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Signaling Roles of Diacylglycerol Kinases

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Abstract Diacylglycerol kinases (DGKs) attenuate diacylglycerol signaling by converting this lipid to phosphatidic acid (PA). The nine mammalian DGKs that have been identified are widely expressed, but each isoform has a unique tissue and subcellular distribution. Their kinase activity is regulated by mechanisms that modify their access to diacylglycerol, directly affect their kinase activity, or alter their ability to bind to other proteins. In many cases, these enzymes regulate the activity of proteins that are modulated by either diacylglycerol or PA. Experiments using cultured cells and model organisms have demonstrated that DGKs have prominent roles in neuronal transmission, lymphocyte signaling, and carcinogenesis. J. Cell. Biochem. 97: 474–484, 2006. © 2005 Wiley-Liss, Inc.

Key words: diacylglycerol kinase; signal transduction; lipid signaling; diacylglycerol; phosphatidic acid; lymphocyte signaling; carcinogenesis

Diacylglycerol (DAG) is a membrane lipid that activates numerous proteins, including conventional and novel protein kinase C (PKC) isoforms [Newton, 1997; Toker, 1998], Ras guanyl nucleotide-releasing proteins (RasGRPs) [Ron and Kazanietz, 1999], and some transient receptor potential channels [Lucas et al., 2003]. DAG also recruits a number of proteins to membrane compartments, including the chimaerins, protein kinase D, and the Munc13 proteins [Ron and Kazanietz, 1999]. Because of its broad effects, the availability diacylglycerol needs to be tightly regulated. DAG is metabolized in three ways: hydrolysis of a fatty acyl chain by diacylglycerol lipase to generate a monoacylglycerol and a free fatty acid, addition of CDP-choline or-ethanolamine to form phosphatidylcholine or phosphatidylethanolamine, or phosphorylation of the free hydroxyl group to produce phosphatidic acid (PA). Under most circumstances, its conversion to PA is the major route for metabolism of signaling DAG. This reaction is catalyzed by a large family of enzymes, the diacylglycerol kinases (DGKs) (Fig. 1). Nine mammalian DGK isoforms have been identified to date. In contrast to mammals, one or only a few DGK isoforms have been identified in organisms such as *Caenorhabditis elegans*, *Drosophila melanogaster*, *Dictyostelium discoideum*, and *Arabidopsis thaliana* [de la Roche et al., 2002; Luo et al., 2004b]. No DGK gene has been identified in yeast, and there is one bacterial diacylglycerol kinase that has little similarity to eukaryotic DGKs. Recently, a mammalian lipid kinase that phosphorylated several types of lipids, including 1-acylglycerol, diacylglycerol, and ceramide was identified and localized predominantly to the mitochondria. It has a catalytic domain homologous to the mammalian DGKs, but otherwise has little similarity [Waggoner et al., 2004; Bektas et al., 2005].

The structural diversity of DGKs in mammals suggests that they have evolved to perform roles in processes specific to higher vertebrates. All of the eukaryotic DGK isoforms identified to date are similar in having a kinase domain consisting of catalytic and accessory domains. Each

Abbreviations used: DGK, diacylglycerol kinase; DAG, diacylglycerol; PA, phosphatidic acid; PKC, protein kinase C; RasGRP, Ras guanyl nucleotide-releasing protein; PH, pleckstrin homology; PI, phosphatidylinositol; SAM, sterile alpha motif; PLD, phospholipase D; PIP_2, phosphaditylinositol-4,5-bisphosphate; mTOR, mammalian target of rapamycin.

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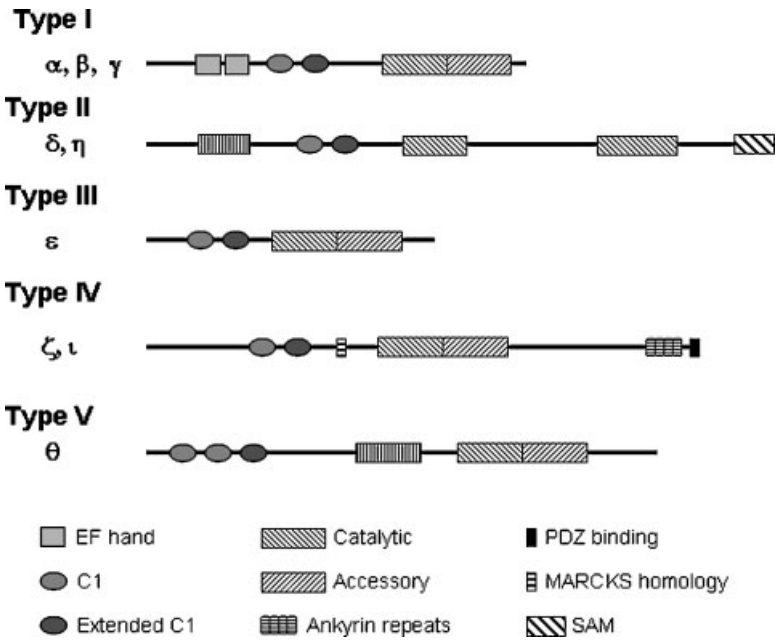

Fig. 1. The mammalian DGK family. Nine mammalian DGKs have been identified. They are divided into five subtypes based on structural similarity. All DGKs have a kinase domain consisting of catalytic and accessory domains. Each DGK also has at least two C1 domains. The C1 domain closest to the catalytic domain has an extended motif. Other motifs shown in this figure regulate DGK activity, subcellular localization, or interaction with other proteins or lipids. Other structural motifs of unknown significance are not included in this figure.

catalytic domain has a consensus ATP binding site with the sequence Gly-X-Gly-X-X-Gly, where X is any amino acid. Mutation of the second glycine to an aspartate [Topham et al., 1998] or alanine [Los et al., 2004], yields an inactive enzyme. The DGK catalytic domains likely function similarly to those of protein kinases by presenting ATP as the phosphate donor. However, structural differences between the DGK and protein kinase catalytic domains suggest that DGK catalytic domains may have access to DAG in lipid bilayers. Protein kinase catalytic domains, in general, do not require such access. Mammalian DGKs δ and η have bipartite catalytic domains, indicating that the catalytic and accessory domains might function as two independent units in a coordinated fashion. In addition to their kinase domains, all eukaryotic DGKs have at least two cysteine-rich regions homologous to the C1A and C1B motifs of PKCs [Hurley et al., 1997]. Whereas structural predictions suggest that these domains likely bind DAG, no DGK C1 domain has been conclusively shown to bind diacylglycerol. The C1 domains of mammalian DGKs α, β, γ, δ, η, and θ have been tested for their ability to bind PDBu, a long-lived DAG analog, but only the C1A domains of DGKs β and γ could successfully bind [Sakane et al., 1996b; Shindo et al., 2001, 2003]. And based on sequence alignments, Hurley et al. [1997] noted that most DGK C1 domains were sufficiently different from those in PKCs that they might not bind DAG. It is possible, though, that the inability of the DGK C1 domains to bind PDBu is due to structural differences between PDBu and DAG, reflecting an exquisite selectivity of DGK C1 domains for authentic DAG, in contrast to the more promiscuous C1 domains of other proteins that also bind phorbol esters like PDBu. It is interesting to note that the C1 domain in DGKs that is closest to the catalytic domain has an extended motif of 15 amino acids that is highly conserved between DGKs and not present in other C1 domains [Houssa and van Blitterswijk, 1998]. The conserved residues in this extended motif are critical for DAG kinase activity [Los et al., 2004], suggesting that this extended C1 domain might have a critical function in binding DAG.

In addition to the C1 and catalytic domains, most DGKs have other structural domains that appear to have regulatory roles. These domains are used to group the mammalian DGKs into five subfamilies. Type I DGKs have calcium-binding EF hand motifs, making these isoforms

more active in the presence of calcium [Yamada et al., 1997]. DGKs with pleckstrin homology (PH) domains at their amino termini are grouped as type II. Though no specific function has been identified for these domains, the PH domain of DGKδ was shown to bind weakly and nonselectively to phosphatidylinositols (PIs) [Takeuchi et al., 1997]. DGKδ also has at its C-terminus a sterile alpha motif (SAM) that is necessary to localize DGKδ to the endoplasmic reticulum where it participates in vesicle trafficking between there and the golgi apparatus [Nagaya et al., 2002]. The SAM domain might also mediate oligomerization of type II DGKs [Murakami et al., 2003]. The type III DGKε has no identifiable regulatory domains, but it does have an unusual specificity toward acyl chains of DAG, strongly preferring an arachidonoyl group at the *sn*-2 position [Tang et al., 1996]. This preference suggests that DGKε might be a component of the biochemical pathway that accounts for the enrichment of PI species with arachidonate. Type IV DGKs have a region homologous to the phosphorylation domain of the MARCKS protein that acts as a nuclear localization signal [Ding et al., 1998; Topham et al., 1998]. These DGKs also have four ankyrin repeats at their C-termini. The type V DGKθ has three C1 domains and a region with weak homology to a PH domain. There is additional complexity in the mammalian DGK family due to alternative splicing that occurs with DGKs β [Caricasole et al., 2001], γ [Kai et al., 1994], δ [Sakane et al., 2002], ζ [Ding et al., 1997], ι [Ito et al., 2004], η [Murakami et al., 2003], and probably others. Their diversity indicates that the mammalian DGKs are an important family of enzymes that regulate a variety of signaling events.

PHOSPHATIDIC ACID PRODUCED BY DGKs HAS SIGNALING FUNCTIONS

The DGK reaction is unusual because not only does it remove the signaling molecule DAG, but the product of the reaction, PA, also has signaling roles. PA can stimulate DNA synthesis and is potentially mitogenic [van Corven et al., 1992]. And PA generated at the plasma membrane helps recruit Raf [Rizzo et al., 2000] and sphingosine kinase 1 [Delon et al., 2004]. It is also involved in vesicle trafficking [Siddhanta and Shields, 1998] and modulates the activity of several enzymes, including PI 5-kinases [Ishihara et al., 1998], PAK1 [Bokoch et al., 1998], Ras-GAP [Tsai et al., 1990], PKCζ [Limatola et al., 1994], and protein phosphatase 1 [Jones and Hannun, 2002]. Although the bulk of signaling PA is thought to be generated via phospholipase D (PLD) enzymes, DGKs likely also contribute to its intracellular concentration. The PA species derived from the DGK and PLD reactions are structurally different because the initial substrates have distinct fatty acid components. PA produced by PLD is derived predominantly from phosphatidylcholine, which is largely composed of saturated and mono-unsaturated fatty acids. PA produced by DGK is derived from DAG, which is enriched in poly-unsaturated fatty acids—particularly arachidonate [Pettitt et al., 1997; Hodgkin et al., 1998]. This is an important distinction because there is good evidence that each PA species—saturated and unsaturated—can differentially activate targets [Hodgkin et al., 1998]. For example, saturated PA species induce MAPK activation to a greater extent than unsaturated PAs. Thus, DGKs might not only regulate DAG signaling, but might also initiate other signaling events by producing PA. Suggesting a signaling role for PA derived from DGKs, Flores et al. [1996] demonstrated evidence in T-lymphocytes that PA produced by DGKα was necessary for progression to S phase of the cell cycle. Thus, DGKs can influence signaling events either by metabolizing DAG or by generating PA.

TISSUE DISTRIBUTION OF THE DGKs

Except for DGKs δ and ε, all mammalian DGK isoforms are expressed in the brain at levels equivalent or higher that in other tissues. They have been detected in a number of different regions of the brain, including the hippocampus (β, γ, ε, ζ, ι, θ), cerebellum (γ, ε, ζ, ι, θ), and olfactory bulb (β, γ, ζ, θ), and in the retina (ε, γ, ι) [Topham and Prescott, 1999]. Their prominent expression in these structures indicates that DGKs are an integral part of central nervous system and visual function. Several DGKs (α, ε, ζ, η) are also expressed in the lung [Klauck et al., 1996; Katagiri et al., 2005], with DGKs ζ and α being expressed in alveolar type II cells [Katagiri et al., 2005]. DGKs are also highly expressed in muscle, with DGKs δ and ζ expressed in striated muscle and DGKs β and ε being expressed in cardiac muscle [Topham

and Prescott, 1999; Abramovici et al., 2003]. Finally, DGKs are also highly expressed in the spleen (α, ζ, δ, η), thymus (α, ζ) and various cultured white blood cells (α, δ, ζ) [Klauck et al., 1996; Topham and Prescott, 1999; Sakane et al., 2002; Zhong et al., 2003]. It is interesting to note that several DGKs can be expressed in the same tissue and even in the same cell. Further, co-expressed DGKs are usually from different subfamilies, strongly suggesting that each DGK subfamily has a specific function.

REGULATION OF DGK ACTIVITY

Although this has not been conclusively demonstrated, DGKs are believed to be quiescent until their activity is required. This is achieved by coordinating their access to DAG with other activating events such as phosphorylations and binding to cofactors. This idea was initially tested by Van der Bend et al. [1994], who assayed DGK activity after either receptor activation—which caused spatially restricted DAG synthesis—or following treatment of cells with exogenous phospholipase C—which caused global DAG synthesis. They observed DGK activity following receptor activation but did not detect significant activity after treating the cells with exogenous phospholipase C. Their data suggested that DGKs are active only in spatially restricted compartments following physiologic generation of DAG.

DGKs gain access to DAG by translocating to a membrane compartments where DAG is generated, and then their activity is further modified by phosphorylations and by binding to cofactors and other proteins. This complexity permits tissue- or cell-specific regulation of each DGK isotype, depending on the availability of cofactors and the type of stimulus that the cell receives. DGKα is one of the better-studied mammalian DGKs and is a good example of the complex regulation of DGKs. It translocates to at least two different membrane compartments in T-lymphocytes depending upon the agonist used to activate the cells: with IL-2, DGKα translocates from the cytosol to a perinuclear region [Flores et al., 1996], while activation of the T-cell antigen receptor causes DGKα to translocate to the plasma membrane [Sanjuan et al., 2001]. The activity of DGKα can be modified by the availability of several cofactors. Calcium is known to bind to EF hand motifs and to stimulate DAG kinase activity in vitro [Yamada et al., 1997]. A mutant DGKα in which the EF hands were deleted was about three times more active in vitro than full-length DGKα [Sakane et al., 1996b; Sanjuan et al., 2001], suggesting that the deleted region inhibited the activity of DGKα and this inhibition was relieved when the EF hands bound calcium. The deleted region also appeared to limit translocation of DGKα, because its deletion caused the protein to constitutively associate with the plasma membrane [Sanjuan et al., 2001]. Membrane lipids also modify its activity: phosphatidylserine and sphingosine activate DGKα in vitro and likely in vivo [Sakane et al., 1989; Yamada and Sakane, 1993]. And DGKα can be phosphorylated by several protein kinases, including some PKC isoforms [Kanoh and Ono, 1986; Schaap et al., 1993], and Src [Cutrupi et al., 2000]. Evidence suggests that phosphorylation by Src enhances DAG kinase activity [Cutrupi et al., 2000]. Thus, depending on the context, the activity of DGKα can be modified in a number of ways, and different combinations might lead to different outcomes.

Like DGKα, other DGK isotypes appear to be regulated by the availability of lipid or protein co-factors, phosphorylations, and by controlling their access to DAG. Members of each DGK subfamily appear to be similarly regulated. But there are subtle differences between subfamily members such as tissue-specific expression patterns, unique binding partners, alternative splicing, and subcellular localization that affect when and where they are active. For example, type II DGKs have a PH domain, a motif that in DGKδ was able to bind to PIs [Takeuchi et al., 1997]. Their binding, however, was relative weak, and others have failed to demonstrate PI binding [Sakane et al., 1996a], suggesting that these domains might not bind lipids in vivo. In addition to a PH domain, type II DGKs also have SAMs at their carboxy-termini that might mediate homo- and hetero-oligomerization of type II DGKs [Murakami et al., 2003]. Oligomerizing does not appear to affect their activity [Murakami et al., 2003], but might affect subcellular localization. Finally, DGKδ is phosphorylated on serines in the PH domain by conventional PKCs and this reduces its translocation to the plasma membrane [Imai et al., 2004]. The activity of types III and IV DGKs can be modified by lipids, sometimes in opposing ways. DGKε is inhibited by PIs and by phosphatidylserine, whereas type IV DGKs are

activated by phosphatidylserine [Thirugnannam et al., 2001]. Type IV DGKs are also strongly regulated by subcellular translocation. These enzymes have a nuclear localization signal similar to the phosphorylation site domain of MARCKS. This domain in the DGKs is phosphorylated by conventional PKCs, which inhibits their nuclear import [Ding et al., 1998; Topham et al., 1998]. The syntrophin family of scaffolding proteins also regulates the subcellular location of DGKζ by associating with the DGKζ carboxy-terminal PDZ binding domain to sequester DGKζ in the cytoplasm [Hogan et al., 2001]. And we have also observed that DGKζ has a strong nuclear export signal (unpublished). Thus, nuclear accumulation of type IV DGKs is exquisitely regulated, suggesting an important nuclear function for these isozymes. Finally, DGKθ, a type V DGK, can be regulated in two ways. First, its translocation to the plasma membrane appears to be induced when PKCs ε or η phosphorylate it [van Baal et al., 2005]. And, second, the activity of DGKθ can be regulated through its association with active RhoA [Houssa et al., 1999]. Binding to RhoA completely inhibits DGKθ kinase activity and is the only known example of direct regulation of DGK activity through a protein–protein interaction.

DGK INHIBITORS

There are two compounds, R59022 and R59949, that inhibit DGK activity in vitro and in vivo. These have been used extensively to examine the consequences of DGK inhibition, but the compounds are poorly characterized with respect to their selectivity for different DGK isoforms and other proteins. Another caveat to consider when these compounds are used in cultured cells is that they are variably inactivated by serum. In the most rigorous study of these inhibitors, Jiang et al. [2000] found that they were relatively selective for type I DGKs, but did not examine all DGK isotypes. They noted that the R59949 likely inhibited DGKα by binding its catalytic domain. Thus, inhibitors are available, but they have not been characterized in great detail.

ACTIVITY OF DGK ISOTYPES IS RESTRICTED TO SPECIFIC CELL COMPARTMENTS

The overlapping distribution of the DGKs suggests that each DGK isotype has a distinct tissue- or cell-specific role. We are only beginning to understand the complexities of spatially restricted DGK function. Most work has focused on the subcellular locations where DGKs are active. Several DGKs localize to the cytoskeleton. Indirect evidence suggests that they may participate in regulating cytoskeleton dynamics. This is not surprising because lipid mediators are known to bind to numerous proteins that associate with the cytoskeleton and dramatically affect their activities. For example, phosphatidylinositol-4,5-bisphosphate (PIP_2) binds to actin capping proteins, which then dissociate from the actin complex, allowing rapid polymerization to occur [Hartwig et al., 1999]. PI 5-kinases generate PIP_2 and, as expected, their overexpression induces abnormally active actin polymerization [Shibasaki et al., 1997; Ishihara et al., 1998]. Interestingly, PA activates PI 5-kinases 8-14-fold [Jenkins et al., 1994; Ishihara et al., 1998]. As such, it would not be surprising if by generating PA, DGKs could regulate actin polymerization. Although evidence suggests that it is the PLDs that associate with and activate PI 5-kinases by generating PA [Divecha et al., 2000; Honda et al., 2000], we demonstrated that DGKζ co-localized and co-immunoprecipitated with PI 5-kinase type Iα and enhanced its activity by generating PA [Luo et al., 2004a]. Consistent with this, Tolias et al. [1998] found that DGK activity was associated with a complex of proteins that included a PI 5-kinase, Rac, Rho, Cdc42, and Rho-GDI. And Yakubchyk et al. [2005] found that DGKζ co-localized and co-immunoprecipitated with Rac and cooperated with it to induce neurite formation in N1E-115 cells.

Houssa et al. [1999] provided further support for a role for DGKs in cytoskeleton dynamics by showing that RhoA associated with DGKθ, but only when RhoA was in its active form. It appeared to bind the catalytic domain of DGKθ, which potentially disrupts DAG kinase activity. But the physiologic significance of this interaction has not been clearly defined. We have observed that several DGK isotypes co-immunoprecipitate with Rho family proteins when overexpressed in cells (unpublished). The physiologic significance of these interactions is not clear, though together, these data suggest that DGKs likely have a prominent role in regulating the cytoskeleton. In a functional study using human platelets, the DGK inhibitor, R59022,

augmented secretion and aggregation [Nunn and Watson, 1987], demonstrating the importance of DGK activity for normal cytoskeleton responses. Their importance in platelets is not surprising because changes in the platelet cytoskeleton are partly mediated by DAG, which rapidly accumulates in platelets treated with thrombin. Work by Bishop and Bell [1986] demonstrated that platelet DGKs are primarily responsible for metabolizing DAG, attenuating downstream signaling and allowing a return to the basal state. But, while their activity in platelets is crucial, no one has conclusively identified which DGK isoforms are present—there are at least three isoforms [Yada et al., 1990]—or rigorously examined their roles. Together, these observations indicate that DGKs are intimately involved cytoskeleton dynamics, but with a few exceptions, the specific functions of DGK isoforms have not been rigorously studied.

In addition to the cytoskeleton, DGKs also appear to prominently function in the nucleus, where there is a PI cycle that is regulated separately from its extranuclear counterpart [Divecha et al., 1993]. DAG produced in the nucleus appears to function separately from extranuclear DAG. For example, some growth factors (e.g., IGF-1) can stimulate transient accumulation of nuclear DAG without causing a measurable change in extranuclear DAG [Leach et al., 1992; Divecha et al., 1993], and several groups have demonstrated that nuclear DAG fluctuates independently of extranuclear DAG during the cell cycle [Banfic et al., 1993; Cocco et al., 1996; Jackowski, 1996]. Its nuclear signaling function has received little attention, but data clearly indicate that in most cases DAG acts to promote cell growth. For example, several groups have demonstrated that nuclear DAG peaks shortly before S phase, suggesting that it may participate in the G_1/S transition [Irvine, 2000]. Supporting this conclusion and emphasizing the importance of nuclear DAG signaling, we demonstrated that overexpressed DGKζ caused cells to accumulate at the G_0/G_1 transition. The effect required both DGK activity and a functional nuclear import signal, suggesting that the effect was caused when DGKζ metabolized nuclear DAG [Topham et al., 1998].

Within the nucleus, DAG signaling, like its extranuclear counterpart, appears to be compartmentalized. And it appears that DAG signaling is not confined to the nuclear envelope, as one would expect, but also occurs at distinct locations within the body of the nucleus [Vann et al., 1997; Boronenkov et al., 1998; Irvine, 2000]. This is not surprising because lipid metabolizing enzymes and DAG signaling have been detected in nuclei stripped of their envelopes [Vann et al., 1997], and immunofluorescence has demonstrated the presence of discrete collections of lipid-modifying enzymes such as PI-kinases within the body of the nucleus [Boronenkov et al., 1998]. And there appear to be numerous pools of nuclear DAG with distinct fatty acid components. For example, in cells induced to differentiate, nuclear DAG with saturated fatty acids decreased while the concentration of unsaturated DAG did not change. And during the cell cycle, the concentration of unsaturated DAG fluctuated while the concentration of saturated DAG did not [D'Santos et al., 1999]. These data strongly suggest the existence of multiple, differentially regulated pools of nuclear DAG. While these data indicate that nuclear DAG signaling is precisely and locally regulated, the targets of this signaling remain unclear. Evidence suggests that PKC isoforms that translocate to the nucleus are activated by DAG, but other as yet unidentified targets are also likely to exist.

Nuclear DGKs appear to have prominent and specific roles. DGKs α, ζ, and ι, translocate to the nucleus, while a fraction of cellular DGKθ is constitutively nuclear [van Blitterswijk and Houssa, 2000]. And different DGK subtypes are confined to specific, separate compartments within the nucleus. For example, DGKα associates with the nuclear envelope [Flores et al., 1996], while DGKs θ and ζ are found in multiple regions in the body of the nucleus [van Blitterswijk and Houssa, 2000]. Movement of proteins in the nucleus largely occurs by random diffusion making it likely that experimental overexpression of DGK isotypes in the nucleus permits them to nonspecifically interfere with the function of other endogenous DGKs. This fact, combined with the lack of isotype-specific DGK inhibitors, has made it difficult to experimentally determine the specific nuclear function of the different DGK isotypes. But it appears that DGKs affect nuclear signaling either by terminating DAG signals or by generating PA. For example, in T-lymphocytes, PA produced by nuclear DGKα appeared to be

necessary for IL-2-mediated progression to S phase of the cell cycle [Flores et al., 1996]. Conversely, as noted above, nuclear DGKζ inhibited progression from G_1 to S phase of the cell cycle, likely by metabolizing DAG [Topham et al., 1998]. And nuclear DGKθ appeared to metabolize nuclear DAG induced by thrombin [Bregoli et al., 2001] and nerve growth factor [Tabellini et al., 2004]. These data suggest unique roles for nuclear DGKs α, ζ, and θ indicating both the complexity and importance of lipid signaling and DGK function in the nucleus.

DGKs CAN REGULATE DAG SIGNALING BY BINDING SPECIFIC PROTEIN PARTNERS

In addition to acting locally at sites of DAG pools, in some cases, DGKs achieve specificity by associating with and inhibiting proteins activated by DAG. For example, we demonstrated that DGKζ associated with RasGRP1, a guanyl nucleotide-exchange factor for Ras [Topham and Prescott, 2001]. RasGRP1 requires DAG in order to activate Ras, and we found that DGKζ regulated the function of RasGRP1 by locally metabolizing DAG. DAG kinase activity was required for the inhibition because a kinase-dead DGKζ that could still associate with RasGRP1, no longer reduced its activity. And this regulation was selective: five other DGK isotypes did not significantly inhibit RasGRP1 activity. Moreover, phorbol esters (DAG analogs) enhanced the association of DGKζ with RasGRP1, suggesting that generation of DAG caused DGKζ to bind to a signaling complex containing RasGRP1. DGKα, a type I DGK, also appeared to inhibit the activity of RasGRP1 by a similar mechanism [Jones et al., 2002; Sanjuan et al., 2003]. And suggesting that DGKs commonly bind DAG-activated proteins to inhibit their activity, DGKζ also associated with and inhibited PKCα [Luo et al., 2003], while DGKι bound and inhibited RasGRP3 [Regier et al., 2005]. Combined, these data demonstrate that DGK activity is not only targeted at sites of DAG production, but is also specifically directed toward a subset of DAG-activated proteins.

In addition to inhibiting proteins activated by diacylglycerol, DGKs also bind to proteins whose activity is modulated by PA. For example, we demonstrated that DGKζ associated with and activated PI 5-kinase type Iα [Luo et al., 2004a], and Avila-Flores et al. [2005] found that DGKζ could also bind and activate the mammalian target of rapamycin (mTOR). As noted above, PA modulates the activity of several protein targets in addition to mTOR and PI 5-kinases, indicating that DGKs might regulate other proteins that bind PA.

MODEL ORGANISMS DEMONSTRATING PHYSIOLOGICAL ROLES OF DGKs

It is clear that DGKs bind and regulate a number of proteins, suggesting that they have important physiological roles in vivo. Several groups have begun examining their functions using a variety of model systems. For example, in *Caenorhabditis elegans*, Nurrish et al. [1999] isolated a strain resistant to serotonin-induced inhibition of locomotion. The mutated gene responsible for the effect, *dgk-1*, was homologous to DGKθ. Their data and observations by Miller et al. [1999] suggested that serotonin signaling negatively regulated synaptic transmission, in part, through DGK-1, which reduced DAG at motor neuron nerve terminals. The DAG would have otherwise recruited UNC-13, a protein that is crucial for acetylcholine release and consequent locomotion.

By deleting a portion of the gene encoding DGKε, Rodriquez de Turco et al. [2001] demonstrated that this DGK was necessary to maintain proper PI signaling. DGKε selectively phosphorylates DAG with an arachidonate in the *sn*-2 position and is the only DGK that prefers to phosphorylate a specific type of DAG. The DGK reaction is the first step in the resynthesis of PIs from signaling DAG. And inositol phospholipids, including the DAG precursor PIP_2, are enriched with arachidonate at the *sn*-2 position [Prescott and Majerus, 1981]. By selectively phosphorylating unsaturated DAG, DGKε is a prime candidate responsible for enriching PIs with unsaturated fatty acids. Compared to DAG with saturated fatty acids, DAG containing unsaturated fatty acids—like arachidonate—more potently activates some signaling proteins, including the PKCs [Hodgkin et al., 1998]. So to maintain the integrity of certain DAG-activated signaling cascades, it is important that PIs have the proper fatty acid composition. Demonstrating its importance in maintaining proper phosphatidylinositol fatty acid composition, brains from DGKε-deficient mice had reduced levels of arachidonate in both

PIP_2 and DAG, compared to wild type mice. To examine the consequences of this change, seizures were induced in the mice using electroconvulsive shock. DGKε-deficient mice had significantly shorter seizures and recovered from the seizures much faster than wild type mice [Rodriquez de Turco et al., 2001]. Their resistance to seizures was presumably caused by altered phosphatidylinositol signaling, demonstrating a critical role for DGKε in maintaining a proper balance of arachidonate-enriched inositol phospholipids.

Zhong et al. deleted the gene encoding DGKζ in mice to examine its role in lymphocyte signaling. As noted above, we had demonstrated that this DGK attenuated Ras signaling by inhibiting RasGRP1. This RasGRP links T-cell receptor signaling to Ras, so Zhong et al. [2003] tested Ras signaling in splenic T-cells and thymocytes. They demonstrated augmented and prolonged signaling in the cells, indicating that DGKζ negatively regulates T-cell receptor signaling. Combined with our work [Topham and Prescott, 2001], it appears to do so by inhibiting the function of RasGRP1.

Inhibition of RasGRPs appears to be a common theme for type IV DGKs. We found that DGKι associated with RasGRP3 and inhibited its activity [Regier et al., 2005]. RasGRP3 can activate either Ras or Rap proteins, and in cell culture experiments we found that DGKι selectively inhibited its activation of Rap. To examine the physiological consequences of this inhibition, we deleted the gene encoding DGKι in mice. Consistent with our cell culture experiments, we found more active Rap in DGKι null cells. Both Ras and Rap can bind to Raf, but in doing so, they cause different signaling outcomes. Whereas Ras activates Raf, Rap forms nonproductive complexes with Raf that then interfere with Ras signaling. We hypothesized that the increased active Rap in the mice would interfere with Ras signaling. To examine this, we crossed the DGKι knockout mice with mice carrying a Ras transgene making them prone to develop skin tumors. Consistent with our hypothesis, we found that DGKι null mice had significantly fewer skin tumors. We also tested whether DGKs ι or ζ regulated other RasGRPs and found the DGKζ inhibited RasGRP3 and RasGRP4 [Regier et al., 2005]. Its inhibition of RasGRP3 appeared to predominantly affect Ras signaling, having the opposite effects compared to DGKι. Thus, type IV DGKs appear to regulate RasGRP proteins, but the inhibition has different physiological outcomes depending on the RasGRP and DGK.

Although no one has studied the function of DGKα by manipulating the gene in a model organism, several studies indicate that this DGK has a prominent role in lymphocyte function. Using cultured lymphocytes, Sanjuan et al. [2001] demonstrated the DGKα translocated to the plasma membrane upon activation of the T-cell receptor. They subsequently found evidence that this DGK attenuated Ras activation when overexpressed in the cells [Sanjuan et al., 2003]. And using DGK inhibitors, Outram et al. [2002] observed that DGKα might promote survival of developing T-cell subsets. Finally, DGKα appeared to regulate secretion of lethal exosomes from T-cells [Alonso et al., 2005]. In addition to its role in lymphocytes, DGKα also appeared to be necessary for both vascular endothelial growth factor [Baldanzi et al., 2004] and hepatocyte growth factor [Cutrupi et al., 2000] signaling and it might function downstream of an anaplastic lymphoma kinase fusion protein commonly found in anaplastic large cell lymphomas [Bacchiocchi et al., 2005]. One must interpret these studies with caution, however, because many of experiments used overexpression of DGKα or the poorly characterized DGK inhibitors. Thus, we await confirmation of its physiological role in a model organism.

CONCLUSIONS

DGKs can influence many signaling pathways because both the substrate and product of the DGK reaction have signaling properties. There are nine mammalian DGKs and evidence suggests that each of them selectively regulates a subset of DAG or PA signaling events. In general, DGKs are regulated by access to DAG and by binding to appropriate cofactors or proteins. These regulatory mechanisms combine to spatially and temporally restrict their activity in cells. Furthermore, some DGKs regulate specific proteins by binding to them and influencing local DAG or PA accumulation. Although the physiological roles of DGKs are not known in great detail, studies in model organisms have demonstrated that DGKs influence neuronal transmission, lymphocyte signaling, and Ras-induced tumorigenesis.

REFERENCES

Abramovici H, Hogan AB, Obagi C, Topham MK, Gee SH. 2003. Diacylglycerol kinase-z localization in skeletal muscle is regulated by phosphorylation and interaction with syntrophins. Mol Biol Cell 14:4499–4511.

Alonso R, Rodriguez MC, Pindado J, Merino E, Merida I, Izquierdo M. 2005. Diacylglycerol kinase alpha regulates the secretion of lethal exosomes bearing Fas ligand during activation-induced cell death of T lymphocytes. J Biol Chem 280:28439–28450.

Avila-Flores A, Santos T, Rincon E, Merida I. 2005. Modulation of the mammalian target of rapamycin pathway by diacylglycerol kinase-produced phosphatidic acid. J Biol Chem 280:10091–10099.

Bacchiocchi R, Baldanzi G, Carbonari D, Capomagi C, Colombo E, van Blitterswijk WJ, Graziani A, Fazioli F. 2005. Activation of alpha-diacylglycerol kinase is critical for the mitogenic properties of anaplastic lymphoma kinase. Blood 106:2175–2182.

Baldanzi G, Mitola S, Cutrupi S, Filigheddu N, van Blitterswijk WJ, Sinigaglia F, Bussolino F, Graziani A. 2004. Activation of diacylglycerol kinase alpha is required for VEGF-induced angiogenic signaling in vivo. Oncogene 23:4828–4838.

Banfic H, Zizak M, Divecha N, Irvine RF. 1993. Nuclear diacylglycerol is increased during cell proliferation *in vivo*. Biochem J 290:633–636.

Bektas M, Payne SG, Liu H, Goparaju S, Milstein S, Spiegel S. 2005. A novel acylglycerol kinase that produces lysophosphadic acid modulates cross talk with EGFR in prostate cancer cells. J Cell Biol 169:801–811.

Bishop WR, Bell RM. 1986. Attenuation of sn-1,2-diacylglycerol second messengers. J Biol Chem 261: 12513–12519.

Bokoch GM, Reilly AM, Daniels RH, King CC, Olivera A, Spiegel S, Knaus UG. 1998. A GTPase-independent mechanism of p21-activated kinase activation. J Biol Chem 273:8137–8144.

Boronenkov IV, Loijens JC, Umeda M, Anderson RA. 1998. Phosphoinositide signaling pathways in nuclei are associated with nuclear speckles containing pre-mRNA processing factors. Mol Biol Cell 9:3547–3560.

Bregoli L, Baldassare JJ, Raben DM. 2001. Nuclear diacylglycerol kinase theta is activated in response to alpha-thrombin. J Biol Chem 276:23288–23295.

Caricasole A, Bettini E, Sala C, Roncarati R, Kobayashi N, Caldara F, Goto K, Terstappen GC. 2001. Molecular cloning and characterization of the human diacylglycerol kinase beta (DGKbeta) gene: Alternative splicing generates DGKbeta isotypes with different properties. J Biol Chem 277:4790–4796.

Cocco L, Capitani S, Maraldi NM, Mazzotti G, Barnabei O, Gilmour RS, Manzoli FA. 1996. Inositol lipid cycle and autonomous nuclear signalling. Advan Enzyme Regul 36:101–114.

Cutrupi S, Baldanzi G, Gramaglia D, Maffe A, Schaap D, Giraudo E, van Blitterswijk WJ, Bussolino F, Comoglio PM, Graziani A. 2000. Src-mediated activation of α-diacylglycerol kinase is required for hepatocyte growth factor-induced cell motility. EMBO J 19:4614–4622.

D'Santos CS, Clarke JH, Irvine RF, Divecha N. 1999. Nuclei contain two differentially regulated pools of diacylglycerol. Curr Biol 9:437–440.

de la Roche MA, Smith JL, Rico M, Carrasco S, Merida I, Licate L, Cote GP, Egelhoff TT. 2002. Dictyostelium discoideum has a single diacylglycerol kinase gene with similarity to the mammalian theta isoforms. Biochem J 368:809–815.

Delon C, Manifava M, Wood E, Thompson D, Krugman S, Pyne S, Ktistakis NT. 2004. Sphingosine kinase 1 is an intracellular effector of phosphatidic acid. J Biol Chem 279:44763–44774.

Ding L, Bunting M, Topham MK, McIntyre TM, Zimmerman GA, Prescott SM. 1997. Alternative splicing of the human diacylglycerol kinase ζ gene in muscle. Proc Natl Acad Sci USA 94:5519–5524.

Ding L, Traer E, McIntyre TM, Zimmerman GA, Prescott SM. 1998. The cloning and characterization of a novel human diacylglycerol kinase, DGKι. J Biol Chem 273: 32746–32752.

Divecha N, Banfic H, Irvine RF. 1993. Inositides and the nucleus and inositides in the nucleus. Cell 74:405–407.

Divecha N, Roefs M, Halstead JR, D'Andrea SD, Fernandez-Borga M, Oomen L, Saqib KM, Wakelam MJO, D'Santos C. 2000. Interaction of the type Iα PIPkinase with phospholipase D: A role for the local generation of phosphatidylinositol 4,5-bisphosphate in the regulation of PLD2 activity. EMBO J 19:5440–5449.

Flores I, Casaseca T, Martinez AC, Kanoh H, Merida I. 1996. 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 271:10334–10340.

Hartwig JH, Barkalow K, Azim A, Italiano J. 1999. The elegant platelet: Signals controlling actin assembly. Thromb Haemost 82:392–398.

Hodgkin MN, Pettitt TR, Martin A, Michell RH, Pemberton AJ, Wakelam MJO. 1998. Diacylglycerols and phosphatidates: Which molecular species are intracellular messenger? Trends Biochem Sci 23:200–204.

Hogan A, Shepherd L, Chabot J, Quenneville S, Prescott SM, Topham MK, Gee SH. 2001. Interaction of γ1-syntrophin with Diacylglycerol Kinase-ζ. J Biol Chem 276:26526–26533.

Honda A, Nogami M, Yokozeki T, Yamazaki M, Nakamura H, Watanabe H, Kawamoto K, Nakayama K, Morris AJ, Frohman MA, Kanaho Y. 2000. Phosphatidylinositol 4-phosphate 5-kinase α is a downstream effector of the small G protein ARF6 in membrane ruffle formation. Cell 99:521–532.

Houssa B, van Blitterswijk WJ. 1998. Specificity of cysteine-rich domains in diacylglycerol kinases and protein kinases C. Biochem J 331:677–680.

Houssa B, de Widt J, Kranenburg O, Moolenaar WH, van Blitterswijk WJ. 1999. Diacylglycerol Kinase θ binds to and is negatively regulated by active RhoA. J Biol Chem 274:6820–6822.

Hurley JH, Newton AC, Parker PJ, Blumberg PM, Nishizuka Y. 1997. Taxonomy and function of C1 protein kinase C homology domains. Protein Sci 6:477–480.

Imai S, Kai M, Yamada K, Kanoh H, Sakane F. 2004. The plasma membrane translocation of diacylglycerol kinase delta 1 is negatively regulated by conventional protein kinase C-dependent phosphorylation at Ser-22 and Ser-26 within the pleckstrin homology domain. Biochem J 382:957–966.

Irvine R. 2000. Nuclear lipid signaling Science's stke (www.stke.org).

Ishihara H, Shibasaki Y, Kizuki N, Wada T, Yazaki Y, Asano T, Oka Y. 1998. Type I phosphatidylinositol-4-phosphate 5-kinases. Cloning of the third isoform and deletion/substitution analysis of members of his novel lipid kinase family. J Biol Chem 273:8741–8748.

Ito T, Hozumi Y, Sakane F, Saino-Saito S, Kanoh H, Aoyagi M, Kondo H, Goto K. 2004. Cloning and characterization of diacylglycerol kinase iota splice variants in rat brain. J Biol Chem 279:23317–23326.

Jackowski S. 1996. Cell cycle regulation of membrane phospholipid metabolism. J Biol Chem 271:20219–20222.

Jenkins GH, Fisette PL, Anderson RA. 1994. Type I phosphatidylinositol 4-phosphate 5-kinases are specifically stimulated by phosphatidic acid. J Biol Chem 269: 11547–11554.

Jiang Y, Sakane F, Kanoh H, Walsh JP. 2000. Selectivity of the diacylglycerol kinase inhibitor R59949 among diacylglycerol kinase subtypes. Biochemical Pharmacology 59:763–772.

Jones JA, Hannun YA. 2002. Tight binding and inhibition of protein phosphatase-1 by phosphatidic acid. J Biol Chem 277:15530–15538.

Jones DR, Sanjuan MA, Stone JC, Merida I. 2002. Expression of a catalytically inactive form of diacylglycerol kinase α induces sustained signaling through RasGRP. FASEB J 16:595–597.

Kai M, Sakane F, Imai S-I, Wada I, Kanoh H. 1994. 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 269:18492–18498.

Kanoh H, Ono T. 1986. Phosphorylation of pig brain diacylglycerol kinase by endogenous protein kinase. FEBS Lett 201:97–100.

Katagiri Y, Ito T, Saino-Saito S, Hozumi Y, Suwabe A, Otake K, Sata M, Kondo H, Sakane F, Kanoh H, Kubota I, Goto K. 2005. Expression and localization of diacylglycerol kinase isoenzymes and enzymatic features in rat lung. Am J Physiol Lung Cell Mol Physiol 288:L1171–L1178.

Klauck TM, Xu X, Mousseau B, Jaken S. 1996. Cloning and characterization of a glucocorticoid-induced diacylglycerol kinase. J Biol Chem 271:19781–19788.

Leach KL, Ruff VA, Jarpe MB, Adams LD, Fabbro D, Raben DM. 1992. α-Thrombin stimulates nuclear diglyceride levels and differential nuclear localization of protein kinase C isozymes in IIC9 cells. J Biol Chem 267:21816–21822.

Limatola C, Schaap D, Moolenaar D, van Blitterswijk WJ. 1994. Phosphatidic acid activation of protein kinase C zeta overexpressed in COS cells: Comparison with other protein kinase C isotypes and other acidic phospholipids. Biochem J 304:1001–1008.

Los AP, van Baal J, de Widt J, Divecha N, van Blitterswijk WJ. 2004. Structure-activity relationship of diacylglycerol kinase theta. Biochim Biophys Acta 1636:169–174.

Lucas P, Ukhanov K, Leinders-Zufall T, Zufall F. 2003. A diacylglycerol-gated cation channel in vomeronasal neuron dendrites is impared in TRPC2 mutant mice. Neuron 40:551–561.

Luo B, Prescott SM, Topham MK. 2003. Association of diacylglycerol kinase ζ with protein kinase C α: Spatial regulation of diacylglycerol signaling. J Cell Biol 160: 929–937.

Luo B, Prescott SM, Topham MK. 2004a. Diacylglycerol kinase z regulates phosphatidylinositol 4-phosphate 5-kinase by a novel mechanism. Cell Signalling 16:891–897.

Luo B, Regier DS, Prescott SM, Topham MK. 2004b. Diacylglycerol kinases. Cellular Signal 16:983–989.

Miller KG, Emerson MD, Rand JB. 1999. $G\alpha_o$ and diacylglycerol kinase negatively regulate the $G\alpha_q$ pathway in *C. elegans*. Neuron 24:323–333.

Murakami T, Sakani F, Imai S, Houkin K, Kanoh H. 2003. Identification and characterization of two splice variants of diacylglycerol kinase η. J Biol Chem 278:34364–34372.

Nagaya H, Wada I, Jia Y-J, Kanoh H. 2002. Diacylglycerol kinase d suppresses ER-to-golgi traffic via its SAM and PH domains. Mol Biol Cell 13:302–316.

Newton AC. 1997. Regulation of protein kinase C. Cell Biology 9:161–167.

Nunn DL, Watson SP. 1987. A diacylglycerol kinase inhibitor, R59022, potentiates secretion by and aggregation of thrombin-stimulated platelets. Biochem J 243: 809–813.

Nurrish S, Segalat L, Kaplan JM. 1999. Serotonin inhibition of synaptic transmission: $G\alpha_o$ decreases the abundance of UNC-13 at release sites. Neuron 24:231–242.

Outram SV, Crompton T, Merida I, Varas A, Martinez AC. 2002. Diacylglycerol kinase a activity promotes survival of CD4+8+ double positive cells during thymocyte development. Immunol 105:391–398.

Pettitt TR, Martin A, Horton T, Liossis C, Lord JM, Wakelam MJO. 1997. Diacylglycerol and phosphatidate generated by phospholipases C and D, respectively, have distince fatty acid compositions and functions. J Biol Chem 272:17354–17359.

Prescott SM, Majerus PW. 1981. The fatty acid composition of phosphatidylinositol from thrombin-stimulated human platelets. J Biol Chem 256:579–582.

Regier DS, Higbee J, Lund KM, Sakane F, Prescott SM, Topham MK. 2005. Diacylglycerol kinase iota regulates ras guanyl-releasing protein 3 and inhibits Rap1 signaling. Proc Natl Acad Sci USA 102:7595–7600.

Rizzo MA, Shome K, Watkins SC, Romero G. 2000. The recruitment of Raf-1 to membranes is mediated by direct interaction with phosphatidic acid and is independent of association with Ras. J Biol Chem 275:23911–23918.

Rodriquez de Turco EB, Tang W, Topham MK, Sakane F, Marcheselli VL, Chen C, Taketomi A, Prescott SM, Bazan NG. 2001. Diacylglycerol kinase e regulates siezure susceptibility and long-term potentiation through arachidonoyl-inositol lipid signaling. Proc Natl Acad Sci USA 98:4740–4745.

Ron D, Kazanietz MG. 1999. New insights into the regulation of protein kinase C and novel phorbol ester receptors. FASEB J 13:1658–1676.

Sakane F, Yamada K, Kanoh H. 1989. Different effects of sphingosine, R59022 and anionic amphiphiles on two diacylglycerol kinase isozymes purified from porcine thymus cytosol. FEBS Lett 255:409–413.

Sakane F, Imai SI, Kai M, Wada I, Kanoh H. 1996a. 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 271:8394–8401.

Sakane F, Kai M, Wada I, Imai S-i, Kanoh H. 1996b. The C-terminal part of diacylglycerol kinase α lacking zinc fingers serves as a catalytic domain. Biochem J 318.

Sakane F, Imai S, Yamada K, Murakami T, Tsushima S, Kanoh H. 2002. Alternative splicing of the human diacylglycerol kinase d gene generates two isoforms differing in their expression patterns and in regulatory functions. J Biol Chem 277:43519–43526.

Sanjuan MA, Jones DA, Izquierdo M, Merida I. 2001. Role of diacylglycerol kinase α in the attenuation of receptor signaling. J Cell Biol 153:207–219.

Sanjuan MA, Pradet-Balade B, Jones DR, Martinez AC, Stone JC, Garcia-Sanz JA, Merida I. 2003. T cell activation in vivo targets diacylglycerol kinase a to the membrane: A novel mechanism for Ras attenuation. J Immunol 170:2877–2883.

Schaap D, van der Wal J, van Blitterswijk WJ, van der Bend RL, Ploegh HL. 1993. Diacylglycerol kinase is phosphorylated in vivo upon stimulation of the epidermal growth factor receptor and serine/threonine kinases, including protein kinase C-ε. Biochem J 289:875–881.

Shibasaki Y, Ishihara H, Kizuki N, Asano T, Oka Y, Yazaki Y. 1997. Massive actin polymerization induced by phosphatidylinositol-4-phosphate 5-kinase in vivo. J Biol Chem 272:7578–7581.

Shindo M, Irie K, Ohigashi H, Kuriyama M, Saito N. 2001. Diacylglycerol kinase gamma is one of the specific receptors of tumor-promoting phorbol esters. Biochem Biophys Res Commun 289:451–456.

Shindo M, Irie K, Masuda A, Ohigashi H, Shirai Y, Miyasaka K, Saito N. 2003. Synthesis and phorbol ester binding of the cysteine-rich domains of diacylglycerol kinase (DGK) isozymes. J Biol Chem 278:18448–18454.

Siddhanta A, Shields D. 1998. Secretory vesicle budding from the trans-golgi network is mediated by phosphatidic acid levels. J Biol Chem 273:17995–17998.

Tabellini G, Billi AM, Fala F, Cappellini A, Evagelisti C, Manzoli L, Cocco L, Martelli AM. 2004. Nuclear diacylglycerol kinase-theta is activated in response to nerve growth factor stimulation in PC12 cells. Cell Signal 16: 1263–1271.

Takeuchi H, Kanematsu T, Misumi Y, Sakane F, Konishi H, Kikkawa U, Watanabe Y, Katan M, Hirata M. 1997. 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 1359:275–285.

Tang W, Bunting M, Zimmerman GA, McIntyre TM, Prescott SM. 1996. Molecular cloning of a novel human diacylglycerol kinase highly selective for arachidonate-containing substrates. J Biol Chem 271:10237–10241.

Thirugnannam S, Topham MK, Epand RM. 2001. Physiological implications of the contrasting modulation of the activities of the ε- and ζ-isoforms of diacylglycerol kinases. Biochemistry 40:10607–10613.

Toker A. 1998. Signaling through protein kinase C. Frontiers in Bioscience 3:d1134–d1147.

Tolias KF, Couvillon AD, Cantley LC, Carpenter CL. 1998. Characterization of a Rac1- and RhoGDI-associated lipid kinase signaling complex. Mol Cell Biol 18:762–770.

Topham MK, Prescott SM. 1999. Mammalian diacylglycerol kinases, a family of lipid kinases with signaling functions. J Biol Chem 274:11447–11450.

Topham MK, Prescott SM. 2001. Diacylglycerol kinase ζ regulates Ras activation by a novel mechanism. J Cell Biol 152:1135–1143.

Topham MK, Bunting M, Zimmerman GA, McIntyre TM, Blackshear PJ, Prescott SM. 1998. Protein kinase C regulates the nuclear localization of diacylglycerol kinase-zeta [see comments]. Nature 394:697–700.

Tsai MH, Yu CL, Stacey DW. 1990. A cytoplasmic protein inhibits the GTPase activity of H-Ras in a phospholipid-dependent manner. Science 250:982–985.

van Baal J, de Widt J, Divecha N, van Blitterswijk WJ. 2005. Translocation of diacylglycerol kinase theta from cytosol to plasma membrane in response to activation of G protein-coupled receptors and protein kinase C. J Biol Chem 280:9870–9878.

van Blitterswijk WJ, Houssa B. 2000. Properties and functions of diacylglycerol kinases. Cellular Signalling 12:595–605.

van Corven EJ, Van Rijswijk A, Jalink K, van der Bend RL, van Blitterswijk WJ, Moolenaar WH. 1992. Mitogenic action of lysophosphatidic acid and phosphatidic acid on fibroblasts. Biochem J 281:163–169.

van der Bend RL, de Widt J, Hilkmann H, van Blitterswijk WJ. 1994. Diacylglycerol kinase in receptor-stimulated cells converts its substrate in a topologically restricted manner. J Biol Chem 269:4098–4102.

Vann LR, Wooding FB, Irvine RF, Divecha N. 1997. Metabolism and possible compartmentalization of inositol lipids in isolated rat liver nuclei. Biochem J 327:569–576.

Waggoner DW, Johnson LB, Mann PC, Morris V, Guastella J, Bajjalieh SM. 2004. MuLK, a eukaryotic multi-substrate lipid kinase. J Biol Chem 279:38228–38235.

Yada Y, Toshihiko O, Kanoh H, Nozawa Y. 1990. Purification and characterization of cytosolic diacylglycerol kinases of human platelets. J Biol Chem 265:19237–19243.

Yakubchyk Y, Abramovici H, Maillet J, Daher E, Obagi C, Parks RJ, Topham MK, Gee SH. 2005. Regulation of neurite outgrowth in N1E-115 cells through PDZ-mediated recruitment of diacylglycerol kinase zeta. Mol Cell Biol 25:7289–7302.

Yamada K, Sakane F. 1993. The different effects of sphingosine on diacylglycerol kinase isozymes in Jurkat cells, a human T-cell line. Biochimica et Biophysica Acta 1169:211–216.

Yamada K, Sakane F, Matsushima N, Kanoh H. 1997. EF-hand motifs of α, β, and γ isoforms of diacylglycerol kinase bind calcium with different affinities and conformational changes. Biochem J 321:59–64.

Zhong X, Hainey EA, Olenchock BA, Jordan MS, Maltzman JS, Nichols KE, Shen H, Koretzky GA. 2003. Enhanced T cell responses due to diacylglycerol kinase ζ deficiency. Nat Immunol 4:882–890.