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Nuclear diacylglycerol kinases: regulation and roles

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1. ABSTRACT

The diacylglycerol-kinases are a family of related lipid kinases. There are currently 10 known isoforms of diacylglycerol kinases that are categorized into five groups based on similarities in their primary sequence. All of these enzymes catalyze the transfer of the γ-phosphate of ATP to one lipid second messenger, diacylglycerol, thereby generating another lipid second messenger, phosphatidic acid. As a result, they are uniquely poised to regulate the relative levels of these two key second messengers. These enzymes show considerable diversity in their cellular and sub-cellular distribution which suggests a great diversity in physiological functions. One sub-cellular compartment that is receiving a considerable attention is the nucleus. A number of DGKs have been found to reside in, or translocate to the nucleus in response to agonists. In this review we focus primarily on the nuclear localization, modulation of intrinsic enzymatic activity, and the potential physiological roles of the six diacylglycerol kinases that have been found in the nucleus: DGK-alpha, DGK-gamma, DGK-delta, DGK-zeta**,** DGK-iota, and DGK-theta.

2. INTRODUCTION

Lipid second messengers have emerged as major signaling components. Among the various aspects of lipid metabolism involved in signaling, the generation and metabolism of two particular lipids, diacylglycerol (DAG) and phosphatidic acid, has begun to receive considerable attention (e.g. see (1)). It is becoming increasingly clear that the relative level of these two lipids, and the intracellular compartment in which they reside, play critical roles in specific signaling pathways. One mechanism that is particularly poised for coordinately regulating the levels of these lipids in specific sub-cellular compartments is via the agonist-induced modulation of the localization and/or activity of diacylglycerol kinases. These enzymes catalyze the transfer of the γ phosphate of ATP to the hydroxyl group of diacylglycerol (DAG) thereby generating phosphatidic acid. Importantly, these enzymes have appreciable basal activities which also implicate housekeeping roles for these enzymes. The question that emerges, therefore, pertains to how the signaling function of diacylglycerol kinases (DGKs) is distinguished from

their housekeeping roles. Modulation of compartmentalization of these enzymes to distinct intracellular membranes and/or enzyme activity in response to agonist stimulation would serve as excellent methods for separating the housekeeping from signaling roles. Indeed, there are numerous studies demonstrating the redistribution of various DGK isoforms in specific cells in response to specific agonists. Importantly, in addition to redistribution, recent studies have appeared that implicate agonist-induced modulation of intrinsic DGK activity.

In this review we focus primarily on the localization of DGKs to the nucleus, modulation of the intrinsic enzymatic activity, and potential physiological roles of these nuclear localized enzymes. A more extensive review of nuclear lipid metabolism, including DGKs, and the physiological roles of this metabolism can be found in some recent excellent reviews and publications (2-5).

3. NUCLEAR LOCALIZATION

There are a number of studies that demonstrate the ability of various agonists to induce the redistribution of DGKs to various sub-cellular compartments. One compartment that is starting to receive some attention is the nucleus. The notion that the nucleus may serve more than a passive role in signaling is not new. In the early 1970s, Prof. Francesco Antonio Manzoli at the University of Bologna demonstrated an interaction between nuclear lipids and DNA and proposed the hypothesis that these interactions had functional consequences. While these studies did not receive a great deal of attention, in the early 1980's Smith and Wells demonstrated the incorporation of radiolabel into specific nuclear phosphatidic acid (PA) and phosphoinositides, phosphatidylinositol 4-phosphate (PIP), and phosphatidylinositol 4,5-bisphosphate (PIP_2) in particular, when isolated rat liver nuclei were incubated in the presence of [gamma^{32}P]ATP (6,7). This observation generated some interest given the new popularity of the "PI Cycle" which was believed to specifically involve an agonist-induced hydrolysis of PIP_2 at the plasma membrane. These and other observations provided strong evidence for an independently regulated nuclear PI-PLC cycle. Over time, two additional major lines of evidence fuelled support for this notion. First, other agonists were identified that stimulated nuclear PI metabolism. Second, studies appeared showing an agonist-induced increase in nuclear DAG and PKC. These studies, and others, eventually led to the intriguing hypothesis that nuclear lipid metabolism played key signalling roles.

Further support for the notion of a physiologically important nuclear lipid metabolism was drawn from studies showing the agonist-specific stimulation of nuclear lipid metabolism. Divecha *et al.* showed that insulin-like growth factor-I (IGF-I), but not bombesin, induced a rapid decrease in the mass of nuclear polyphosphoinositides, increase in nuclear DAG, and nuclear translocation of protein kinase C (PKC) in Swiss 3T3 cells. Bombesin did, however, stimulate the canonical PI cycle in non-nuclear membranes. These data provided compelling evidence for the existence of agonist-regulated nuclear lipid metabolism that was distinct from the well-established PI cycle.

As indicated above, initial studies on nuclear lipid metabolism focused on the involvement of the phosphoinositide cycle as described above (2). Soon, other studies appeared that showed the nuclear DAGs could be derived from another phospholipid, phosphatidylcholine (5,8-14). While these DAGs could be generated during sphingomyelin production, the generally accepted mechanism for the production of nuclear DAGs from phosphatidylcholine involves the activation of a phospholipase D (PLD) activity followed by dephosphorylation of the resulting phosphatidic acid by a lipid phosphate phosphatase ((15) and see Figure 1. In support of this notion, investigators have found a nuclear PLD (8,16-20). These data provided evidence that in addition to a separate nuclear PI cycle, there is also there is also an agonist-induced regulation of phosphatidylcholine metabolism in the nucleus. It should be noted that the data above strongly suggest that the nuclear DAG from either source is generated at or within the nucleus, and not from extranuclear compartments but this has not been definitively determined.

Regardless of where the DAG is generated, or how it arrives at the nucleus, one common aspect of agonist-induced levels of DAG is that it is transient. The mechanism for this transient nature may be in part due to a down modulation of the pathway and/or the activity of the enzyme(s) responsible for generating the DAG, such as a PI-PLC or PLD. The other mechanism that is starting to receive some attention is via agonist-induced redistribution of the enzymes and/or modulation of the enzyme activities responsible for metabolizing the DAG. There are two enzymes that could potentially metabolize DAGs: diacylglycerol lipase (DGL) and DGK. DGL catalyzes the hydrolysis of a terminal fatty acid from DAG leading the production of a free fatty acid and 2-monoacylglycerol. While this enzyme has been implicated as a major DAG metabolizing enzyme in some cell types, such as smooth muscle cells (21-23), the evidence implicating DGKs in modulating signaling systems is becoming increasingly more compelling. To date, six of the ten known DGK isoforms have been identified to reside or translocate to the nucleus in response to an agonist depending on the cell type and agonist (see Figure 2). These include: DGK-alpha, DGK-gamma, DGK-delta, DGK-zeta**,** DGK-iota, and DGK-theta**.**

DGK-alpha is one of the most studied nuclear DGKs. This isoform has been found in the nuclei of rat thymocytes and peripheral T-lymphocytes, CTLL-2 cells, and CHO-K1 cells (24,25). The translocation of DGK-alpha has been observed to be agonist-dependent. In T-cells, IL-2 stimulates the translocation of DGK-alpha to the nuclear matrix while activation of the T-cell receptor leads to translocation of DGK-alpha to a perinuclear region (24,25). In CHO-K1 cells, treatment cells with arachidonic acid leads to an association of DGK-alpha with the plasma membrane and Golgi, while purinergic stimulation results in the translocation of DGK-alpha to the plasma membrane

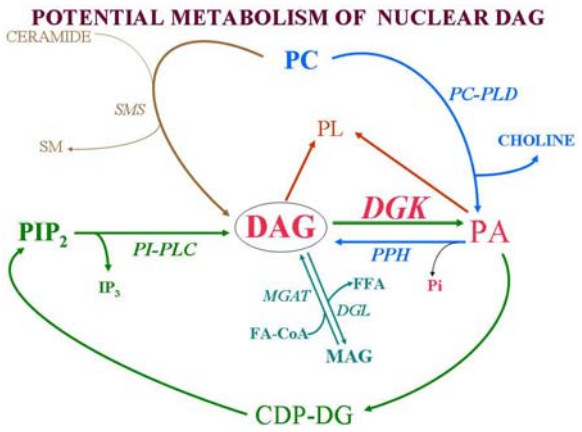

Figure 1. Pathways for DAG generation. See text for details.

only. It should be noted that DGK-alpha translocation to the nucleus is independent of increases in intracellular calcium (25).

While less is known about the nuclear localization of DGK-gamma than DGK-alpha, Matsubara and coworkers described the presence of DGK-gamma in the nucleus of CHO-K1 cells, as well as COS-7, NIH3T3, and SH-SY5Y cells (26). Based on observed changes in the behavior of several mutants of DGK-gamma, these investigators suggested that translocation is independent of catalytic activity, but found that the C1 domain is necessary for the translocation of the enzyme to the nucleus. There are no data supporting an agonist-induced translocation of DGK-gamma to the nucleus.

Very little is known about nuclear DGK-delta. Again, only one report, from our laboratory, has shown the localization of DGK-delta in the nuclei of IIC9 fibroblasts. This isoform is constitutively present at the nuclear envelope, and there are currently no indications that the distribution is altered by agonist (i.e. thrombin) stimulation.

DGK-zeta is a Type IV isoform whose nuclear localization has been rather extensively studied. Goto and Kondo provided the first indication that this enzyme may be trafficked to the nucleus when they discovered a nuclear localization sequence (NLS) within the myristoylated alanine-rich C-kinase substrate (MARKS) domain near the second cysteine-rich domain (27). Interestingly, this NLS is bipartite with an intervening sequence of ten amino acids. When this isoform was transfected into COS-7 cells, western blot analysis and immunohistochemistry provided confirmed that this enzyme was present in the nucleus (27). Confirmation that this localization may be physiological relevant was suggested by data showing the nuclear localization of endogenous DGK-zeta in neurons (28), alveolar epithelium and macrophages (29). Surprisingly, these studies also showed the mechanism of nuclear localization was cell-type dependent. For example, the ankyrin repeats in this isoform appear to be essential for nuclear localization in neurons but not COS-7 cells. Prescott and coworkers showed that phosphorylation of DGK-zeta within the MARCKS domain of the enzyme by PKC-alpha regulated its localization to the nucleus. It is important to note, however, that DGK-iota, another Type IV DGK, contains a similar NLS within a MARCKS domain, but thus far has not been observed to localize to the nucleus. This suggests the presence of other domains or auxiliary components that are involved in localizing DGK-zeta, but not DGK-iota**,** to the nucleus. In addition to an NLS, DGK-zeta contains a nuclear export sequence (NES) (27) that may be involved in regulating the nuclear localization of this enzyme (28,30).

DGK-iota is currently the only other known member of theType IV DGKs. However, in contrast to DGK-zeta, DGK-iota has not been shown to translocate to the nucleus in the same manner in spite of the existence of a putative NLS and additional similarities to the secondary structure of DGK-zeta. During the original identification of this isoform, Ding *et. al* reported that about 25-33% of the human isoform was localized in the nucleus when transfected into COS-7 cells (31). These data are in contrast, however, to those showing that none of the three known DGK-iota splice variants localize to the cytoplasm when overexpressed in hippocampal neurons (32) or C2C12 rat myoblasts (33). Further work will be required to determine whether this isoform is found in the nucleus of other cells and/or whether it translocates to the nucleus of any cell when challenged with an agonist.

DGK-theta is an interesting and unique isoform of the DGK family. This isoform is the only member of the Type V category and is distinguished by the fact that, (a) it contains three, instead of two, cysteine-rich domains, (b) it has a PH domain localized to the middle of the protein instead of near the N-terminus, (c) there is a proline rich domain near the N-terminus, and (d) it is the only isoform known to be inhibited by a small GTPase, RhoA. DGK-theta localizes to specific membranes following agonist stimulation (34-36). Interestingly, this isoform localizes to the nucleus of rat arterial smooth muscle and endothelial cells, and various cell lines such as MDA-MB-453, MCF-7, PC12, IIC9 fibroblasts, and HeLa cells (see (37)) and in some cases the nuclear localization is regulated (25,35).

3.1. Sub-nuclear localization of DGKs

Perhaps on of the most unexpected findings regarding the nuclear localization of DGKs is that, where it has been examined, the enzymes appear to be localized to the nuclear envelope or nuclear matrix. For example, DGK-^{alpha} translocates to the nuclear matrix of rat thymocytes and peripheral T-lymphocytes when these cells are stimulated with concanavalin A or anti-T-cell receptor antibody (25). DGK-theta also appears to localize to nuclear matrix of a number of cell lines, and there is intriguing evidence that DGK-zeta and DGK-theta localize to speckle domains (35,37-39). These domains are specialized regions of the nucleus that are enriched in small ribonucleoprotein particles and various splicing factors. Electron microscopy indicates that DGK-theta, PIP2 and PLCbeta1 associate with electron-dense particles containing inter-chromatin granules (ICG). Interestingly, these speckle domains can be labeled with an antibody

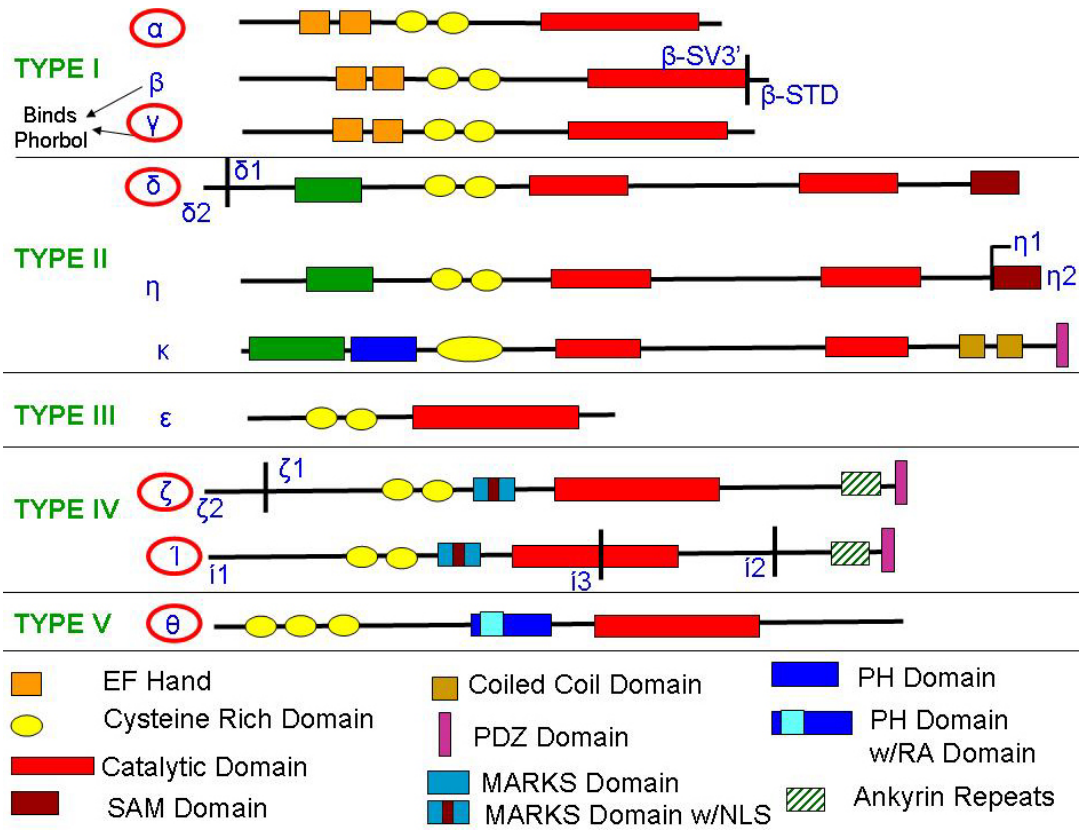

Figure 2. DGK isoforms. Splice Variants are indicated by vertical lines within the linear structure. Red Circles indicate those isoforms found to translocate or reside in nuclei. See text for details.

directed against the SC-35 splicing factor (40) suggesting these domains are regions involved in this process. A subsequent study by Hozumi and coworkers showed that endogenous DGK-zeta also localizes to speckle-like domains in neuronal cells using confocal microscopy, and that this pool of enzyme is resistant to removal by detergent, indicating that it is associated with the particulate fraction (28).

4. MODULATION OF ENZYMATIC ACTIVITY

Most of the data pertaining to the regulation of nuclear DGKs has focused on regulating the localization of the enzyme to this compartment. The intrinsic activity of other lipid signaling enzymes appears to be modulated in an agonist-dependent manner (e.g. see (41-45)) so it is quite possible that the enzymatic activities of DGKs are similarly modulated. One challenge to understanding the enzymatic regulation of DGKs within the nuclear matrix is that the physical form of the lipids within the nuclear matrix remains unknown. This information will be critical to understanding the essential enzymological parameters that may be involved in regulating the DGKs within the nucleus. There are, however, some data that provide clues as to how nuclear DGKs may be regulated.

For example, Jiang *et al* showed that removal of the EF hands from DGK-alpha results in a constitutively active enzyme. These data suggest that the N-terminus auto-inhibits the enzyme, and that calcium binding to the EF hands leads to a conformational change that relieves this inhibition (46). The lipid composition of the membrane may also affect activity. DGKs alpha, beta, gamma, zeta, iota, and theta are all strongly activated *in vitro* by anionic phospholipids (35,47-50). Interestingly, the product, phosphatidic acid has been shown to activate DGK-theta and its *Dictyostelium* homolog DGKA (44,51). In all cases, the increase in activity is due to an increase in *V*max. The mechanism(s) underlying this activation is unclear but has important consequences for the sub-cellular activation in that localization of the enzyme to sites where anionic lipids such as phosphatidylserine and phosphoinositides are concentrated may be critical for agonist-induced regulation of the enzyme.

Understanding the kinetic parameters of DGKs requires recognition of the nature of these enzymes. Except for DGK-epsilon (50,52), none of the DGKs are integral membrane proteins and, therefore, they likely have transient associations with the DAG substrate-containing membranes. When the catalytic event occurs at the hydrophobic/hydrophilic interface, as it is usually assumed, these enzymes are considered "interfacial enzymes". As a consequence, understanding the enzymology requires an understanding of the binding parameters that include both binding to the three-dimensional membrane and binding to

the substrate, which is constrained to the two-dimensional membrane surface.

A complete treatment of interfacial enzymology is beyond the scope of this review, but some kinetic parameters have been identified. In a recent study, we calculated a "composite" Km which included the binding of DGK-theta to a hydrophobic interface and substrate binding during the catalysis. We showed that the apparent "composite" KM of DGK-theta increases, i.e. is negatively affected, following thrombin stimulation in the presence of low bulk substrate concentrations. These data suggest that, in IIC9 fibroblasts, alpha-thrombin stimulation is an antagonist of DGK-theta. Interestingly, the composite KM is decreased, i.e. positively affected, as the bulk substrate concentration increases, indicating a thrombin-regulated change in membrane binding affinity that appears to be dependent on diacylglycerol concentration. Furthermore, we found phosphatidic acid to be a more efficient activator than phosphatidylserine. These data support a hypothesis to explain why the DAG levels are transient (44). According to this hypothesis, thrombin stimulation "slows" the enzyme to allow a build up of DAG suitable for its role as a second messenger. The continual increase in DAG concentration leads to an increase in the phosphatidic acid product which leads to an increase in DGK-theta activity. This increased activity accelerates the reduction of cellular DAG levels, returning them to normal levels. Further studies are in progress to test this hypothesis.

5. POTENTIAL PHYSIOLOGICAL ROLES

While the physiological roles of nuclear DGKs, and nuclear lipid metabolism, are not defined in great detail, there are compelling data supporting the notion that these enzymes play a crucial role in a number of processes. Numerous studies have shown an increase in nuclear DAGs that are required for the recruitment of PKC to the nucleus (5,12) and that the PKC and/or DGK is essential for cell cycle transitions. For example, nuclear DAG, PKC and DGK may be necessary for the transition from G1 into S phase of the cell cycle (see (5,53)) , while others have suggested it may be critical for the transition from G2 to M phase (54,55).

DGK-zeta is perhaps the best studied DGK in terms of determining a physiological role for its nuclear localization. Topham *et al* provided the first and some of the most compelling evidence in support of the notion that nuclear localization of DGK-zeta is important by demonstrating that expression of a constitutively nuclear localized DGK-zeta reduced the levels of nuclear DAG levels and attenuated the growth of A172 cells (56). Furthermore, when cells were transfected with either a kinase-dead DGK-zeta, or a DGK-zeta that was mutated to prevent its localization to the nucleus, the cells accumulated in the Go/G1 phase of the cell cycle (56). van Blitterswijk and co-workers have shown that hyperphosphorylation of pRB binds and activates DGK-zeta. They also showed that sequestration of E2F alone will not arrest cells suggesting that activation of DGK-zeta may be important (57,58).

The study by Matsubara *et. al* (26) suggested a role for nuclear DGK-^{gamma}. These investigators over-expressed this isoform which, over time, localized to the nucleus of COS-7, NIH3T3, and SH-SY5Y cells. Over-expression of a kinase-dead mutant in COS-7 lead to an increase in cell size, slower growth and extended S phase implicating this isoform, presumably at the nucleus, in regulation of the cell cycle. These data, however, must be reconciled with those from other data, e.g. nuclear DGK-zeta that indicates expression of an active DGK in the nucleus slows the cell cycle (see above), and that nuclear DAG levels increased coordinately with increases in DNA synthesis during liver regeneration *in vivo* (59). Importantly, there is *in vivo* evidence suggesting a role for nuclear DGK-zeta. In some interesting studies by Goto and co-workers, using a transient forebrain ischemic model, they showed that DGK-zeta is translocated from the nucleus to the perikaryal cytoplasm only in the CA1 area of pyramidal hippocampal neurons during the early phase of ischemia (30). Further, the DGK-zeta remains in the cytoplasm and the level decreases during reperfusion. This decline in nuclear DGK-zeta likely results in a decrease in nuclear DAG metabolism which would result in sustained nuclear DAG signaling. It is suggested that this may play be involved in modulating the DAG/PA-dependent induction of hypoxia-inducing factor 1alpha (HIF1alpha) (60,61).

Another DGK isoform that appears to play important physiological roles in the nucleus is DGK-theta. This isoform has been shown to localize to nuclei in an agonist-dependent manner in fibroblasts and PC12 neuronal cells (35,37,39). As indicated above, DGK-theta appears to localize to intranuclear particles containing ICG and splicing factors suggesting this isoform may be involved in transcriptional and pre-mRNA processing (35,37-39). Given its abundance in neuronal cells, and its localization to nuclei in these cells, it is tempting to speculate that this isoform will have physiological roles specific to neurons, but more research is needed.

The above data, combined with data showing that PKCbII phosphorylates nuclear lamin B and PKC-epsilon phosphorylates histone H1, supports the notion that nuclear DGKs may regulate these activities by modulating the level of DAG which activates PKC. It is also possible that the DGK product, phosphatidic acid, is an important nuclear messenger. This lipid has been shown to inhibit PP1 protein phosphatase which localizes to the nucleus and may be involved in regulating HIV-1 transcription (62,63). While it's not clear whether this phosphatidic acid is derived from a DGK or PLD, it does lend support to the notion that nuclear DGK may be a critical player in regulating the cell cycle and cytokinesis.

6. CONCLUDING REMARKS

Since the early work of Prof. Francesco Antonio Manzoli at the University of Bologna and the intriguing studies by Smith and Wells, interest in, and evidence for, a separate and independently regulated metabolism of nuclear lipids involved in signalling cascades has received

increasing attention. DGKs clearly will play an important role in these pathways, largely by regulating the relative levels of nuclear DAG and phosphatidic acid. The precise nature of their roles and mechanisms of regulation, however, remain elusive. It seems likely that these enzymes, by modulating substrate and product levels, will play a role in regulating the cell cycle and a number of nuclear processes. This regulation will probably involve the regulation of chromatin remodelling, alterations of the matrix or nuclear lamina, or modulation of gene transcription. There is obviously a need for more research which holds great promise for some very exciting discoveries.

7. ACKNOWLEDGEMENTS

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