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QUARTERLY

Minireview

Lipids and signal transduction in the nucleus[✪]

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During the last few years a growing amount of data has accumulated showing phospholipid participation in nuclear signal transduction. Very recent data strongly support the hypothesis that signal transduction in the nucleus is autonomic. Local production of inositol polyphosphates, beginning with the activation of phospholipase C is required for their specific function in the nucleus. Enzymes which modify polyphosphoinositols may control gene expression. Much less information is available about the role of other lipids in nuclear signal transduction. The aim of this minireview is to stress what is currently known about nuclear lipids with respect to nuclear signal transduction.

The existence of signal transduction in the nucleus is still an open question (for reviews see [1–7]). The most frequent opponents' question is – for what reason? Yet, kinases, phospholipases, phosphatases, inositol derivatives, IP_3 and ryanodine receptors – compo-

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Abbreviations: cADPr, cyclic ADP-ribose; Cho, choline; ChoP, choline phosphate; CT, CTP:phosphocholine cytidyltransferase; DAG, diacylgycerol; DAGK, diacylgycerol kinase; ER, endoplasmic reticulum; IGF, insulin-like growth factor; IP_3, inositol 1,4,5-trisphosphate; IP_4, inositol 1,3,4,5-tetrakisphosphate; IP_5, inositol 1,3,4,5,6-pentakisphosphate; IP_6, inositol 1,2,3,4,5,6-hexakisphosphate; Ipk, inositol kinase; LysoPA, lysophosphatidic acid; LysoPC, lysophosphatidylcholine; nSMase, neutral sphingomyelinase; PA, phosphatidic acid; PC, phosphatidylcholine; PI, phosphatidylinositol; PI3K, phosphoinositide 3-kinase; PIP, phosphatidylinositol 4-phosphate; PIP_2, phosphatidylinositol 4,5-bisphosphate; PIPK, phosphatidylinositolphosphate kinase; PI-PLC, phosphatidylinositol phospholipase C; PKC, protein kinase C; PLC, phospholipase C; PLD, phospholipase D; PS, phosphatidylserine; PSS, base-exchange enzyme.

nents of the well known phosphoinositides cycle have been found in the nucleus, suggesting that this organelle has Ca^{2+} signaling similar to, but most probably separate, from the cytoplasm [2–6, 8, 9]. Moreover, accumulating data indicate that nuclear signaling does not repeat the signal transduction pathways leading from plasma membrane receptors [6]. Most recently, three genes (*PLC1, IPK1*, and *IPK2/ARG82*) have been described in yeast and vertebrate cells which account for the pathway converting PIP_2 to IP_3, IP_4, IP_5 and IP_6 [6]. They encode specific PI-PLC and two inositol kinases (Ipk), one of which, Ipk2, phosphorylates IP_3 to IP_4 and IP_5; the second one (Ipk1) phosphorylates IP_5 to IP_6. Mutation in any of these proteins blocks export of mRNA. Interestingly, Ipk2 is identical to Arg82, a regulator of the ArgR·Mcm1 transcription complex. This finding shows that inositol polyphosphates regulate gene expression [6, 8]. The subcellular localization of Ipk2 and Ipk1 in the nucleus and at the nuclear envelope further suggests that these enzymes constitute a nuclear signaling pathway [8].

The next question is whether second messengers that are generated cytoplasmatically can penetrate the nuclear envelope [9]. The prevailing data suggest that extracellular signals activate proteins which enter nuclei by nuclear pores and bind to their intranuclear receptors – proteins, lipids or nucleic acids, generating the response. Among these proteins are phospholipases, kinases and phosphatases [3, 5, 10]. Other data have shown that the nucleus has the possibility to liberate Ca^{2+} from the nuclear envelope into the nucleoplasm [2, 4]. These data have shown that both IP_3 receptors and ryanodine receptors are present in the inner nuclear membrane [2, 4, 9, 11]. These findings strongly support the hypothesis that the nucleus has a separate possibility from the cytoplasm to regulate calcium level. In the nucleus, PI-PLC cleaving PIP_2 generates IP_3 which in turn may liberate calcium from the nuclear envelope, and free DAG, which – in some cases together with Ca^{2+} – stimulate different isoforms of PKC. Many different isoforms of PKC have been found in the nucleus [12]. Some of them are translocated into the nucleus from the cytoplasm, after agonist stimulation, others seem to reside in the nucleus. Signal transduction *via* PKC is regulated by its subcellular localization [13, 14]. PKC binds to DAG and PS domains in membranes and probably dissociates after DAG phosphorylation or PKC autophosphorylation [14]. Does the same mechanism of PKC activation function in the nucleus? The answer is still unknown.

Another problem of lipid participation in signal transduction in the nuclei, which has not yet been solved, concerns lipid synthesis within, or their transport into nuclei, and lipid location within this organelle. The nuclear envelope is not the only place in the nucleus where lipids are present. Lipids and lipoproteins have also been found in the nuclear matrix. Soluble enzymes that metabolize nuclear lipids may be transported from the cytoplasm through nuclear pore complexes (NPC) or may shuttle through the NPC between the nucleoplasm and the cytoplasm [3, 5, 10, 12].

PHOSPHATIDYLINOSITOL AND PHOSPHATIDYLINOSITOL POLYPHOSPHATES SYNTHESIS IN THE NUCLEUS

It is unresolved what the source of PI in the nucleus is. Only one report shows that PI synthesis from CDP-diacylglycerol and L-*myo*-inositol occurs in nuclei prepared from the cerebral cortex of 15-day-old rabbits. On the other hand, the α isoform of phosphatidylinositol transfer protein is present in the nucleus and this suggests that PI can be transported into this organelle [3, 5]. Lateral diffusion of PI from ER membranes, the main site of PI synthesis, to the nuclear envelope is possible but has not been shown.

Phosphatidylinositol polyphosphates are synthesized in the nucleus (Fig. 1). Isolated rat liver nuclear envelopes and rat liver nuclear matrix synthesize PIP and PIP_2. The presence of PIP and PIP_2 in the nucleus was also shown using monoclonal antibodies [5].

In mammalian cells, PIPKs, the type I and type II isoforms, distinct from cytoplasmic PIPKs, incorporating phosphate to the 4th and 5th position of the inositol ring are concentrated in the nucleus. An active PI3K was also identified in isolated rat liver nuclei by *in vitro* labeling with [γ-^{32}P]ATP. The products of this enzyme, i.e. inositides phosphorylated in the 3 position of the inositol ring, may act as second messengers themselves. Translocation of PI3K and generation of phosphatidylinositol 3,4,5-trisphosphate in the nucleus was detected in various types of cells [5, 15–18]. Kinases synthesizing various phosphatidylinositol polyphosphates are differentially localized in the nucleus. In rat liver and NIH 3T3 fibroblast nuclei, peripheral lamina is the exclusive site of phosphatidylinositol-4-kinase activity, whereas phosphatidylinositol-4-phosphate 5-kinase is preferentially associated with the internal matrix. PIPKs and PIP_2 are not associated with invaginations of the nuclear envelope. As was shown, both compounds are localized to speckles containing pre-mRNA processing factors [5].

The role of phosphatidylinositol polyphosphates in the nucleus is intensively studied. PIP_2 has been located mainly in the heterochromatin and nuclear matrix of Madine-Darby canine kidney and murine erythroleukemia cells [5]. PIP_2 is probably tightly bound to the nucleoskeleton. Phosphorylation of histone 1 by PKC decreases the amount of PIP_2 bound to the histone. On the other hand, the inhibition of RNA transcription caused by histone 1 can be reversed by PIP_2. PI and other acidic lipids have no such effect [5].

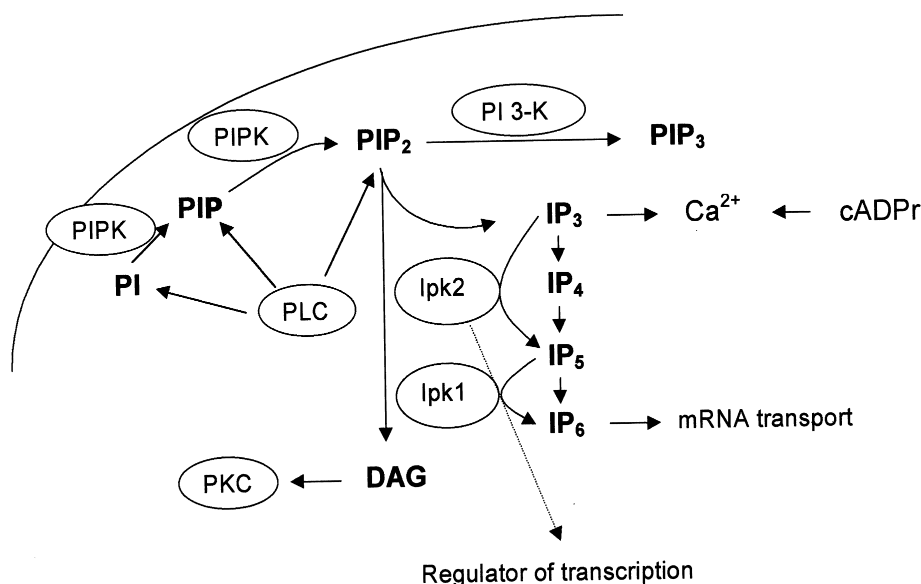

Figure 1. Inositol lipid metabolism in the nucleus.

PLC CLEAVES PHOSPHATIDYLINOSITIDES IN THE NUCLEUS

Phosphoinositides are hydrolyzed to inositol phosphates and DAG by PLC. The β-isoforms of PLC, activated by GTP-binding proteins, have been found in the nuclei [19]. Nuclei of NIH 3T3 cells contained all four isozymes of the β-family of PI-PLC [20]. PLC γ-isoforms,

which are activated by tyrosine kinases, Tau protein, arachidonic acid or phosphatidic acid, and PLC isoform δ, with an unknown mechanism of activation, were also detected in the nucleus [3, 5, 21, 22].

The classical G proteins have not been found in the nucleus. Thus, the mechanism of nuclear PLC activation is not known [3, 5]. However, ARL4, an ADP-ribosylation factor-like protein that is developmentally regulated has been recently found in nuclei and nucleoli [23].

Does PLC shuttle between the nucleus and the cytoplasm? PLC isoforms are translocated from the cytosol to the nucleus during HL-60 cell differentiation. IGF activates PLC-γ1 in the cytoplasm and selectively PLC-β1 in the nuclei of various tissue cultures [20]. Antisense RNA against PLC-β1 completely abolishes the mitogenic effect of IGF. Immunofluorescence data show that the PLC-δ4 isoform of the enzyme is detectable within the nuclei depending on the cell cycle [5]. A recent report shows that PLC-δ4 is expressed in the nuclei of Swiss 3T3 cells treated with serum [21] but PLC-δ4 mRNA is distributed abundantly in hepatoma, *src*-transformed and glioma C6 cells suggesting an important role of this enzyme in cell proliferation [22].

The activity of all PLC isozymes is regulated by Ca^{2+}. It has been shown that PLC from rat liver nuclei uses PI, PIP and PIP_2 as a substrate, depending on this cation concentration. Various inositol phosphates play a role in the modulation of calcium concentration in the nuclei [3, 5]. The presence of an IP_3 receptor has been described in the nuclei [24]. Another receptor connected with the regulation of calcium concentration — ryanodine receptor — is also present in this organelle [2]. Cyclic ADP-ribose, the ligand of this receptor, is synthesized by an enzyme located in the inner nuclear membrane [9, 11]. These data may indicate that calcium concentration can be regulated in the nuclear matrix [2, 4].

Nuclear DAG liberated by phosphoinositide degradation seems to activate some isoforms of PKC in the nucleus. DAG downstream signaling can be terminated by DAGK [3, 5]. DAGK-ζ was found in the nucleus and its nuclear localization is regulated by PKC [25].

DOES PLC HYDROLYSE PHOSPHATIDYLCHOLINE IN THE NUCLEUS?

It has recently been found that newly synthesized endonuclear phosphatidylcholine species are characterized by a high degree of diacyl/alkylacyl chain saturation and are co-located with CDP-choline pathway enzymes [26]. Membrane-free nuclei retain all three CDP-choline pathway enzymes. It is proposed that endonuclear PC synthesis may regulate nuclear accumulation of PC-derived lipid second messengers, however, saturated nuclear PC may play an additional role in regulating chromatin structure.

Latest data show the presence of PC-PLC in the nuclei [27]. PLC, acting on PC, produces DAG and ChoP. PC-specific PLC activity was found in nuclear membranes and in the chromatin fraction of rat liver hepatocytes. The enzyme in the chromatin fraction differs from that of the nuclear membrane in pH optimum and K_m. The proposed role of the nuclear enzyme is to produce DAG that may activate PKC (Fig. 2).

The second product of the enzyme is choline phosphate. ChoP is the substrate of CT a major regulatory enzyme in PC synthesis in mammalian cells. CT is translocated to the nuclear envelope upon activation by treatment with oleate or PLC [28]. Phosphorylated CT was found in the nuclear matrix in a soluble form. On the other hand, during cell quiescence, CT was confined to the nucleus and the shuttling of the enzyme between the nuclei and the ER is correlated with the activation of the enzyme — not with its phosphorylation [29].

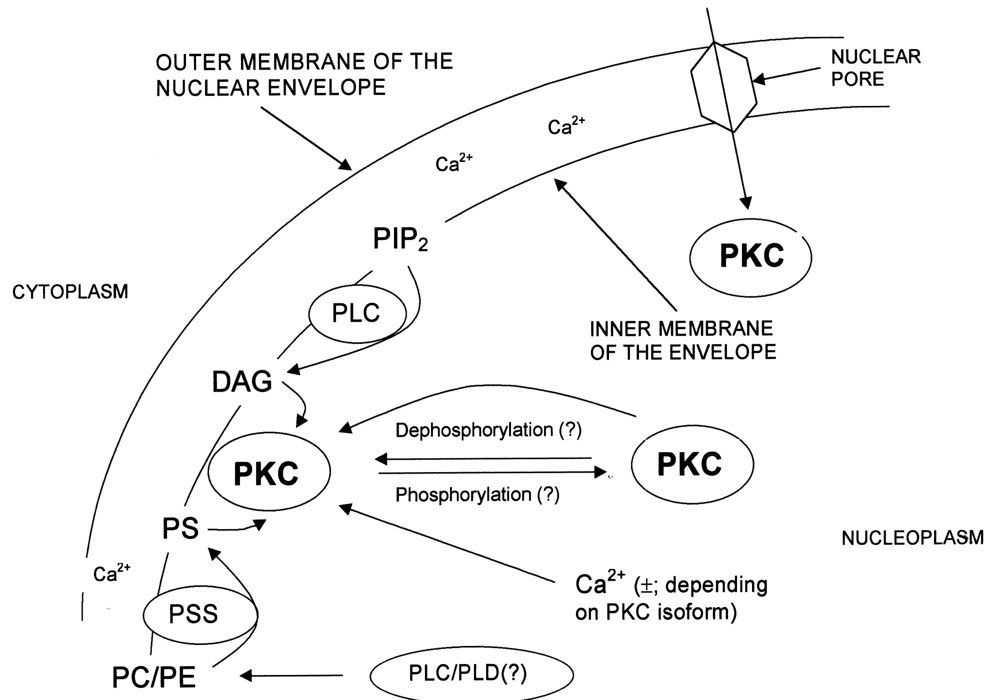

Figure 2. Proposed activation of PKC by lipids in the nucleus.

HYDROLYSIS OF PHOSPHATIDYLCHOLINE BY PLD IN THE NUCLEUS

PLD hydrolyses PC and forms PA and Cho. PA in the cells is a key intermediate in lipid metabolism. PA can also be synthesized from DAG by DAGK. PA in the cells stimulates protein kinases, PI(4)-kinase, PLC-γ, increases GTP-binding to Ras, activates Raf and mitogen-activated protein kinase. Moreover, PLD is involved in forming stress fibers and the budding of coated vesicles from Golgi membranes [30]. However, its role in the nuclei is unknown. PA can be hydrolysed to DAG by phosphatidate phosphohydrolase, which was shown in the nuclei from Madine-Darby canine kidney cells [31]. In neuronal nuclei, LysoPA has been found. LysoPA is an inhibitor of nuclear LysoPC lysophospholipase, which helps to maintain a fairly constant level of nuclear LysoPC [32].

Choline, the second product of PC-PLD-dependent hydrolysis, can be used as a substrate for PC *de novo* synthesis or for the base-exchange reaction. The activity of the base-exchange enzyme was reported to be present in hepatocyte nuclei [33].

In the nuclear envelope, ADP-ribosylation-dependent PLD activities and oleate-dependent activities have been found [34, 35]. PLD activity in the nuclei is regulated by PKC isozymes, Rho family proteins and ADP-ribosylation factors. On the other hand, in murine macrophages nuclear PLD activity was maximally stimulated in the presence of both GTPγS and ARF1. In contrast, it was not affected by RhoA either alone or in combination with GTPγS and ATP [36].

PLD participates in processes connected with membrane vesiculation. One can speculate that it also takes part in nuclear envelope vesiculation during mitosis, meiosis or apoptosis. It has been found that PLD activity and DAG production in the nucleus of HL-60 human promyelocytic leukemia cells is stimulated by camptothecin, a pro-apoptotic drug [37]. The association of PLD1 with the detergent-insoluble cytoskeletal fraction has also been reported [35].

The second isoform of PLD that has been found in the nuclei of rat brain, oleate-dependent PLD, is *in vitro* inhibited by acidic phospholipids like phosphatidylglycerol, PS, cardiolipin, PIP_2 and PA [38]. The main product of PLD in rat brain neuronal nuclei is DAG and this suggests the presence of phosphatidate phosphohydrolase in this organelle [39]. The role of this isoform of PLD is unknown [35].

IS PHOSPHATIDYLSERINE IN THE NUCLEUS OBLIGATORY FOR PKC ACTIVATION?

Almost all PKC isoforms have been found in the nuclei of different cells. All of them need PS for activation [13, 40]. PS synthesis in mammalian cells occurs during serine base-exchange reaction in the presence of calcium [41, 42]. During this reaction, free Cho or ethanolamine is liberated. We have recently shown that PS synthesis occurs in the inner membrane of the envelope of nuclei isolated from rat liver [43]. However, how PS level is regulated in the nuclear membrane and in the nuclear matrix, where PS is also present, is still unknown [5, 43].

In various cell types, PKC, in response to the activation of cell surface receptors, is directed to the plasma membrane by two membrane targeting domains, named the C1 and C2 regions [13]. This is followed by the return of the enzyme to the cytoplasm, a process shown most recently to require PKC autophosphorylation [14]. It was also demonstrated that multiple PKC isoforms exhibit increases in tyrosine phosphorylation in response to oxidative stress and that these tyrosine-phosphorylated PKCs are persistently stimulated, remaining catalytically active *in vitro* in the absence of cofactors [44].

PKC isozymes shuttle between the cytoplasm and the nuclear matrix during cell differentiation and during the cell cycle [12]. Among them is an atypical PKC subfamily, unresponsive to Ca^{2+} and DAG [12]. In murine erythroleukemia cells the PKC-theta isozyme is recruited on to the mitotic spindle in dividing cells and specifically associates with centrosome and kinetochore structures. In phorbol ester treated cells PKC-theta is translocated from the nuclear to the cytosolic compartment, an event that is accompanied by phosphorylation of the PKC molecule and is followed by its down-regulation [45]. The mechanism of PKC activity regulation in the nuclei by its binding to and dissociation from specific lipids domains, and its autophosphorylation, remains to be elucidated.

DOES SPHINGOLIPID SIGNALING OCCUR IN THE NUCLEUS?

While sphingomyelinase activity has been detected in the nucleus, it is not known whether sphingolipid signal transduction occurs in this organelle. Mg^{2+}-dependent, nSMase in the nuclei of rat ascites hepatoma cells has been demonstrated. Another nSMase, Mg^{2+}-independent, was found associated to either the nuclear envelope or the nuclear matrix in hepatocyte nuclei [5]. It is worth noting that a chromatin-bound nSMase, different from that present at the nuclear membrane, has also been identified [5]. Nuclear sphingomyelin protects RNA from RNase action. It was shown recently that in radiation-induced apoptosis nuclear sphingomyelinase was activated which resulted in the generation of ceramide and apoptotic features [46]. *In vitro* experiments have shown that sphingolipids can increase calcium concentrations in isolated rat liver and brain nuclei [47, 48].

REMARKS

Increasing amounts of data show the importance of lipid metabolism in nuclear signal transducing networks. Especially the role of

the inositide cycle in nuclear signal transduction is now being extensively studied. The first new data showing a potential role of inositol kinases as regulators of gene expression in yeast are now awaiting confirmation in mammalian cells. However, a role of lipids in the nuclei, other than inositol-derivatives, remains to be established. This new exciting area of research is still at the very beginning.

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