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Review

Diacylglycerol kinases in nuclear lipid-dependent signal transduction pathways

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Abstract. Several independent groups have shown that lipid-dependent signal transduction systems operate in the nucleus and that they are regulated independently from their membrane and cytosolic counterparts. A sizable body of evidence suggests that nuclear lipid signaling controls critical biological functions such as cell proliferation and differentiation. Diacylglycerol is a fundamental lipid second messenger which is produced in the nucleus. The levels of nuclear diacylglycerol fluctuate during the cell cycle progression, suggesting that such a molecule has important regulatory roles. Most likely, nuclear diacylglycerol serves as a chemoattractant for some isoforms of protein kinase C that migrate to the nucleus in response to a variety of agonists. The nucleus also contains diacylglycerol kinases, i.e. the enzymes that, by converting diacylglycerol into phosphatidic acid, terminate diacylglycerol-dependent events. A number of diacylglycerol kinases encoded by separate genes are present in the mammalian genome. This review aims at highlighting the different isotypes of diacylglycerol kinases identified at the nuclear level as well as at discussing their potential function and regulation.

Key words. Diacylglycerol; phosphoinositides; phosphatidylcholine; phosphatidic acid; nucleus; phospholipase C; phospholipase D; signal transduction.

Introduction

There is now solid evidence that lipid-dependent signaling pathways operate within the nucleus ([reviewed in [1–5]). Diacylglycerol (DAG) is a key second messenger which is generated at the nuclear level along these pathways. Data coming from independent laboratories have shown that nuclear DAG can derive from either phosphoinositides or phosphatidylcholine (PC) [6, 7]. DAG kinases catalyze phosphorylation of DAG to yield phosphatidic acid (PA). DAG kinases are key modulators of levels of DAG and potential terminator of DAG-dependent events, such as the activation of some protein kinase C (PKC) isoforms and thereby the signaling pathways downstream of PKC. The DAG-dependent PKC isozymes include conventional PKC-α, -β_I, -β_{II} and -γ and novel PKC -δ, -ε, -η, -θ and -μ. In contrast, atypical PKC-ζ and -ι/-λ do not require DAG for their activation [8, 9]. Indeed, conventional and novel PKC isozymes are capable of binding DAG by means of two cysteine-rich zinc-finger regions within their C_1 domain. Atypical PKCs lack one of the two cysteine-rich zinc-finger regions and therefore do not bind (and cannot be activated by) DAG [9].

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Recent findings have indicated that DAG kinases are present in the nucleus where they may be involved in regulating the amount of intranuclear DAG. In some cases, the activity of nuclear DAG kinases has been demonstrated to be critical for the control of cell proliferation [10, 11]. This review concentrates on the subnuclear localization, functions and possible mechanisms of activation of the various types of DAG kinases that have been described in association with the nucleus.

DAG kinases

A plethora of extracellular stimuli (growth factors, hormones, neurotransmitters) cause a transient rise in the levels of intracellular DAG. The two main sources for DAG are phosphoinositides and PC [8]. Phosphoinositide-specific phospholipase C (PI-PLC) hydrolyzes phosphatidylinositol 4,5-bisphosphate [PtdIns(4,5)P_2] to yield DAG and inositol 1,4,5-trisphosphate [Ins(1,4,5)P_3]. Phospholipase D (PLD) catalyzes the hydrolysis of the terminal diester bond of glycerophospholipids (mostly PC), resulting in the formation of PA plus a related base. PA is subsequently converted into DAG by a PA-phosphohydrolase [8].

DAG is mostly known as a second messenger which allosterically activates some PKC isoforms that play key roles in several critical cell responses [9]. However, other molecular targets of DAG have been identified, such as α- and β-chimaerins, the guanine nucleotide exchange factor vav, and guanyl nucleotide-exchange factors for Ras and Rap [12, 13]. These findings indicate that DAG might be involved in controlling the Ras and Rho family of proteins. DAG derived from PI is polyunsaturated, whereas DAG produced through the PLD pathway is monounsaturated and saturated. It is believed by some investigators that only polyunsaturated DAG is the real second messenger which activates PKC isoforms, whereas monounsaturated and saturated DAG would have no signaling functions (reviewed in [8, 14]). Some results lend credit to such a hypothesis [16]. However, findings by other groups do not support such a theory, because there are reports of PC-derived DAG which activated PKC isoforms [17]. On the other hand, available evidence suggests that only PI-derived DAG, not PC-derived DAG, is converted into PA by DAG kinases [17]. PA is believed to elicit many biological responses by itself. For example, PA may play a role in cytoskeletal organization by inducing actin polymerization and stress fiber formation. It is also involved in the regulation of enzymes such as phosphatidylinositol 5-kinases, PAK1, PKC-ζ, and Ras [12, 14, 15, 17, 18]. Moreover, both DAG and PA are important intermediates in de novo biosynthesis of lipids [19].

The control of steady-state cellular levels of DAG is crucial to cellular physiology. DAG signaling must be short lived because persistently high levels of DAG induce malignant transformation. The transforming activity of DAG has been attributed most often to persistent activation of PKC isoforms that are clearly involved in tumorigenesis [e. g. 9]. DAG kinases metabolize DAG by converting it to PA. Since they can attenuate local accumulation of signaling DAG, DAG kinases play a pivotal role in many biological responses such as cell proliferation, differentiation, survival and apoptosis (see fig. 1).

The family of DAG kinases is well conserved amongst most species. Nine mammalian isoforms of DAG kinase, organized into five classes, have thus far been cloned and their complementary DNAs (cDNAs) characterized: class I comprises the α, β and γ isozymes; class II the δ and η; class III the ε isoform; class IV the ζ and ι; class V the θ [12, 17, 18]. All of the mammalian DAG kinases share a conserved catalytic domain in the COOH-terminal region and at least a pair of cysteine-rich motifs (DAG kinase-θ has three) similar to the C1A and C1B motifs of PKC but lacking certain consensus residues present in phorbol ester-binding proteins. It is possible that these cysteine-rich domains bind DAG and present it to the catalytic domain. Nevertheless, DAG kinase isotypes can be distinguished by the presence of additional domains that conceivably confer to each isozyme specific functions in biological processes, sensitivity to different regulatory mechanisms and a differential intracellular localization. Indeed, these motifs are likely to play a role in lipid-protein and protein-protein interactions in various signaling pathways. Class I DAG kinases contain a pair of EF hand-like domains (that bind Ca^{2+}) in their NH_2-terminal half. Class II isotypes have a pleckstrin homology-like domain at their NH_2-terminal portion, whereas class IV isozymes display COOH-terminal repeats and a region homologous to the phosphorylation site of the well-established PKC substrate 'myristoylated alanine-rich C-kinase substrate' (MARCKS) (see also below). Class V DAG kinase has a pleckstrin homology domain located in the middle of its sequence. This domain overlaps with a Ras-associating domain. Class III DAG kinase is the only isotype which has no domains with obvious regulatory functions. A thorough description of the molecular structure of various mammalian DAG kinase isotypes is beyond the scope of this article, and the reader is referred to excellent and comprehensive reviews on this issue for an update of our current knowledge about the structure of this family of signal transduction enzymes [12, 17, 18].

The specificity of DAG kinases has been a matter of debate. Most of the isotypes do not show significant selectivity in vitro towards DAG of different fatty acyl composition. Only DAG kinase-ε strongly prefers DAG species with an arachidonoyl at the sn-2 position both in vitro and in vivo [12, 16]. For this reason, it has been suggested that such an isotype might be the component of the PI cycle

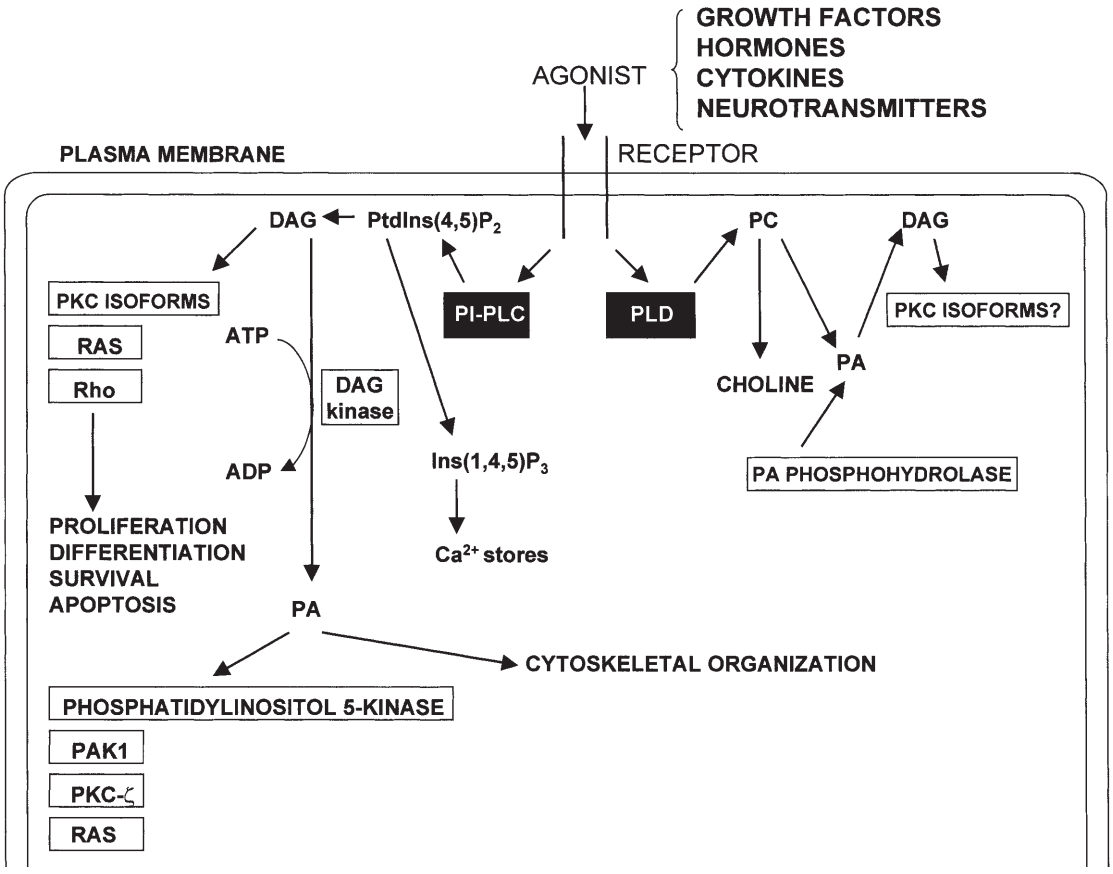

Figure 1. Schematic drawing outlining the pathways leading to the generation of DAG and PA on cell stimulation with agonists, as well as the potential targets of these two intracellular messengers.

which accounts for the enrichment of inositol lipids with arachidonate [12, 18]. Interestingly, DAG kinase-ε is inhibited by PtdIns(4,5)P_2 in vitro, thus providing a potential negative-feedback mechanism to control inositol lipid synthesis [12]. Nevertheless, DAG kinase-ε has a limited tissue distribution, and according to some authors, cannot fulfill this function broadly [18]. Specificity of DAG kinases may, however, be constrained by their subcellular location and compartmentalization or substrate accessibility [12].

Nuclear DAG kinase isoforms

In 1983, Smith and Wells [20] reported the presence of DAG kinase activity in nuclear envelopes prepared from rat liver cells. However, the first, indirect demonstration of the existence of DAG kinase activity in envelope-deprived nuclei came in 1987, when Cocco et al. [21] showed that demembranated nuclei of murine erythroleukemia (MEL) cells incorporated in vitro radiolabel from [γ-^{32}P] ATP into phosphatidylinositol 4-phosphate and into PtdIns(4,5)P_2. Nevertheless, PA also became phosphorylated, an indication that a nuclear DAG kinase was active. It took 5 years before a subsequent investigation showed that intranuclear DAG kinase activity was enriched in the internal nuclear matrix fraction prepared from NIH 3T3 cells [22]. It is worth recalling here that the nuclear matrix is a dynamic structure, mostly composed of nonhistone proteins, which has been proposed to serve as a sort of nuclear skeleton or scaffold [23–26]. Many of the enzymes related to nuclear lipid-dependent signaling pathways are found associated with the nuclear matrix, which conceivably plays a critical role in the regulation of these complex networks [27]. The presence of DAG kinase in demembranated rat liver cell nuclei was subsequently reported by Previati et al. [28]. These authors performed a very detailed investigation on the properties of nuclear DAG kinase when compared with the same activity associated with a microsomal fraction. They found that the nuclear enzyme, which represented 4.5% of total cell activity, was inhibited by detergents such as octylglucoside, CHAPS and Triton X-100, whereas deoxycholate had a stimulatory effect. While in isolated rat liver nuclei DAG kinase activity was enhanced by phospholipids such as PA,

phosphatidylethanolamine, phosphatidylserine (PS), PI and phosphatidylglycerol, in the microsomal fraction these lipids inhibited DAG kinase. Ceramide also stimulated the nuclear activity, whereas it had no effect on the microsomal fraction-associated enzyme. Nuclear, but not microsomal, DAG kinase activity was completely inhibited by the pharmacological inhibitor R59022. These results suggested that the nuclear enzyme was different from the microsomal one. It should be considered that R59022 only inhibits class I DAG kinase isotypes, while an in vitro sensitivity to octylglucoside and Triton X-100 is quite typical of the α isotype [12]. Therefore, we might speculate that the isoform present in membrane-deprived rat liver nuclei was DAG kinase-α.

However, in these very early reports there was no clear indication of the DAG kinase isozyme(s) present within the nucleus, because at that time our knowledge about the isotypes was quite limited. Due to the progessive identification of the different isoforms and the availability of specific antibodies, in the following years it became clear that several DAG kinase isoforms may be nucleus associated.

DAG kinase-α and -γ

DAG kinase-α was found in the nuclear matrix of rat thymocytes and peripheral T lymphocytes by means of immunostaining or immunochemical techniques [29]. It was demonstrated that in response to either concanavalin A or anti-T-cell receptor antibody, the α isozyme of DAG kinase translocated (slowly) to and associated with the nuclear matrix. A significant nuclear accumulation of PA was also detected. However, these results conflict with the findings reported by others [30] who showed that DAG kinase-α was intranuclear in resting human T lymphocytes and migrated to the perinuclear region in response to interleukin-2.

In CHO-K1 cells both DAG kinase-α and -γ localized to the nucleus. In response to arachidonic acid, the α (but not the γ) isozyme showed a dot-like accumulation within the nucleus [31]. It should be emphasized that in this investigation, DAG kinase-α was transiently overexpressed as a hybrid fused to green fluorescent protein (GFP). This labeling technique is considered far more reliable than fluorescent immunostaining because no artifacts due to fixation or cross-reaction of the antibodies are generated. Moreover, this paper was the first demonstrating that more than one DAG kinase isoform can localize in the nucleus of the same cell type.

Our group has reported the presence of a DAG kinase activity in the nuclei of Swiss 3T3 cells. Although we did not characterize the nuclear isoform(s) by means of specific antibodies, we found that the activity was strongly inhibited by the pharmacological inhibitor R59949 [32]. R59949 is a specific and powerful inhibitor of DAG kinase-α, while other tested isoforms are either not or poorly inhibited [33, 34]. Thus, we might assume that the activity we measured in the nucleus of Swiss 3T3 cells was due to DAG kinase-α.

DAG kinase-ζ and -ι

DAG also kinase-ζ localizes to the nucleus, as shown by immunofluorescence experiments in which it was overexpressed in COS-7 cells either as a FLAG epitope-tagged cDNA [35] or as a hybrid protein fused to GFP [36]. In A172 cells, a glioblastoma-derived line which constitutively expresses DAG kinase-ζ, 15–30% of total cellular DAG kinase-ζ was located in the nucleus [36].

DAG kinase-ζ possesses a bipartite nuclear targeting motif located close to the second zinc-finger-like sequence in its regulatory domain. As for other proteins that localize to the nucleus [37], the motif consists of a cluster of two adjacent basic amino acids separated by 10 amino acids from a second cluster, in which 3 of the next 5 amino acids are also basic. This motif of DAG kinase-ζ is similar to the phosphorylation-site domain of the MARCKS protein [38]. In COS-7 cells overexpressing a cDNA lacking the sequence of DAG kinase-ζ homologous to the phosphorylation-site domain of MARCKS, the enzyme was almost entirely excluded from the nucleus. This motif contains a serine residue (Ser 265) which is phosphorylated by PKC-α and -γ. In A172 cells overexpressing these PKC isozymes, the amount of DAG kinase-ζ present in the nucleus was reduced, whereas treatment with phorbol esters (that downregulate the amount of the two PKC isoforms) resulted in enhanced intranuclear localization of DAG kinase-ζ as well as an increase in nuclear DAG kinase activity. Localization of DAG kinase-ζ in the nucleus was thus shown to be dynamically regulated by phosphorylation of its MARCKS homology domain by some PKC isoforms, even though it is not clear whether or not these phosphorylative events took place in the nucleus or in the cytoplasm [37]. The same group reported similar results regarding the other member of class IV, i.e. DAG kinase-ι [39].

DAG kinase-θ and -δ

DAG kinase-θ is another isozyme which has been found in the nucleus of several cell types, including rat arterial smooth muscle and endothelial cells, human MelJuso melanoma cells, COS-7 cells and IIC9 cells [12, 13, 40]. Quiescent IIC9 fibroblasts also express DAG kinase-δ in the nucleus, but only the -θ was activated in response to α-thrombin (see later) [13].

In table 1 we summarize the DAG kinase isotypes identified so far at the nuclear level.

Table 1. DAG kinase isoforms present in the nucleus.

Isoform	Tissue or cell line	References
n.d.	NIH 3T3 cells	22
n.d. (α?)	rat liver	28
α	rat thymocytes, rat T lymphocytes, human T cell line CTLL-2, CHO-K1 cells	29, 30, 31
n.d. (α?)	Swiss 3T3 cells	32
γ	CHO-K1 cells	31
δ	IIC9 cells	13
ζ	COS-7 cells, A172 cells, Hela cells	35, 36, 65
ι	COS-7 cells	39
θ	rat arterial smooth muscle and endothelial cells, human MelJuso melanoma cells, COS-7 cells, IIC9 cells	12, 13, 40

n.d., not determined.

Subnuclear localization of DAG kinases

As illustrated above, early reports showed the association of DAG kinase activity with the nuclear matrix. However, recent results obtained by immounostaining [12, 13, 31] have indicated that both DAG kinase-α and -θ are concentrated in dots resembling the speckle domains of the nucleus. These domains are structures containing elements of both the transcriptional and pre-messenger RNA (mRNA) processing machinery, including RNA polymerase II and the splicing factor SC-35 [41–43]. However, it should be emphasized that so far nobody has conclusively demonstrated that the dots containing DAG kinase isoforms are indeed speckles. This would require, for example, double immunostaining with antibodies to a DAG kinase and a well-established marker of the speckles, such as SC-35. In fact, the nucleus contains several other types of domains, collectively called nuclear bodies [41, 44]. In any case, should these structures indeed prove to be speckles, the association will appear extremely interesting, because recent results have shown that several elements of the PI cycle are present within these nuclear domains. These elements include the α and β isoforms of type II phosphatidylinositol kinase [45], PtdIns(4,5)P_2 [45, 46] and phosphoinositide 3-kinase [47]. Moreover, we would like to recall that nuclear speckles correspond, at the ultrastructural level, to interchromatin granule clusters [48–50]. Interestingly, PI-PLCβ1, the enzyme which hydrolyzes PtdIns(4,5)P_2, yielding DAG and Ins(1,4,5)P_3, was detected by immunoelectron microscopy in the interchromatin granules [e.g. 51]. A number of protein kinases, such as SRPK1, CLK/STY and casein kinase Iα, are also associated with the speckles where they phosphorylate non-snRNP (non-small nuclear ribonucleoprotein) splicing factors [52–54]. Furthermore, protein phosphatases are detected in the nuclear speckles [55]. The functions of the PI cycle elements present in the speckle domains remain to be elucidated, even though recent evidence points to the likelihood that they may be involved in some steps of pre-mRNA processing [46].

Functions of nuclear DAG kinases

The evidence so far collected indicates that at least in some cases, nuclear DAG kinases regulate the levels of DAG in the nucleus. There are numerous examples of treatment with agonists that cause a rise in the intranuclear DAG mass (reviewed in [1–5]). The function of nuclear DAG seems to be the attraction and/or the activation of DAG-dependent PKC isoforms such as βII and α [56–60].

In A172 cells exposed to epidermal growth factor (EGF), it was shown that nuclear DAG mass rose two- to threefold above the baseline, whereas total cell levels were unchanged [36]. As a step towards the clarification of the function of DAG kinase in the nucleus, the group of Prescott [36] compared nuclear DAG levels in control A172 cells with levels in cells with a high amount of nuclear DAG kinase-ζ (i.e. those incubated with phorbol esters, see above). When A172 cells were exposed to EGF for 10 min, nuclear DAG mass in cells not pretreated with phorbol esters increased about 2.5-fold above the basal levels, whereas nuclear DAG levels in cells pretreated with phorbol esters increased only 1.3-fold. Furthermore, in cells overexpressing an inducible DAG kinase-ζ, the doubling time increased about twofold over controls. In addition, cells transfected with cDNAs encoding either a kinase-dead mutant or a mutant that did not localize to the nucleus accumulated in the G_0/G_1 phase of the cell cycle. Thus, these findings convincingly demonstrated that DAG kinase-ζ is important for the regulation of cell proliferation through the control of nuclear DAG mass, and both its enzymatic activity and localization in the nucleus were found to be essential to this end. These data appeared very intriguing, but they were mainly obtained with transfected cells overexpressing DAG kinase-ζ. Therefore, we set out to confirm them by analyzing endogenous DAG kinase activity after stimulating quiescent Swiss 3T3 cells with insulin-like growth factor-I (IGF-I). Indeed, this is a classic experimental model in which there is a rise in nuclear (but not whole cell) DAG mass [56, 59]. Exposure to IGF-I resulted in the stimulation of nuclear DAG kinase activity, but not of the same activity present in whole-cell homogenate. An inverse relationship between nuclear DAG mass and DAG kinase activity levels was shown by time-course analysis. If 3T3 cells

were preincubated with two DAG kinase pharmacological inhibitors, R59022 and R59949, the IGF-I-dependent rise in nuclear DAG kinase activity was blocked, and intranuclear levels of DAG remained elevated for a longer period than in control cells. Also, nuclear PKC-α activity stayed higher in cells treated with the DAG kinase inhibitors than in untreated cells. Furthermore, the two pharmacological inhibitors markedly potentiated the mitogenic effect of IGF-I. Thus, our findings have confirmed that nuclear DAG kinase plays a key role in regulating the levels of DAG present in the nucleus and that DAG is a key molecule for the mitogenic effect which IGF-I exerts on Swiss 3T3 cells. Moreover, we have shown that the effect of IGF-I on cell proliferation is conceivably mediated by nuclear PKC-α [32].

Stimulation of nuclear DAG kinase activity has recently been reported in α-thrombin-stimulated IIC9 cells by the group of Raben [13]. In this system, previous results have indicated that α-thrombin produces an increase in nuclear DAG. α-thrombin activates a nuclear PLD, which, however, is responsible for an increase in PA, and not DAG, levels. The authors hypothesized that nuclear DAG was generated by the enigmatic enzyme PC-PLC [61]. Since immunoblotting analysis showed that nuclei prepared from IIC9 cells contained two DAG kinase isoforms, δ and θ, experiments were carried out to determine which isoform was modulated by α-thrombin. While high concentrations of PS (an established inhibitor of DAG kinase-δ) inhibited the basal nuclear activity, they had no effect on the stimulated nuclear activity. Moreover, constitutively active RhoA (which is known to inhibit DAG kinase-θ, see [12]) inhibited the α-thrombin-evoked rise in nuclear DAG kinase activity. This activity was also sensitive to a monoclonal antibody directed to a sequence (amino acids 677–683) present in the catalytic domain of DAG kinase-θ. Immunofluorescence analysis with the same monoclonal antibody revealed migration of DAG kinase-θ to the nucleus in response to α-thrombin treatment. Overall, these results strongly indicated that DAG kinase-θ was responsible for the increased nuclear DAG kinase activity which followed stimulation of quiescent IIC9 cells with α-thrombin. The function of DAG kinase-θ in IIC9 cell nuclei would be to increase PA production early (3–5 min) after challenge with α-thrombin. Afterwards (from 10 min to 1 h of stimulation), activated PLD would ensure PA production. RhoA, which once translocated in the nucleus, inhibits DAG kinase-θ, activates PLD and thus acts as a switch from one type of PA production to another [13]. The reason for this switch is unclear, even though we may imagine that depending on the source, PA is generated in different subnuclear domains and/or possesses distinct molecular species of fatty acids. A limitation of such an interpretation is that DAG generated in IIC9 cell nuclei in response to α-thrombin derives from PC [62]. Thus if DAG kinase-θ utilizes such a PC-derived DAG to generate PA, that would be an exception to the rule that PC-originated DAG is not a substrate for DAG kinases. However, given that the nucleus possesses signal transduction pathways that are somehow different from those operating at the cell periphery [63], it would not be totally surprising if a PC-derived DAG were a substrate for a nuclear DAG kinase.

The function of PA (if any) in the nucleus is completely unknown. Functional studies carried out in vivo are necessary to address such an issue, and the model represented by IIC9 cells stimulated by α-thrombin may be extremely useful in this sense. Certainly, we need to know more about nuclear PA, also taking into account that its synthesis has been reported to be in relationship with the cell cycle in MEL (murine erythroleukemia) cells [6]. Since the nuclear matrix is considered by some investigators to be the equivalent of the cytoskeleton, it might be that nuclear PA is involved in regulating some aspects of the structure of this nuclear scaffold.

Regulation of nuclear DAG kinase activity

The activity of DAG kinases must be tightly regulated to allow DAG and PA to perform their proper functions. However, our knowledge about the mechanisms of activation of the various DAG kinase isotypes present in the cytoplasm is quite limited. Since the isotypes possess different domains in their primary structure, the mechanism of activation is also likely to differ among the isozymes. Membrane association (for example of DAG kinase-θ) is necessary but not sufficient for the activation [40]. Most likely there must be other activation steps, such as phophorylation and/or interaction with small GTP-binding proteins [12]. In this connection, both DAG kinase-α and -θ can be phosphorylated by different PKC isoforms, even though, at present, it seems that phosphorylation by PKC does not affect DAG kinase activity [12]. Moreover, DAG kinase-α can be phosphorylated on tyrosine in vivo, e. g. after stimulation of the EGF receptor [34]. As far as interactions with small GTP-binding proteins are concerned, DAG kinase-θ specifically does so with the active form of RhoA, while the ζ isotype interacts with Rac-1 [12]. When RhoA binds DAG kinase-θ, the kinase activity is blocked, most likely because the interaction leads to masking of the catalytic domain of the θ isotype.

Moreover, unsaturated fatty acids inhibit growth factor-induced DAG kinase-α, but have no effect on basal activity [64]. Thus it seems that we know more about the possible mechanisms inactivating DAG kinases than about those stimulating their activity. Compartmentalization could also play a role in the regulation of DAG kinases. Indeed, it is known that DAG kinase utilizes only DAG generated by receptor stimulation but not DAG randomly

produced at the plasma membrane by treating cells with an exogenous PLC [12].

How does this limited knowledge about the regulation of cytoplasmic DAG kinases fit into the issue of nuclear DAG kinase isotypes? We know that phosphorylation by PKC of DAG kinase-ζ blocks its nuclear localization, but conceivably it has nothing to do with its activity. However, phosphorylation of DAG kinase-ζ is not the only mechanism which controls its nuclear localization, because very recently it has been shown that an interaction with γ1-syntrophin also plays a role in regulating the subcellular localization of this isotype [65]. The interaction takes place between the COOH-terminal portion of DAG kinase-ζ and the PDZ (postsynaptic density protein-95/discs large/zona occludens-1) domain of γ1-syntrophin. Proteins containing PDZ domains are emerging as key players in targeting of membrane proteins and spatial control of intracellular signaling. In cells, the two proteins form a stable complex which can translocate to the nucleus. Disruption of the interaction between DAG kinase-ζ and the PDZ domain of γ1-syntrophin altered the intracellular localization of both proteins: DAG kinase-ζ accumulated in the nucleus, whereas γ1-syntrophin remained in the cytoplasm. These intriguing new findings also explain an earlier observation [35] showing that the COOH-terminal region of DAG kinase-ζ influenced the nuclear localization of the enzyme.

As discussed above, nuclear DAG-θ can be blocked by active RhoA, while the δ isotype is sensitive to PS. Thus their regulation may be similar to what happens in the cytosol. The nucleus also contains phospholipase A2 ([reviewed in [2]), which could release fatty acids to influence DAG kinase activity.

Compartmentalization is conceivably of fundamental importance at the nuclear level to ensure correct termination of DAG signaling, given that in intact nuclei exogenously added DAG kinase phosphorylates only the detergent-resistant, nuclear matrix-associated DAG species derived from PI hydrolysis but not DAG from PC (which constitutes the bulk of nuclear DAG and is located in the nuclear envelope). However, disruption of nuclei leads to phosphorylation of all DAG species [6, 66]. Therefore, we may speculate that in the nucleus inactive DAG kinase occurs in a complex with PI-PLC (located in the nuclear speckle domains?), and upon cell stimulation with an agonist, DAG produced through inositol lipid hydrolysis is converted to PA by an activated DAG kinase. However, since these above-outlined data were obtained with an exogenous enzyme, we do not know whether or not they reflect what happens in vivo, and further studies are required to address this possibility.

Concluding remarks and future perspectives

Even though we are only in the very beginning of investigating nuclear DAG kinases, we have collected valuable information about the isotypes present in the nucleus. For at least one isoform we know in detail the mechanisms that regulate its nuclear localization. We also have solid evidence that in some experimental models nuclear DAG kinases control the amount of DAG mass within the nucleus, which has marked consequences on the cell proliferation rates. In contrast, we know very little about regulation of activity of DAG kinases operating in the nuclear environment. However, we have now many tools to considerably further our understanding of this family of enzymes. Every effort should be made to identify the sequences that control nuclear localization of the other isotypes. The use of kinase-deficient mutants and highly specific antibodies should allow us to study the function and localization of individual isozymes in more detail. Very recent results have shown that two splicing variants of DAG kinase-β exist, and future findings might show that this also applies to other isotypes [68]. It might be that some of these splicing variants will be found to be mainly nuclear, as it happens for the b form of PI-PLCβ1 [e.g. 69, 70].

Forced overexpression of isotypes with deletions in their putative regulatory domains should provide important data about the mechanisms controlling the activity of nuclear DAG kinases. If some DAG kinases are indeed localized in the speckle domains of the nucleus, a further critical step will be to identify the interacting partners important for activation and inhibition. In this connection, a very useful technique is the yeast two-hybrid system, which has already allowed the unexpected identification of a protein (PIKE) fundamental for the regulation of phosphoinositide 3-kinase within the nucleus [67]. The presence of more than one isotype in the nucleus of the same cell suggests that DAG kinases operate in distinct signal transduction pathways that need to be identified. Another fundamental issue will be clarifying the role(s) of nuclear PA.

Our understanding at the molecular level of nuclear lipid-dependent signaling networks is at present evolving quite rapidly. A better knowledge of nuclear DAG kinase isozymes appears desirable, mostly because if it happens that they are indeed activated in a distinct manner from their cytosolic counterparts and regulated in a way peculiar to the nuclear compartment, rationale drug design should be able to selectively inhibit the relevant nuclear isotypes while sparing those operating at theplasma membrane.

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