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Nuclear Lipids: New Functions for Old Molecules?

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Abstract It is becoming increasingly evident that stimulation of nuclear lipid metabolism plays a central role in many signal transduction pathways that ultimately result in various cell responses including proliferation and differentiation. Nuclear lipid metabolism seems to be at least as complex as that existing at the plasma membrane. However, a distinctive feature of nuclear lipid biochemical pathways is their operational independence from their cell periphery counterparts. Although initially it was thought that nuclear lipids would serve as a source for second messengers, recent evidence points to the likelihood that lipids present in the nucleus also fulfil other roles. The aim of this review is to highlight the most intriguing advances made in the field over the last year, such as the production of new probes for the in situ mapping of nuclear phosphoinositides, the identification of two sources for nuclear diacylglycerol production, the emerging details about the peculiar regulation of nuclear phosphoinositide synthesizing enzymes, and the distinct possibility that nuclear lipids are involved in processes such as chromatin organization and pre-mRNA splicing. J. Cell. Biochem. 88: 455–461, 2003.

Key words: diacylglycerol; protein kinase C; phospholipase C; phospholipase D; phosphoinositide 3-kinase

In response to a plethora of stimuli bioactive lipid metabolites are generated in a receptor-mediated manner at the plasma membrane. These lipid molecules activate signaling pathways that ultimately may elicit nuclear responses [Divecha and Irvine, 1995]. However, over the last decade abundant evidence has accumulated highlighting that the nucleus is the site for an active autonomous lipid metabolism, which is regulated independently from that of the plasma membrane [D'Santos et al., 2000; Cocco et al., 2001a; Martelli et al., 2001]. Indeed, many agonists that stimulate the membrane metabolism do not activate the one present in the nucleus and vice versa. In other cases, if an agonist stimulates both, it does so in a temporally distinct manner. Since there are lipids that resist washing of nuclei with detergents, it should be assumed that these molecules are not components of the nuclear envelope, but are actually within the nucleus, most likely not in a bilayer membrane but

Abbreviations used: DAG, diacylglycerol; DMSO, dimethylsulfoxide; GFP, green fluorescent protein; GST, glutathione-S-transferase; IGF-I, insulin-like growth factor-I; LTA, large T antigen; NGF, nerve growth factor; PA, phosphatidic acid; PC, phosphatidylcholine; PH, pleckstrin homology; PKC, protein kinase C; PI, phosphoinositide; PI-PLC, PI-specific phospholipase C; PI3K, phosphoinositide 3-kinase; PLD, phospholipase D; PIP kinase, phosphatidylinositol phosphate kinase; pRB, retinoblastoma susceptibility gene product; PtdIns(3,4)P_2, phosphatidylinositol (3,4)-bisphosphate; PtdIns(4,5)P_2, phosphatidylinositol (4,5)-bisphosphate; PtdIns(3,4,5)P_3, phosphatidylinositol (3,4,5)-trisphosphate.

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instead forming proteolipid complexes with proteins that remain to be identified [Divecha et al., 2000]. Indeed, several nuclear proteins possess phosphoinositide (PI)-binding sequences [Cocco et al., 2001a]. A wealth of results supports the hypothesis that nuclear lipids generate second messengers [Divecha et al., 2000; Martelli et al., 2001]. Nevertheless, recent experimental evidence indicates that nuclear lipids might be involved in other functions ranging from chromatin remodelling to pre-mRNA splicing [Osborne et al., 2001; Rando et al., 2002]. This article discusses the most new findings about the localization of nuclear lipids, the source of lipid second messengers in the nucleus, the regulation of lipid-synthesizing enzymes within this highly peculiar organelle, and new possible roles played by nuclear lipid molecules.

NEW PROBES FOR IN SITU VISUALIZATION OF NUCLEAR LIPIDS

Although the presence of lipids in isolated nuclei has been reported since the 1960 [reviewed in Cocco et al., 2001b], these findings were generally met with scepticism and disbelief and dismissed as being the result of cytoplasmic contamination [Divecha et al., 2000; Cocco et al., 2001a]. This was rightly so, because any isolated intracellular organelle is always likely to suffer from different degree of contamination with other organelles. However, the availability of complementary DNAs and antibody probes to the enzymes that modify these lipids allowed for the conclusion that they are unequivocally localized in the nucleus using techniques that do not require organelle isolation, such as immunofluorescent staining. This put the issue of contamination to rest, at least as far as enzymes were concerned, and opened up new investigation into putative nuclear targets [Divecha et al., 2000; Cocco et al., 2001a]. Nevertheless, the contention was still open surrounding the exact localization of nuclear lipids. Very recently, however, antibodies have been generated that are specific for PIs and suitable for immunocytochemical staining [e.g., Prestwich et al., 2002]. One of these antibodies allowed to map phosphatidylinositol (4,5)-bisphosphate (PtdIns(4,5)P_2) to the speckle domains of the nucleus that contain components of both the transcriptional and pre-mRNA processing machinery, including RNA polymerase II and the splicing factor SC-35 [Osborne et al., 2001]. Furthermore, the use of the same antibody revealed the presence of PtdIns(4,5)P_2 in the nucleoplasm. Treatment with RNase, but not with DNase, substantially reduced immunostaining, suggesting the existence of an interaction between PtdIns(4,5)P_2 and RNA. Immunoprecipitation experiments showed an association between PtdIns(4,5)P_2 and the hyperphosphorylated form of RNA polymerase II as well as with Sm proteins, but not with hnRNP A1.

Another elegant approach which was recently employed to localize the subcellular distribution of PtdIns(4,5)P_2 entailed the use of the specific lipid-binding domains known as pleckstrin homology (PH). These domains characterize several proteins that interact with PIs [Lemmon and Ferguson, 2000]. The PH domain of PI-specific phospholipase C (PI-PLC) δ_1 was fused to glutathione-S-transferase (GST) in an on-section electron microscopy labeling approach to avoid transfection procedures [Watt et al., 2002]. These authors reported that the PI-PLC δ_1PH-GST probe, besides labeling the plasma membrane and the intracellular membranes, was also detected in electron-dense structures within the nucleus. Quantitative analysis demonstrated that nuclear labeling was 17–21% of total. This new technique offers some advantages over the method, which requires the use PH domains tagged with green fluorescent protein (GFP) in combination with confocal microscopy. The PH-GFP method has a limited resolution (200 nm) and, more importantly, uses the overexpression of PH domains in the cytosol, which may have deleterious effects on cell function either by sequestering PIs or by promoting functionally important protein–protein interactions. However, the exploitation of GFP-tagged probes allows for their detection in living, unfixed cells [Balla et al., 2000].

THERE ARE TWO SOURCES FOR NUCLEAR DIACYLGLYCEROL (DAG)

The best documented lipid second messenger in the nucleus is DAG, which can derive from either PIs or phosphatidylcholine (PC) [Divecha et al., 2000; Neri et al., 2002a]. In case of PIs, a PI-PLC hydrolyzes PtdIns(4,5)P_2 to yield DAG and inositol 1,4,5-trisphosphate. When DAG derives from PC, there is first the activation of a phospholipase D (PLD) which produces

phosphatidic acid (PA) and choline from PC. Then, a PA phosphohydrolase synthesizes DAG (Fig. 1). What is the role of this nuclear DAG? Several papers have related the generation of nuclear DAG with the attraction to the nucleus of DAG-dependent protein kinase C (PKC) isoforms. This has been demonstrated in IIC9 cells stimulated with α-thrombin [Jarpe et al., 1994], in Swiss 3T3 cells treated with insulin-like growth factor-I (IGF-I) [Neri et al., 1998] and during the G_2/M phase of the cell cycle of HL-60 [Sun et al., 1997] or U-937 human leukemia cells [Deacon et al., 2002]. In these experimental models, the source of nuclear DAG could be either PIs or PC. The existence of two separate pools of nuclear DAG suggested that this lipid second messenger might be involved in distinct pathways that lead to different cell responses. However, a conclusive demonstration that in the same cell line different stimuli activate distinct phospholipases present in the nucleus and that this differential activation was responsible for attracting to the organelle specific, DAG-dependent PKC isoforms, was lacking. We very recently provided evidence that, in the HL-60 cells, a rise in PI-derived DAG levels occurs in response to a proliferating stimulus represented by IGF-I which activates a nuclear PI-PLCβ1. This increase in DAG mass is responsible for PKC-β_{II} translocation to the nucleus [Neri et al., 2002b]. It is still unclear how nuclear PI-PLCβ1 is activated in response to IGF-I stimulation of HL-60 cells (Fig. 1). However, in Swiss 3T3 cells treated with IGF-I, PI-PLCβ1 is phosphorylated and activated by p42/44 MAP kinase which translocates from cytoplasm to nucleus [Xu et al., 2001]. In contrast, in response to

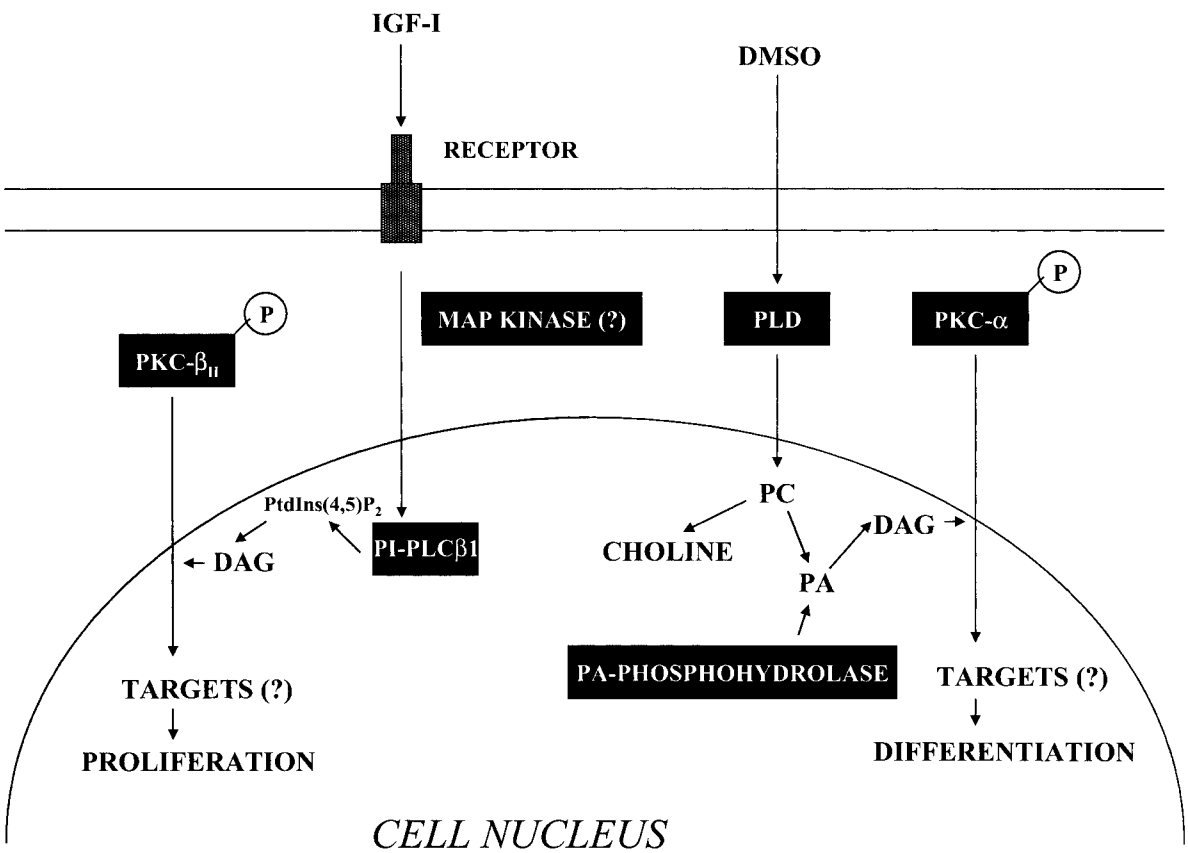

Fig. 1. In HL-60 leukemia cells treatment with IGF-I leads to activation of a nuclear PI-PLCβ1. It is still unclear how this activation is achieved, but it might be through phosphorylation by p42/44 MAP kinase, in analogy with Swiss 3T3 cells. Nuclear PI-PLCβ1 hydrolyzes PtdIns(4,5)P_2 yielding DAG. This DAG attracts to the nucleus phosphorylated PKC-β_{II} isozyme which conceivably phosphorylates substrates that are important for cell proliferation. If HL-60 cells are exposed to DMSO, a 90 kDa PLD traslocates to the nucleus to generate PA from PC. PA is then converted to DAG by a PA phosphohydrolase. PC-derived DAG is responsible for nuclear translocation of phosphorylated PKC-α which probably targets proteins critical for differentiation along the granulocytic lineage.

dimethylsulfoxide (DMSO, i.e., a stimulus towards granulocytic differentiation), we observed in HL-60 cells a rise in nuclear DAG levels derived from PC and a translocation of PKC-α to the nucleus. How is this DAG generated? We found that DMSO causes migration to the nucleus of a 90 kDa PLD distinct from PLD1 or PLD2 (Fig. 1). The presence of this type of PLD has also been reported in granulocytes [Horn et al., 2001]. The mechanisms through which PLD translocation to the nucleus takes place are at presently completely unknown. There are other experimental models, however, in which DMSO was demonstrated to be able to activate a PLD activity [see Neri et al., 2002b, and references therein]. Therefore, we can now postulate the existence in the cell nucleus of two independently regulated DAG sources, related to distinct stimuli and capable of recruiting to the nucleus different PKC isozymes. DAG derived from PIs is polyunsaturated, whereas DAG produced through the PLD pathway is mono-unsaturated and saturated [Neri et al., 2002a]. At present, great controversy surrounds the issue of whether or not both forms of DAG can activate PKC isoforms [Deacon et al., 2002]. Our results seem to indicate that it is the different fatty acid composition of DAG the factor which drives to the nucleus distinct PKC isoforms, even though further experiments are necessary to conclusively address this issue. In any case, our findings are in complete agreement with those of others who also showed nuclear translocation of PKC-β_{II} isoform to be dependent on PI-derived DAG [Sun et al., 1997; Deacon et al., 2002]. An unresolved issue is how PKC isoforms located in the cytoplasm "sense" DAG generated in the nucleus. It was proposed that nuclear DAG could "trap" PKC during a transient visit to the nucleus [Divecha et al., 1991]. However, this would entail a rapid and continuous cycling of PKC in and out of the nucleus, and therefore seems unlikely because a massive migration of PKC isozymes to the nuclear compartment usually takes place in a short time after stimulation [e.g., Neri et al., 1998]. Since targeting of PKC isozymes to distinct sites of action is crucial for their role, it appears very interesting that nuclear DAG derived from different lipid precursors is different with respect to not only its structure but also its intranuclear localization [D'Santos et al., 1999; Jones et al., 2002]. This may well represent a mechanism for the distinct spatial and temporal activation of specific PKC isoforms within the nucleus. In this connection, however, it should be emphasized that the interactions between lipid second messengers and PKC cannot fully explain the fine regulation of this peculiar protein kinase in the nuclear compartment. Because there usually are multiple PKC isozymes within a cell, the differential subcellular localization has been proposed to explain the specificity of different isoforms. The localization appears to be mediated in part by association of each PKC isozyme with specific anchoring proteins, referred to as PKC-binding proteins [Mochly-Rosen and Gordon, 1998; Jaken and Parker, 2000]. The identification and characterization of nuclear PKC-binding proteins has only recently begun [Martelli et al., 2002; Rosenberger et al., 2002; Tabellini et al., 2002], but useful information will hopefully be collected in a short amount of time.

THE REGULATION OF NUCLEAR PHOSPHOINOSITIDE SYNTHESIZING ENZYMES

Phosphoinositide 3-kinases (PI3Ks) are a family of enzymes, subdivided in three classes (I, II, and III), that synthesize 3-phosphorylated phosphoinositides, such as phosphatidylinositol (3,4,5)-trisphosphate (PtdIns(3,4,5)P_3) [Vanhaesebroeck and Waterfield, 1999; Vanhaesebroeck et al., 2001]. These peculiar inositides are not substrate for any known phospholipases but act as second messengers by themselves, impinging on a plethora of cellular functions [Vanhaesebroeck et al., 2001]. Increases in nuclear class IA PI3K protein and activity have been reported in, for example, nerve growth factor (NGF)-stimulated PC12 cells [Neri et al., 1999]. Nuclear PtdIns(3,4,5)P_3 seems to be the driving force that attracts to the nucleus PKC-ζ, in response to NGF stimulation of PC12 cells [Neri et al., 1999; White et al., 2002] or C2-ceramide treatment of rat hepatocytes [Calcerrada et al., 2002]. Activation of nuclear class IA PI3K is controlled by PIKE, a nuclear GTPase that enhances PI3K activity [Ye et al., 2000]. This activation by PIKE requires the p85 regulatory subunit of PI3K, and is thus different from the Ras-activation of PI3K that occurs at the plasma membrane through the p110 catalytic subunit of PI3K [Vanhaesebroeck et al., 2001]. Intriguing data have emerged very recently, demonstrating that

the SH3 domain of nuclear PI-PLCγ1 acts as a guanine nucleotide exchange factor (GEF) of PIKE [Ye et al., 2002]. These findings account for the earlier surprising observation that the catalytic domain of PI-PLCγ1 is not required for a mitogenic activity, whereas the SH3 domain is essential [Bae et al., 1998]. Thus, the regulation of nuclear PI3K remains an exciting and entirely new area for exploration.

Another nuclear enzyme involved in phosphoinositide synthesis that appears to have a peculiar regulation is type I phosphatidylinositol phosphate kinase (PIP kinase). This enzyme phosphorylates phosphatidylinositol (4) phosphate on the 5′ position of the inositol ring, yielding PtdIns(4,5)P_2 [Clarke et al., 2001]. Recent evidence has shown that the retinoblastoma susceptibility gene product (pRB) interacts both in vitro and in vivo with type I PIP kinase and this interaction stimulates the kinase activity in an in vitro assay [Divecha et al., 2002]. Since the interaction was blocked by the large T antigen (LTA), the authors took advantage of cell lines overexpressing a temperature-sensitive mutant of LTA. At 32°C this mutant was stable in the nucleus and repressed pRB function, whereas increasing temperature to 39°C led to its degradation. It was found that at 32°C there was a fourfold increase in the mass of nuclear PtdIns(4,5)P_2 as compared to the levels at 39°C. No changes in the mass of PtdIns(4,5)P_2 at the plasma membrane was measured at the two temperatures. Therefore, the data strongly suggested that, in vivo, pRB is capable of regulating the levels of PtdIns(4,5)P_2 in the nucleus through its interaction with type I PIP kinase.

ADDITIONAL ROLES FOR NUCLEAR LIPIDS

There is no doubt that nuclear lipids are a source for second messengers. However, there are promising data regarding other functions of PtdIns(4,5)P_2 in the nucleus. PtdIns(4,5)P_2 can influence chromatin structure by facilitating the interaction between the nuclear matrix and the chromatin remodeling complex referred to as BAF [Zhao et al., 1998]. What are the molecular mechanisms that regulate such an interaction? Recent findings have shown that PtdIns(4,5)P_2 enhances actin binding by the BAF complex [Rando et al., 2002]. Since there are reports indicating that actin is a nuclear matrix protein [reviewed in Pederson, 2000], PtdIns(4,5)P_2 is an attractive candidate for a matrix localization signal for the BAF complex. The BAF complex is composed of several proteins including actin, BAF53 and Brg1. A full BAF complex was required for PtdIns(4,5)P_2 binding and stabilization of actin filaments. In addition, it was found that Brg1 interacted with actin using at least two separate domains and PtdIns(4,5)P_2 could selectively displace actin from one of these sites, thus relieving capping of BAF53 and actin by the Brg1 C terminus [Rando et al., 2002]. However, the physiological relevance of this targeting mechanism of actin to the nuclear matrix remains to be explored.

Another exciting possibility has emerged for an intranuclear function of PtdIns(4,5)P_2. Given that PtdIns(4,5)P_2 is localized in the speckle domains of the nucleus [Osborne et al., 2001] it might be involved in pre-mRNA splicing. Indeed, if PtdIns(4,5)P_2 was removed by immunoprecipitation from HeLa cell nuclear extracts, a specific inhibition of pre-mRNA splicing in the extracts ensued [Osborne et al., 2001]. However, it remains to be established exactly what PtdIns(4,5)P_2 is interacting with in this context (most likely proteins or RNA) and thus whether its involvement in pre-mRNA splicing is direct or indirect with some components of the nuclear matrix (as discussed previously).

It might be that PtdIns(4,5)P_2 binds nuclear matrix proteins and serves as a structural interface between the enzymatic core of the spliceosome and the matrix itself. In any case, what is slowly beginning to emerge is that nuclear PtdIns(4,5)P_2 does not have just one function (i.e., generation of second messengers), in analogy with the multiple roles this lipid plays in the cytoplasm [Hinchliffe et al., 1998].

CONCLUDING REMARKS AND FUTURE PERSPECTIVES

The availability of various new tools for phospholipid research has resulted in several important discoveries in the field of nuclear lipids, particularly over the last five years. Hopefully, the pace of this research will accelerate with the advent of even better tools to study individual phospholipids.

In this connection, every effort should be made to identify the proteins nuclear lipids interact with, because this information will be of great help in understanding the functions of

these lipids. The initial identification should take advantage of the straightforward overlay assays that have been developed recently [e.g., Dowler et al., 2000; reviewed in Drøbak and Heras, 2002] coupled to proteome analysis. Furthermore, these experiments should clarify what is the physicochemical form of intranuclear lipids, an issue which is poorly understood at present. Another area likely to receive considerable attention is represented by 3-phosphorylated inositol lipids, because they are now emerging as possible major players in nuclear signaling, but it may not be only PtdIns(3,4,5)P_3 that should be considered for a nuclear function. Indeed, phosphatidylinositol (3,4)-bisphosphate (PtdIns(3,4)P_2) has been detected in nuclei in vivo by means of a specific monoclonal antibody [Yokogawa et al., 2000], and the enzyme which preferentially synthesizes PtdIns(3,4)P2, class II PI3K C2α, has been immunolocalized to the nuclear speckles of HeLa cells [Didichenko and Thelen, 2001]. The results briefly reviewed here have set new stages for nuclear lipid molecules; the task at hand is to confirm their physiological relevance, to dissect the complexities of the various nuclear signaling pathways and, ultimately, to elucidate the downstream targets. These and other future investigations will certainly highlight the multiple emerging roles played by lipids in the extremely complex microenvironment of the nucleus.

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