-rich DAG, which is produced by the hydrolysis of PtnIns(4,5)P₂. As noted above, there are lower levels of arachidonate-rich PtnIns(4,5)P₂ species in DGK ϵ deficient cells, so it is not surprising that DGK ϵ -KO mice generate less arachidonoyl-DAG following electroconvulsive shock and consequently have mild seizures compared to WT mice. Together these data indicate that DGK ϵ modulates neuronal plasticity and epileptogenesis and could be targeted to minimize seizures.

6. DGK ζ functions in several subcellular compartments

DGK ζ , with its ankyrin repeats and MARCKS homology domain, is a type IV DGK. It also possesses a carboxy-terminal PDZ binding domain that plays a prominent role in its subcellular localization. DGK ζ , unlike DGK ϵ , does not appear to have marked specificity for different species of DAG, suggesting that it regulates biological events by virtue of its subcellular localization and by the company that it keeps. As such, the mechanisms that modulate where DGK ζ localizes in cells, how it gets there, and what it does upon arrival are characterized in more detail than for most other DGKs.

6.1 Subcellular localization

In the basal state, DGK ζ , like most DGKs, resides in the cytoplasm. But upon appropriate cellular events, it translocates to different parts of the cell in order to perform its biological role. DGK ζ functions in several subcellular regions, the best characterized of which are the nucleus, plasma membrane, and cytoskeleton. Surprisingly, its translocation to each of these compartments is mediated predominantly through its MARCKS homology domain and its PDZ binding domain.

The nucleus has a PtdIns cycle and several groups have demonstrated that nuclear DAG fluctuates independently of extranuclear DAG during the cell cycle [93]. Nuclear DAG was shown to peak shortly before S phase [94], and most data support the conclusion that nuclear DAG promotes cell growth. We demonstrated that DGK ζ translocates to the nucleus and that its MARCKS homology domain is a major nuclear localization signal [32,95]. Moreover, its nuclear import is modulated by PKC isoforms which phosphorylate the MARCKS homology domain to reduce its nuclear accumulation[32]. An additional level of regulation occurs when syntrophins, which are scaffolding proteins, anchor DGK ζ in the cytoplasm by associating with its PDZ binding domain [51]. DGK ζ also possesses a nuclear export signal that, when mutated, causes DGK ζ to accumulate in the nucleus (M.K.T. unpublished observations). This exquisite regulation of its nuclear localization suggests that DGK ζ has an important biological function in the nucleus. Indeed, it appears to modulate the cell cycle by metabolizing DAG [32]. It should be noted, however, that other DGKs also translocate to the nucleus and they probably have equally important and distinct roles there[96]. Specificity of function for DGKs in the nucleus would not be surprising because diverse stimuli lead to generation of nuclear DAG that, like its extranuclear counterpart, appears to be compartmentalized [97].

In addition to the nucleus, DGK ζ also localizes at the plasma membrane. Its translocation there is mediated, in part, by phosphorylation of the MARCKS homology domain. A mutant DGK ζ in which serines within this domain were changed to alanine did not translocate to the plasma membrane upon activation of the M1 muscarinic receptor (M1R), a G protein-coupled receptor (GPCR) [98]. Conversely, a mutant in which these serines were altered to aspartate to mimic phosphorylation was partly localized to the plasma membrane [99]. In addition to the MARCKS homology domain, the C1 domains were also necessary for translocation [98]. Collectively, these data suggested that upon activation of GPCRs, DGK ζ becomes phosphorylated on serine residues within the MARCKS homology domain and then translocates to the plasma membrane to metabolize the DAG that is generated upon receptor activation. Indeed, Nelson et al. showed that DGK ζ translocated to the plasma membrane after M1R activation in order to metabolize DAG [39]. Its translocation required binding to β -arrestins—which are scaffolding proteins that also bind GPCRs—and blocking this interaction attenuated DAG metabolism as measured by the generation of PA. The binding site on DGK ζ for β -arrestins mapped to the C1 domains, which is consistent with data showing that the C1 domains were required for DGK ζ to translocate to the plasma membrane. Since β -arrestins bind other GPCRs and even other DGKs, this mechanism is likely broadly applied to limit DAG signalling initiated by many different GPCR agonists. Indeed, over-expressing DGK ζ enhanced decay of ERK phosphorylation following activation of another GPCR, the gonadotropin-releasing hormone receptor [100].

In addition to associating closely with the plasma membrane, DGK ζ also interacts with cytoskeletal elements near the plasma membrane at the leading edge of migrating cells[38, 56]. This interaction appears to depend on its binding to syntrophins through the PDZ binding domain of DGK ζ [38]. Additionally, phosphorylation of DGK ζ by ERK proteins in a carboxy-terminal, proline-rich region is important [99]. The biological function of DGK ζ at the leading edge of migrating cells is not yet clear, but DGK ζ binds to both Rac1 [38] and RasGRP1

[56] and co-localizes with these proteins at the leading edge of cells, suggesting that it might regulate their activity by metabolizing DAG or by generating PA.

6.2 Interactions with specific proteins

Upon translocation to an intracellular compartment, DGK ζ achieves specificity of function by associating with signalling complexes to regulate the activity of resident proteins. DGK ζ modulates these proteins by either metabolizing DAG to terminate its signalling or by producing PA to promote signalling driven by this lipid. For example, in A172 cells, DGK ζ binds to RasGRP1—a Ras exchange factor that is activated by DAG [56]. Genetic deletion of DGK ζ leads to excessive Ras activity in T cells[57] and embryo fibroblasts[58]. Together, these data suggest that DGK ζ binds to RasGRP1 to inhibit its activity and, thus, modulate Ras signalling. In A172 cells, it was also demonstrated that DGK ζ binds and inhibits the activity of PKC α by metabolizing DAG [59]. Conversely, DGK ζ binds to phosphatidylinositol 5-kinase type Ia [62] in order to activate this protein by generating PA, and it might activate mTOR by the same mechanism[63]. Additionally, DGK ζ binds to other proteins such as the retinoblastoma protein [101] and Rac1 [38], but its function in these signalling complexes remains unclear.

6.3 Factors that regulate the activity level of DGK ζ

Several factors can modulate the kinase activity of DGK ζ , including anionic phospholipids, which augment its activity, and calcium, which reduces its activity [84]. Phosphorylations within DGK ζ can also alter its level of activity. For example PKC α phosphorylation in the MARCKS homology domain reduces DGK activity [60]. It is appealing to speculate that this phosphorylation, which occurs within a cluster of cationic amino acids, might reduce the ability of DGK ζ to bind to anionic phospholipids, which augment its activity. DGK ζ also binds to active Src, and its activity appears to be augmented by this event [100]. But at this point there is no evidence that Src phosphorylates DGK ζ , suggesting that its association with Src causes a conformational change in DGK ζ that promotes its catalytic activity. Supporting this mechanism of activation, DGK ζ is also activated upon binding to the retinoblastoma protein [101]. Collectively, these data indicate that the activity levels of DGK ζ are exquisitely modulated by a variety of important biological events.

6.4 Studies in genetically modified mice

The gene encoding DGK ζ has been disrupted in mice and studies have shown that this DGK has a critical function in lymphocytes, which have hyperactive responses [57] when DGK ζ is absent. Additional defects have been identified in mast cells [102] and macrophages [103] isolated from the mice, and it appears that these defects are caused either by elevated DAG levels or by reduced levels of PA. DGK ζ is expressed in most tissues in mice, suggesting that other defects will eventually be uncovered in organ systems that have not yet been studied in detail.

As a converse approach to study the biological function of DGK ζ , a transgene encoding DGK ζ was expressed in mouse myocardium [104]. Its over-expression protected the heart after experimental infarction by reducing left ventricular remodelling. Survival of the DGK ζ transgenic mice was substantially improved, and the authors speculated that the protection afforded by DGK ζ was due to attenuated GPCR signalling, which is consistent with the *in vitro* studies noted above. The DGK ζ transgene also protected mice in an experimental model experimental cardiac hypertrophy [105]. However, one must interpret the experiments in transgenic mice with caution because most DGKs bind to β -arrestins when they are over-expressed [39], raising the possibility that the protection afforded by the DGK ζ transgene might simply be an artefact of its over-expression. Indeed, as noted above, transgenic expression of DGK ϵ in the myocardium also protected mice from experimental cardiac hypertrophy[92]. The

reciprocal experiment should be performed in DGK ζ knockout mice to definitively demonstrate the protective effects of DGK ζ following myocardial ischemia. Together, these data indicate that DGK ζ has important biological roles in several cell types, but the full spectrum of its function still remains to be identified.

7.0 Summary and Conclusions

DGKs represent a diverse family of lipid kinases with representatives in organisms from bacteria to humans. In higher eukaryotes, DGKs modify signalling events through their ability to either metabolize DAG or generate PA. In general, DGKs reside in the cytoplasm in quiescent cells, but translocate to specific subcellular compartments where they regulate specific signalling events by associating with signalling complexes that contain proteins whose activity is modulated by either DAG or PA. Although we have a relatively firm grasp of their general function and modes of regulation, we lack information about the full spectrum of their biological activities.

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Abbreviations used

DAG	diacyl glycerol
DGK	diacyl glycerol kinase
ER	endoplasmic reticulum
GFP	green fluorescent protein
GPCR	G-Protein Coupled Receptors
KO	knock-out
MEF	mouse embryo fibroblasts
PA	phosphatidic acid
PKC	protein kinase C
PLC	phospholipase C
PtnIns	phosphatidylinositol

PtnIns(45)P₂, phosphatidylinositol-(4,5)-bisphosphate**WT**

wild-type

References

1. Rhee SG, Bae YS. Regulation of phosphoinositide-specific phospholipase C isozymes. *J Biol Chem* 1997;272:15045–15048. [PubMed: 9182519]
2. Newton AC. Regulation of protein kinase C. *Cell Biology* 1997;9:161–167.
3. Parekh DB, Ziegler W, Parker PJ. Multiple pathways control protein kinase C phosphorylation. *EMBO Journal* 2000;19:496–503. [PubMed: 10675318]
4. Toker A. Signaling through protein kinase C. *Frontiers in Bioscience* 1998;3:d1134–1147. [PubMed: 9792904]
5. Ebinu JO, Bottorff DA, Chan EYW, Stang SL, Dunn RJ, Stone JC. RasGRP, a ras guanyl nucleotide-releasing protein with calcium-and diacylglycerol-binding motifs. *Science* 1998;280:1082–1086. [PubMed: 9582122]
6. Tognon CE, Kirk HE, Passmore LA, Whitehead IP, Der CJ, Kay RJ. Regulation of RasGRP via a phorbol ester-responsive C1 domain. *Mol Cell Biol* 1998;18:6995–7008. [PubMed: 9819387]
7. Kawasaki H, Springett GM, Shinichiro T, Canales JJ, Harlan P, Blumenstiel JP, Chen EJ, Bany A, Mochizuki N, Ashbacher A, Matsuda M, Housman DE, Graybiel AM. A Rap guanine nucleotide exchange factor enriched highly in the basal ganglia. *Proc Natl Acad Sci USA* 1998;95:13278–13283. [PubMed: 9789079]
8. Yamashita S, Mochizuki N, Ohba Y, Tobiume M, Okada Y, Sawa H, Nagashima K, Matsuda M. CalDAG-GEFIII Activation of Ras, R-Ras, and Rap1. *J Biol Chem* 2000;275:25488–25493. [PubMed: 10835426]
9. Clyde-Smith J, Silins G, Gartside M, Grimmond S, Etheridge M, Apolloni A, Hayward N, Hancock JF. Characterization of RasGRP2, a Plasma Membrane-targeted, Dual Specificity Ras/Rap Exchange Factor. *J Biol Chem* 2000;275:32260–32267. [PubMed: 10918068]
10. Yang Y, Wong GW, Krilis SA, Madhusudhan MS, Sali A, Stevens RL. RasGRP4, a New Mast Cell-restricted Ras Guanine Nucleotide-releasing Protein with Calcium- and Diacylglycerol-binding Motifs. *J Biol Chem* 2002;277:25756–25774. [PubMed: 11956218]
11. Reuther GW, Lambert QT, Rebhun JF, Caligiuri MA, Quilliam LA, Der CJ. RasGRP4 Is a Novel Ras Activator Isolated from Acute Myeloid Leukemia. *J Biol Chem* 2002;277:30508–30514. [PubMed: 11880369]
12. Lucas P, Ukhanov K, Leinders-Zufall T, Zufall F. A Diacylglycerol-Gated Cation Channel in Vomeronasal Neuron Dendrites Is Impaired in TRPC2 Mutant Mice. *Neuron* 2003;40:551–561. [PubMed: 14642279]
13. Ron D, Kazanietz MG. New insights into the regulation of protein kinase C and novel phorbol ester receptors. *FASEB J* 1999;13:1658–1676. [PubMed: 10506570]
14. Merida I, Avila-Flores A, Merino E. Diacylglycerol kinases: at the hub of cell signalling. *Biochem J* 2008;409:1–18. [PubMed: 18062770]
15. Nurrish S, Segalat L, Kaplan JM. Serotonin inhibition of synaptic transmission: G α_o decreases the abundance of UNC-13 at release sites. *Neuron* 1999;24:231–242. [PubMed: 10677040]
16. Miller KG, Emerson MD, Rand JB. G α_o and diacylglycerol kinase negatively regulate the G $q\alpha$ pathway in *C. elegans*. *Neuron* 1999;24:323–333. [PubMed: 10571227]
17. Masai I, Hosoya T, Kojima SI, Hotta Y. Molecular cloning of a Drosophila diacylglycerol kinase gene that is expressed in the nervous system and muscle. *Proc Natl Acad Sci USA* 1992;89:6030–6034. [PubMed: 1321433]
18. Masai I, Okazaki A, Hosoya T, Hotta Y. 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* 1993;90:11157–11161. [PubMed: 8248222]

19. Harden N, Yap SF, Chiam MA, Lim L. A *Drosophila* gene encoding a protein with similarity to diacylglycerol kinase is expressed in specific neurons. *Biochem J* 1993;289:439–444. [PubMed: 8380995]
20. Katagiri T, Mizoguchi T, Shinozaki K. Molecular cloning of a cDNA encoding diacylglycerol kinase (DGK) in *Arabidopsis thaliana*. *Plant Mol Biol* 1996;30:647–653. [PubMed: 8605313]
21. Gomez-Merino FC, Brearley CA, Ornatowska M, Abdel-Halim ME, Zanol MI, Mueller-Roeber B. AtDGK2, a Novel Diacylglycerol Kinase from *Arabidopsis thaliana*. *J Biol Chem* 2004;279:8230–8241. [PubMed: 14665624]
22. Topham MK. Signaling roles of diacylglycerol kinases. *J Cell Biochem* 2006;97:474–484. [PubMed: 16288460]
23. Badola P, Sanders CR II. *Escherichia coli* diacylglycerol kinase is an evolutionarily optimized membrane enzyme and catalyzes direct phosphoryl transfer. *J Biol Chem* 1997;272:24176–24182. [PubMed: 9305868]
24. Han GS, O'Hara L, Siniossoglou S, Carman GM. Characterization of the yeast DGK1-encoded CTP-dependent diacylglycerol kinase. *J Biol Chem* 2008;283:20443–53. [PubMed: 18458076]
25. Han GS, O'Hara L, Carman GM, Siniossoglou S. An unconventional diacylglycerol kinase that regulates phospholipid synthesis and nuclear membrane growth. *J Biol Chem* 2008;283:20433–42. [PubMed: 18458075]
26. Sakane F, Imai S, Kai M, Yasuda S, Kanoh H. Diacylglycerol kinases: why so many of them? *Biochim Biophys Acta* 2007;1771:793–806. [PubMed: 17512245]
27. Sakane F, Imai SI, Kai M, Wada I, Kanoh H. Molecular cloning of a novel diacylglycerol kinase isozyme with a pleckstrin homology domain and a C-terminal tail similar to those of the EPH family of protein tyrosine kinases. *J Biol Chem* 1996;271:8394–8401. [PubMed: 8626538]
28. Klauck TM, Xu X, Mousseau B, Jaken S. Cloning and characterization of a glucocorticoid-induced diacylglycerol kinase. *J Biol Chem* 1996;271:19781–19788. [PubMed: 8702685]
29. Imai H, Kai M, Yasuda S, Kanoh H, Sakane F. Identification and characterization of a novel type II diacylglycerol kinase, DGK kappa. *J Biol Chem* 2005;280:39870–39881. [PubMed: 16210324]
30. Sanjuan MA, Jones DA, Izquierdo M, Merida I. Role of diacylglycerol kinase α in the attenuation of receptor signaling. *J Cell Biol* 2001;153:207–219. [PubMed: 11285286]
31. Nagaya H, Wada I, Jia YJ, Kanoh H. Diacylglycerol Kinase δ Suppresses ER-to-Golgi Traffic via Its SAM and PH Domains. *Mol Biol Cell* 2002;13:302–316. [PubMed: 11809841]
32. Topham MK, Bunting M, Zimmerman GA, McIntyre TM, Blackshear PJ, Prescott SM. Protein kinase C regulates the nuclear localization of diacylglycerol kinase-zeta [see comments]. *Nature* 1998;394:697–700. [PubMed: 9716136]
33. Los AP, van Baal J, de Widt J, Divecha N, van Blitterswijk WJ. Structure-activity relationship of diacylglycerol kinase theta. *Biochim Biophys Acta* 2004;1636:169–174. [PubMed: 15164764]
34. Hurley JH, Newton AC, Parker PJ, Blumberg PM, Nishizuka Y. Taxonomy and function of C1 protein kinase C homology domains. *Protein Science* 1997;6:477–480. [PubMed: 9041654]
35. Shindo M, Irie K, Ohigashi H, Kuriyama M, Saito N. Diacylglycerol kinase gamma is one of the specific receptors of tumor-promoting phorbol esters. *Biochem Biophys Res Commun* 2001;289:451–456. [PubMed: 11716494]
36. Shindo M, Irie K, Masuda A, Ohigashi H, Shirai Y, Miyasaka K, Saito N. Synthesis and Phorbol Ester Binding of the Cysteine-rich Domains of Diacylglycerol Kinase (DGK) Isozymes. *J Biol Chem* 2003;278:18448–18454. [PubMed: 12621060]
37. Sakane F, Kai M, Wada I, Imai S-i, Kanoh H. The C-terminal part of diacylglycerol kinase α lacking zinc fingers serves as a catalytic domain. *Biochem J* 1996;318
38. Yakubchik Y, Abramovici H, Maillet J, Daher E, Obagi C, Parks RJ, Topham MK, Gee SH. Regulation of neurite outgrowth in N1E-115 cells through PDZ-mediated recruitment of diacylglycerol kinase zeta. *Mol Cell Biol* 2005;25:7289–7302. [PubMed: 16055737]
39. Nelson CD, Perry SJ, Regier DS, Prescott SM, Topham MK, Lefkowitz RJ. Targeting of diacylglycerol degradation to M1 muscarinic receptors by -arrestins. *Science* 2007;315:663–666. [PubMed: 17272726]
40. Sakane F, Yamada K, Kanoh H, Yokoyama C, Tanabe T. Porcine diacylglycerol kinase sequence has zinc finger and E-F hand motifs. *Nature* 1990;344:345–348. [PubMed: 2156169]

41. Goto K, Kondo H. Molecular cloning and expression of a 90-kDa diacylglycerol kinase that predominantly localizes in neurons. *Proc Natl Acad Sci USA* 1993;90:7598–7602. [PubMed: 7689223]
42. Kai M, Sakane F, Imai S-i, Wada I, Kanoh H. Molecular cloning of a diacylglycerol kinase isozyme predominantly expressed in human retina with a truncated and inactive enzyme expression in most other human cells. *J Biol Chem* 1994;269:18492–18498. [PubMed: 8034597]
43. Yamada K, Sakane F, Matsushima N, Kanoh H. EF-hand motifs of α β and γ isoforms of diacylglycerol kinase bind calcium with different affinities and conformational changes. *Biochem J* 1997;321:59–64. [PubMed: 9003401]
44. Takeuchi H, Kanematsu T, Misumi Y, Sakane F, Konishi H, Kikkawa U, Watanabe Y, Katan M, Hirata M. Distinct specificity in the binding of inositol phosphates by pleckstrin homology domains of pleckstrin, RAC-protein kinase, diacylglycerol kinase and a new 130 kDa protein. *Biochimica et Biophysica Acta* 1997;1359:275–285. [PubMed: 9434133]
45. Murakami T, Sakani F, Imai S, Houkin K, Kanoh H. Identification and Characterization of Two Splice Variants of Diacylglycerol Kinase η . *J Biol Chem* 2003;278:34364–34372. [PubMed: 12810723]
46. Sakane F, Imai S, Yamada K, Murakami T, Tsushima S, Kanoh H. Alternative Splicing of the Human Diacylglycerol Kinase δ Gene Generates Two Isoforms Differing in Their Expression Patterns and in Regulatory Functions. *J Biol Chem* 2002;277:43519–43526. [PubMed: 12200442]
47. Tang W, Bunting M, Zimmerman GA, McIntyre TM, Prescott SM. Molecular cloning of a novel human diacylglycerol kinase highly selective for arachidonate-containing substrates. *J Biol Chem* 1996;271:10237–10241. [PubMed: 8626589]
48. Prescott SM, Majerus PW. The fatty acid composition of phosphatidylinositol from thrombin-stimulated human platelets. *J Biol Chem* 1981;256:579–582. [PubMed: 7451460]
49. Bunting M, Tang W, Zimmerman GA, McIntyre TM, Prescott SM. Molecular cloning and characterization of a novel human diacylglycerol kinase ζ . *J Biol Chem* 1996;271:10230–10236. [PubMed: 8626588]
50. Ding L, Traer E, McIntyre TM, Zimmerman GA, Prescott SM. The cloning and characterization of a novel human diacylglycerol kinase, DGK ι . *J Biol Chem* 1998;273:32746–32752. [PubMed: 9830018]
51. Hogan A, Shepherd L, Chabot J, Quenneville S, Prescott SM, Topham MK, Gee SH. Interaction of γ 1-syntrophin with Diacylglycerol Kinase- ζ . *J Biol Chem* 2001;276:26526–26533. [PubMed: 11352924]
52. Houssa B, Schaap D, van der Wal J, Goto K, Yamakawa A, Shibata M, Takenawa T, van Blitterswijk WJ. Cloning of a novel human diacylglycerolkinase (DGK θ) containing three cysteine-rich domains, a proline-rich region and a pleckstrin homology domain with overlapping ras-associating domain. *J Biol Chem* 1997;272:10422–10428. [PubMed: 9099683]
53. Houssa B, de Widt J, Kranenburg O, Moolenaar WH, van Blitterswijk WJ. Diacylglycerol Kinase θ binds to and is negatively regulated by active RhoA. *J Biol Chem* 1999;274:6820–6822. [PubMed: 10066731]
54. Topham MK, Prescott SM. Mammalian diacylglycerol kinases, a family of lipid kinases with signaling functions. *J Biol Chem* 1999;274:11447–50. [PubMed: 10206945]
55. Pawson T, Scott JD. Signaling through scaffolding, anchoring, and adaptor proteins. *Science* 1997;278:2075–2080. [PubMed: 9405336]
56. Topham MK, Prescott SM. Diacylglycerol kinase ζ regulates Ras activation by a novel mechanism. *J Cell Biol* 2001;152:1135–1143. [PubMed: 11257115]
57. Zhong X, Hainey EA, Olenchok BA, Jordan MS, Maltzman JS, Nichols KE, Shen H, Koretzky GA. Enhanced T cell responses due to diacylglycerol kinase ζ deficiency. *Nat Immunol* 2003;4:882–890. [PubMed: 12883552]
58. Regier DS, Higbee J, Lund KM, Sakane F, Prescott SM, Topham MK. Diacylglycerol kinase ι regulates ras guanyl-releasing protein 3 and inhibits Rap1 signaling. *Proc Natl Acad Sci (USA)* 2005;102:7595–7600. [PubMed: 15894621]
59. Luo B, Prescott SM, Topham MK. Association of diacylglycerol kinase ζ with protein kinase C α : spatial regulation of diacylglycerol signaling. *J Cell Biol* 2003;160:929–937. [PubMed: 12629049]

60. Luo B, Prescott SM, Topham MK. Protein Kinase C α Phosphorylates and Negatively Regulates Diacylglycerol Kinase ζ . *J Biol Chem* 2003;278:39542–39547. [PubMed: 12890670]
61. Tolia KF, Couvillon AD, Cantley LC, Carpenter CL. Characterization of a Rac1- and RhoGDI-associated lipid kinase signaling complex. *Molecular and Cellular Biology* 1998;18:762–770. [PubMed: 9447972]
62. Luo B, Prescott SM, Topham MK. Diacylglycerol kinase ζ regulates phosphatidylinositol 4-phosphate 5-kinase by a novel mechanism. *Cell Signalling* 2004;16:891–897. [PubMed: 15157668]
63. Avila-Flores A, Santos T, Rincon E, Merida I. Modulation of the mammalian target of rapamycin pathway by diacylglycerol kinase-produced phosphatidic acid. *J Biol Chem* 2005;280:10091–10099. [PubMed: 15632115]
64. Rodriguez de Turco EB, Tang W, Topham MK, Sakane F, Marcheselli VL, Chen C, Taketomi A, Prescott SM, Bazan NG. Diacylglycerol kinase ϵ regulates seizure susceptibility and long-term potentiation through arachidonoyl-inositol lipid signaling. *Proc Natl Acad Sci USA* 2001;98:4740–4745. [PubMed: 11287665]
65. Crotty T, Cai J, Sakane F, Taketomi A, Prescott SM, Topham MK. Diacylglycerol kinase δ regulates protein kinase C and epidermal growth factor receptor signaling. *Proc Natl Acad Sci (USA)* 2006;103:15485–15490. [PubMed: 17021016]
66. Olenchok BA, Guo R, Carpenter JH, Jordan M, Topham MK, Koretzky GA, Zhong XP. Disruption of diacylglycerol metabolism impairs the induction of T cell anergy. *Nat Immunol* 2006;7:1174–1181. [PubMed: 17028587]
67. Chianale F, Cutrupi S, Rainero E, Baldanzi G, Porporato PE, Traini S, Filigheddu N, Gnocchi VF, Santoro MM, Parolini O, van Blitterswijk WJ, Sinigaglia F, Graziani A. Diacylglycerol Kinase- $\{\alpha\}$ Mediates Hepatocyte Growth Factor-induced Epithelial Cell Scatter by Regulating Rac Activation and Membrane Ruffling. *Mol Biol Cell* 2007;18:4859–4871. [PubMed: 17898083]
68. Sakane F, Yamada K, Imai S-i, Kanoh H. Porcine 80-kDa diacylglycerol kinase is a calcium-binding and calcium/phospholipid-dependent enzyme and undergoes calcium-dependent translocation. *J Biol Chem* 1991;266:7096–7100. [PubMed: 1849900]
69. Abe T, Lu X, Jiang Y, Boccone CE, Qian S, Vattam KM, Wek RC, Walsh JP. Site-directed mutagenesis of the active site of diacylglycerol kinase α : calcium and phosphatidylserine stimulate enzyme activity via distinct mechanisms. *Biochem J* 2003;375:673–80. [PubMed: 12908872]
70. Fanani ML, Topham MK, Walsh JP, Eband RM. Lipid modulation of the activity of diacylglycerol kinase α - and ζ -isoforms: activation by phosphatidylethanolamine and cholesterol. *Biochemistry* 2004;43:14767–14777. [PubMed: 15544347]
71. Cipres A, Carrasco S, Merino E, Diaz E, Krishna M, Falck JR, Martinez C, Merida I. Regulation of Diacylglycerol Kinase α by Phosphoinositide 3-Kinase Lipid Products. *J Biol Chem* 2003;278:35629–35635. [PubMed: 12832407]
72. Merino E, Avila-Flores A, Shirai Y, Moraga I, Saito N, Merida I. Lck-dependent tyrosine phosphorylation of diacylglycerol kinase α regulates its membrane association in T cells. *J Immunol* 2008;180:5805–15. [PubMed: 18424699]
73. Baldanzi G, Cutrupi S, Chianale F, Gnocchi V, Rainero E, Porporato P, Filigheddu N, van Blitterswijk WJ, Parolini O, Bussolino F, Sinigaglia F, Graziani A. Diacylglycerol kinase- α phosphorylation by Src on Y335 is required for activation, membrane recruitment and Hgf-induced cell motility. *Oncogene*. 2007
74. Fukunaga-Takenaka R, Shirai Y, Yagi K, Adachi N, Sakai N, Merino E, Merida I, Saito N. Importance of chroman ring and tyrosine phosphorylation in the subtype-specific translocation and activation of diacylglycerol kinase α by D- α -tocopherol. *Genes Cells* 2005;10:311–9. [PubMed: 15773894]
75. Jiang Y, Sakane F, Kanoh H, Walsh JP. Selectivity of the Diacylglycerol Kinase Inhibitor R59949 among Diacylglycerol Kinase Subtypes. *Biochemical Pharmacology* 2000;59:763–772. [PubMed: 10718334]
76. Flores I, Casaseca T, Martinez-A C, Kanoh H, Merida I. Phosphatidic acid generation through interleukin 2 (IL-2)-induced α -diacylglycerol kinase activation is an essential step in IL-2-mediated lymphocyte proliferation. *J Biol Chem* 1996;271:10334–10340. [PubMed: 8626603]

77. Baldanzi G, Mitola S, Cutrupi S, Filigheddu N, van Blitterswijk WJ, Sinigaglia F, Bussolino F, Graziani A. Activation of diacylglycerol kinase alpha is required for VEGF-induced angiogenic signaling in vivo. *Oncogene* 2004;23:4828–4838. [PubMed: 15122338]
78. Cutrupi S, Baldanzi G, Gramaglia D, Maffe A, Schaap D, Giraudo E, van Blitterswijk WJ, Bussolino F, Comoglio PM, Graziani A. Src-mediated activation of α -diacylglycerol kinase is required for hepatocyte growth factor-induced cell motility. *EMBO J* 2000;19:4614–4622. [PubMed: 10970854]
79. Alonso R, Rodriguez MC, Pindado J, Merino E, Merida I, Izquierdo M. Diacylglycerol kinase alpha regulates the secretion of lethal exosomes bearing Fas ligand during activation-induced cell death of T lymphocytes. *J Biol Chem* 2005;280:28439–28450. [PubMed: 15870081]
80. Zha Y, Marks R, Ho AW, Peterson AC, Janardhan S, Brown I, Praveen K, Stang S, Stone JC, Gajewski TF. T cell anergy is reversed by active Ras and is regulated by diacylglycerol kinase- α . *Nat Immunol* 2006;7:1166–73. [PubMed: 17028589]
81. Olenchok BA, Guo R, Carpenter JH, Jordan M, Topham MK, Koretzky GA, Zhong XP. Disruption of diacylglycerol metabolism impairs the induction of T cell anergy. *Nat Immunol* 2006;7:1174–81. [PubMed: 17028587]
82. Postle AD, Dombrowsky H, Clarke H, Pynn CJ, Koster G, Hunt AN. Mass spectroscopic analysis of phosphatidylinositol synthesis using 6-deuterated-myo-inositol: comparison of the molecular specificities and acyl remodelling mechanisms in mouse tissues and cultured cells. *Biochem Soc Trans* 2004;32:1057–9. [PubMed: 15506962]
83. Milne SB, Ivanova PT, Armstrong MD, Myers DS, Lubarda J, Shulga YV, Topham MK, Brown HA, Epand RM. Dramatic differences in the roles in lipid metabolism of two isoforms of diacylglycerol kinase. *Biochemistry* 2008;47:9372–9. [PubMed: 18702510]
84. Thirugnannam S, Topham MK, Epand RM. Physiological Implications of the Contrasting Modulation of the Activities of the ϵ - and ζ -Isoforms of Diacylglycerol Kinases. *Biochemistry* 2001;40:10607–10613. [PubMed: 11524004]
85. Walsh JP, Suen R, Lemaitre RN, Glomset JA. Arachidonoyl-diacylglycerol kinase from bovine testis. Purification and properties. *J Biol Chem* 1994;269:21155–21164. [PubMed: 8063736]
86. Walsh JP, Suen R, Glomset JA. Arachidonoyl-diacylglycerol kinase. Specific in vitro inhibition by polyphosphoinositides suggests a mechanism for regulation of phosphatidylinositol biosynthesis. *J Biol Chem* 1995;270:28647–53. [PubMed: 7499383]
87. Decaffmeyer M, Shulga YV, Dicu AO, Thomas A, Truant R, Topham MK, Brasseur R, Epand RM. Determination of the topology of the hydrophobic segment of mammalian diacylglycerol kinase epsilon in a cell membrane and its relationship to predictions from modeling. *J Mol Biol* 2008;383:797–809. [PubMed: 18801368]
88. Kobayashi N, Hozumi Y, Ito T, Hosoya T, Kondo H, Goto K. Differential subcellular targeting and activity-dependent subcellular localization of diacylglycerol kinase isozymes in transfected cells. *Eur J Cell Biol* 2007;86:433–44. [PubMed: 17599647]
89. Dicu AO, Topham MK, Ottaway L, Epand RM. Role of the hydrophobic segment of diacylglycerol kinase epsilon. *Biochemistry* 2007;46:6109–17. [PubMed: 17455907]
90. Blobel G. Intracellular protein topogenesis. *Proc Natl Acad Sci U S A* 1980;77:1496–500. [PubMed: 6929499]
91. Yahagi H, Takeda M, Asaumi Y, Okumura K, Takahashi R, Takahashi J, Ohta J, Tada H, Minatoya Y, Sakuma M, Watanabe J, Goto K, Shirato K, Kagaya Y. Differential regulation of diacylglycerol kinase isozymes in cardiac hypertrophy. *Biochem Biophys Res Commun* 2005;332:101–8. [PubMed: 15896305]
92. Niizeki T, Takeishi Y, Kitahara T, Arimoto T, Ishino M, Bilim O, Suzuki S, Sasaki T, Nakajima O, Walsh RA, Goto K, Kubota I. Diacylglycerol kinase-epsilon restores cardiac dysfunction under chronic pressure overload: a new specific regulator of G α (q) signaling cascade. *Am J Physiol Heart Circ Physiol* 2008;295:H245–55. [PubMed: 18487437]
93. Irvine, R. September 52000. Science's stke (www.stke.org)
94. Banfic H, Zizak M, Divecha N, Irvine RF. Nuclear diacylglycerol is increased during cell proliferation in vivo. *Biochem J* 1993;290:633–636. [PubMed: 8457189]
95. Goto K, Kondo H. A 104-kDa diacylglycerol kinase containing ankyrin-like repeats localizes in the cell nucleus. *Proc Natl Acad Sci USA* 1996;93:11196–11201. [PubMed: 8855332]

96. Luo B, Regier DS, Prescott SM, Topham MK. Diacylglycerol Kinases. *Cellular Signal* 2004;16:983–989.
97. D'Santos CS, Clarke JH, Irvine RF, Divecha N. Nuclei contain two differentially regulated pools of diacylglycerol. *Curr Biol* 1999;9:437–440. [PubMed: 10226027]
98. Santos T, Carrasco S, Jones DR, Merida I, Eguinoa A. Dynamics of diacylglycerol kinase zeta translocation in living T-cells. Study of the structural domain requirements for translocation and activity. *J Biol Chem* 2002;277:30300–9. [PubMed: 12015310]
99. Abramovici H, Hogan AB, Obagi C, Topham MK, Gee SH. Diacylglycerol Kinase-z Localization in Skeletal Muscle Is Regulated by Phosphorylation and Interaction with Syntrophins. *Mol Biol Cell* 2003;14:4499–4511. [PubMed: 14551255]
100. Davidson L, Pawson AJ, Lopez de Maturana R, Freestone SH, Barran P, Millar RP, Maudsley S. Gonadotropin-releasing hormone-induced activation of diacylglycerol kinase-zeta and its association with active c-src. *J Biol Chem* 2004;279:11906–16. [PubMed: 14707140]
101. Los AP, Vinke FP, de Widt J, Topham MK, van Blitterswijk WJ, Divecha N. The retinoblastoma family of proteins bind to and activate diacylglycerol kinase zeta. *J Biol Chem* 2005;281:858–866. [PubMed: 16286473]
102. Olenchok BA, Guo R, Silverman MA, Wu JN, Carpenter JH, Koretzky GA, Zhong XP. Impaired degranulation but enhanced cytokine production after Fc epsilonRI stimulation of diacylglycerol kinase zeta-deficient mast cells. *J Exp Med* 2006;203:1471–80. [PubMed: 16717114]
103. Liu CH, Machado FS, Guo R, Nichols KE, Burks AW, Aliberti JC, Zhong XP. Diacylglycerol kinase zeta regulates microbial recognition and host resistance to *Toxoplasma gondii*. *J Exp Med* 2007;204:781–92. [PubMed: 17371930]
104. Niizeki T, Takeishi Y, Arimoto T, Takahashi H, Shishido T, Koyama Y, Goto K, Walsh RA, Kubota I. Cardiac-specific overexpression of diacylglycerol kinase zeta attenuates left ventricular remodeling and improves survival after myocardial infarction. *Am J Physiol Heart Circ Physiol* 2007;292:H1105–12. [PubMed: 17071729]
105. Niizeki T, Takeishi Y, Kitahara T, Arimoto T, Koyama Y, Goto K, Mende U, Kubota I. Diacylglycerol kinase zeta rescues G alpha q-induced heart failure in transgenic mice. *Circ J* 2008;72:309–17. [PubMed: 18219172]

Biography

Richard M. Epand is currently a Professor in the Department of Biochemistry and Biomedical Sciences at McMaster University in Hamilton, Ontario, Canada. Dr. Epand received his A.B. from the Johns Hopkins University and his Ph.D. in the Department of Biochemistry of Columbia University under the direction of Professor I.B. Wilson. Richard Epand then did postdoctoral studies with Professor Harold Scheraga at Cornell University and with Professor Leloir at the Instituto de Investigaciones Bioquimicas in Buenos Aires. Dr. Epand has held academic positions at the University of Guelph before joining McMaster.

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Richard Epand current research interests in the general area of the functions of biological membranes. Studies range from the properties of proteins in membranes to the study of membrane components in living cells.

Figure 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.

Figure 2. Model of spatial DGK function

Evidence indicates that DGKs bind and modulate 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.

Figure 3. D18-Q42 models of the native DGK ϵ calculated by PepLook

Top: Snapshot of the 99 best structures provided by PepLook (left). View of the best model of each population (Prime's structure in pink and model 2 in blue) (right). Bottom: Local RMS (window of 9 residues) along the sequence. The reference structure is the Prime. The red plot represents the mean values of all models of the Prime's subpopulation, the blue one represents the mean value for the all models of the second subpopulation. The standard deviations clearly indicate that the two populations are homogenous. (Taken from [87])

Figure 4. D18-Q42 models of the P32A DGK ϵ calculated by PepLook

Calculated conformations of P32A-DGK ϵ 18–42. (A) Snapshot of the 99 best structures provided by PepLook. (B) View of the best model of each population (Prime's structure in pink and representative of 2nd population in blue). (C) Local RMS (window of 9 residues) along the sequence. The reference structure is the Prime. The red plot represents the mean values of all models of the Prime's subpopulation, the blue one represents the mean value for the all models of the second subpopulation. The standard deviations clearly indicate that the Prime's populations are homogenous and that the second population is less homogenous. Taken from [87].