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Nuclear Lipid Metabolism and Signaling

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Evidence has been accumulating that nuclear lipid metabolism is involved in the regulation of nuclear functions. Here I describe an autonomous nuclear lipid signaling that has been found to be associated with the metabolism of such lipids as phosphoinositides, choline phospholipids, and the acylation and deacylation cycle. Some lipid signals from the plasma membrane ultimately reach the nucleus and regulate the nuclear function. In this case, however, generated lipids and their metabolites may not directly act on the nuclear factors involved in nuclear function. The unique and direct effects of nuclear lipids and their metabolites on nuclear factors are also discussed.

Key words: nucleus, phospholipase A_2, phospholipase C, phospholipase D, sphingomyelinase.

The generally accepted model of lipid signaling involves the generation of bioactive lipid metabolites in response to stimuli in a receptor-directed manner at the plasma membrane. These metabolites activate signaling cascades that are connected to the genes in nucleus. In recent years, however, evidence has accumulated that the nucleus is the site for active autonomous lipid metabolism for either synthesis or hydrolysis (*1–3*). Lipids play both functional and structural roles in the nucleus as well as in the plasma membrane.

Lipid metabolism in the nucleus may be regulated independently from that of the plasma membrane. Some extracellular stimuli have been reported to induce the generation of lipid signaling molecules only in the nucleus but not in the plasma membrane (*3, 4*).

How have eukaryotes developed two pathways of lipid signaling? Divecha *et al.* proposed that the nuclear lipid cycle first evolved to regulate nuclear function in response to environmental messages, and then it was duplicated at the plasma membrane in order to allow cross-talking between extracellular signals and the genome in multicellular organisms (*4*).

The purpose of this review is to show the existence of an autonomous nuclear lipid signaling, to specify the nuclear lipid signaling, and to propose its possible functions.

Phosphoinositide metabolism in the cell nucleus

The cell nucleus contains a complete enzyme system for phosphoinositide (PI) metabolism: phosphatidylinositol (PtdIns) synthetase (*5*), PI-specific phospholipase C (PI-PLC) (*3*), PtdIns 4-kinase (*6*), PtdIns 4-monophosphate 5-kinase (*6*), and PI 3-kinase (*7*). Among these, PI-PLC and PI 3-kinase have been studied extensively (Table I). Recently, Faenza *et al.* reported that overexpression of nuclear PLCβ1 induces and activates cyclin D3–cdk 4 complex, which promotes the progression of the G1 phase (*8*).

(1) Nuclear PI-PLC and signaling. We found a PI-PLC activity in the nucleus of rat liver cells that increased at S-phase during regeneration after partial hepatectomy (*9*). We purified the PI-PLC (tentatively designated as N4) which appears uniquely in the nuclei of proliferating cells, *i.e.*, regenerating hepatocytes or hepatoma (*10*). Liu *et al.* cloned a novel PI-PLC, PLCδ4, which is exclusively present in growing cell nuclei (*11*). PLCδ4 is expressed remarkably and appears exclusively in growing cell nuclei. PLCδ4 is expressed in regenerating liver and hepatoma as well as src-transformed cells. In serum-stimulated Swiss 3T3 cells, the level of nuclear PLCδ4 dramatically increases at G1/S transition, and thereafter attenuates till the next G1-phase. In contrast, the cytosolic enzymes PLCγ1 and δ1 do not fluctuate during the cell cycle. At present, PLCδ4 is the only isoform known to be specific to the nucleus, though no typical nuclear localization signal (NLS) has been identified in the molecule. The positively charged amino acid residues in the 'pleckstrin homology domain' are suggested to play a role in its nuclear localization (*1*).

While PLCδ4 is newly synthesized in response to extracellular stimuli, PI-PLCβ isoforms are constitutively present in the nuclei of quiescent cells or resting tissues. PI-

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Abbreviations: ARF, ADP-ribosylation factor; ATRA, all-*trans* retinoic acid; Car, cardiolipin; Cer, ceramide; CT, CTP:phosphocholine cytidyltransferase; D3-PI, 3-phosphorylated phosphoinositide; DAG, diacylglycerol; DMSO, dimethylsulfoxide; IGF-1, insulin-like growth factor-1; IP_3, inositol 1,4,5-trisphosphate; NGF, nerve growth factor; NLS, nuclear localization signal; nSMase, Mg^{2+}-dependent, neutral sphingomyelinase; PA, phosphatidic acid; PC, phosphatidylcholine; PC-PLC, PC-specific phospholipase C; tentative PC-PLC, PC:ceramide phosphocholine transferase; PE, phosphatidylethanolamine; PLD, phospholipase D; PC-PLD, PC-specific PLD; PDGF, platelet-derived growth factor; PG, phosphatidylglycerol; PIKE, phosphoinositide kinase enhancer; PTEN, phosphatase and tensin homolog; PI(s), phosphoinositide(s); PLC, phospholipase C; PI-PLC, PI-specific PLC; PKC, protein kinase C; $PLA(s)_2$, phospholipase(s) A_2; PS, phosphatidylserine; PtdIns, phosphatidylinositol; PtdIns(4)P, PtdIns 4-monophosphate; PtdIns(4,5)P_2, PtdIns 4,5-bisphosphate; PtdIns(3,4)P_2, PtdIns 3,4-bisphosphate; PtdIns(3,4,5)P_3, PtdIns 3,4,5-trisphosphate; SH3 domain, src homology 3 domain; SM, sphingomyelin; SMS, sphingomyelin synthase; Sph, sphingosine; SPHK, sphingosine kinase.

TABLE I. **Response of nuclear phosphoinositide-metabolic enzymes to extracellular stimuli.**

Enzyme	Stimulus	Cell or tissue stimulated	Responses of nuclear enzyme: Activity	Responses of nuclear enzyme: Mass	Mass of substrate or product in nucleus	Cell response
PI-PLCs	Partial hepatectomy	Rat liver	T[a] ↑[b] (9,10)[d] (S phase)	↑ (10) (S phase)		Liver regeneration
PI-PLCδ4	Serum	Swiss 3T3		↑ ↓[c] (S/M phase) (next G1 phase)		Onset of DNA replication
PI-PLCβ1	IGF-I	Swiss 3T3	T ↑ (13,14) (2 min)	↔[e] (13,15) (2 min)	↑ (16,17) DAG; T ↓ (15) (2 min) PtdIns(3,4,5)P3	Proliferation Onset of DNA replication G0/G1 to S-phase transition
	Insulin	Swiss 3T3	T ↑ (18) (2 min)		↑ (18) (2 min) DAG	Proliferation
	DMSO	Friend cells	↓ (23) (2 d)	(23) ↓ ↓ (15) (4 d)	↓ (23) (2 d) DAG; ↑ (15) (4 d) PtdIns(4,5)P2	Erythrocytic differentiation
PI-PLCβ1b	Interleukin-1α	Saos-2	T ↑ (19, 20) (2 min)		↓ (19, 20) ↓ PtdIns(4)P1 PtdIns(4,5)P2	Cessation of proliferation and increase in the number of cells G1 phase
	Interleukin-2	Human natural killer cells (primary)	T ↑ (21) (within 1 h)		↑ (21) DAG	Proliferation
PI-PLCβ2, β3, γ1, γ2	ATRA	HL-60		↑ ↑ ↑ ↑ (24) β2 β3 γ1 γ2 (2 d)		Granulocytic differentiation
	Vitamin D3	HL-60		↑ ↑ ↑ (25) ↔ β2 β3 γ1 γ2 (2 d) (3 d) (4 d)		Monocytic differentiation
PI-PLCγ1	Partial hepatectomy	Rat liver		↑ (30) (22 h)		Liver regeneration
PI 3-kinase (p85-p110)	Interleukin-1	Saos-2		↑ (42) (5 min)		Bone resorption
	ATRA	HL-60	↑ (38) (4 d)	↑ (38, 29,39) (4 d)		Granulocytic differentiation
	Vitamin D3	HL-60		↑ (25) (until 4 d)		Monocytic differentiation
	H_2O_2	293T			↑ ↑ (42, 43) PtdIns (3,4)P2 PtdIns (3,4,5)P3	
	NGF	PC-12	↑ (40) (9 min)	↑ (40) (9 min)	↑ (41,40) (9 min) PtdIns(3,4,5)P3	Neural differentiation
	PDGF	NIH3T3			↑ (41) PtdIns(3,4,5)P3	Proliferation
PI 3-kinase (C2β)	Partial hepatectomy	Rat liver	↑ (44) (22 h)			Liver regeneration

[a] Transient [b] Increase [c] Decrease [d] Reference number [e] No change

PLCβ isoforms translocate into nuclei, mediated by the long C-terminal containing a lysine cluster (*12*). PI-PLCβ1 present in nuclei is quickly activated in quiescent Swiss 3T3 cells, within 2 min after the mitogenic stimulation with insulin-like growth factor I (IGF-I) (*13, 14*), and then returns to the low level within 30 min without any changes in protein mass (*13, 15*). As a result, IGF-I increases diacylglycerol (DAG) (*16, 17*) at the expense of PtdIns 4,5-bisphosphate [PtdIns(4,5)P_2] (*15*) in the nucleus, but not in the plasma membrane (*4*).

Nuclear PI-PLCβ1 is also activated by insulin (*18*) or interleukins (*19–21*), while cytoplasmic PLCs do not respond to them (*19, 21*). Treatment of cells with these mitogens causes phosphorylation of nuclear PI-PLCβ1 at serine 982 (*18, 21*), and a specific inhibitor for MAP kinase strongly inhibits both phosphorylation and enzyme activation (*18*). When serine 982 of PI-PLCβ1 is replaced by glycine, IGF-I fails to activate the metabolic cycle of nuclear PI in the cells overexpressing this mutant enzyme (*22*). Obviously, the activation of the nuclear PI-PLCβ1 strictly depends on the serine phosphorylation through the MAP kinase pathway. However, an *in vitro* study showed that the MAP kinase-mediated phosphorylation does not increase the enzyme activity of purified PI-PLCβ1 (*7*). Therefore, an unknown protein factor that binds to the phosphorylated domain could be involved in the activation of PI-PLCβ1.

Levels of the nuclear PLCβ family are affected by differentiation-inducing agents. Dimethylsulfoxide (DMSO) decreases the activity of PI-PLCβ1 in nuclei of Friend erythro-leukemic cells during differentiation, associated with decrease of the protein mass of PI-PLCβ1 (*15, 23*). Concomitantly, the cell population in the G1 phase of the cell cycle increases (*23*). On the contrary, PLCβ2 and PLCβ3 increase in the nuclei when HL-60 promyelocytic leukemic cells are induced to differentiate by all *trans*-retinoic acid (ATRA) (*24*) or vitamin D3 (*25*). These changes of PLCβ isoforms appear very slowly at 2 to 4 days after cell treatment.

The PLCγ family is also present in nuclei in spite of its lacking an NLS (*26, 27*). During ATRA-induced differentiation of HL60 cells, the PLCγ isoform increases in nuclei and forms an immunoprecipitable complex with an oncoprotein, Vav, that possesses an NLS. In the nuclei of differentiated HL-60 cells, Vav phosphorylated at its tyrosine residue increases (*28, 29*). Therefore, PLCγ could be transported into nuclei as a complex with Vav.

Nuclear PLCγ1 markedly decreases at 3 and 16 h after hepatectomy, and then increases at 22 h, while the level of cytoplasmic PLCγ1 is not affected by liver regeneration (*30*).

PI-signaling on plasma membranes has been studied extensively: PI-PLC activation by external stimuli leads to a rise in diacylglycerol (DAG) level, which activates protein kinase C (PKC), one of the key enzymes in intracellular signaling cascades. DAG is then converted into phosphatidic acid. A similar but distinct PI-cycle is also recognized in the nucleus. DAG is generated by the activated PLCβ1 in nuclei after IGF-I stimulation (*16, 17*). DAG activates PKC-α and also induces its nuclear translocation (*17*). Nuclear PKC-α phosphorylates PLCβ1 at serine 887, resulting in inactivation of PLCβ1 (*31*) that has been activated by the first phosphorylation at serine 982 (*18, 21*). On the other hand, IGF-I treatment of quiescent Swiss 3T3 cells induces nuclear DAG kinase activity that converts DAG into phosphatidic acid (*16*). Since DAG is a stimulator of PKC-α, the enhanced nuclear DAG kinase terminates the PKC-α action (*16*). In other words, the systems of switch-on and switch-off for PLCβ1 are both generated sequentially by the IGF-I stimulation.

DAG kinase-ζ is a nuclear protein possessing an NLS in the region homologous to the phosphorylation domain of the MARCKS protein (*32*). Interestingly, the phosphorylation of the serine residues in the MARCKS-homology region by PKC regulates the intracellular location of DAG kinase-ζ: the unphosphorylated form is in the nucleus, whereas the phosphorylated form is extranuclear.

Besides its role in terminating the nuclear PI cycle, nuclear PKC acts as a mitotic lamin kinase (*33–36*). The activated nuclear PI-PLC generates DAG to activate β_{II} PKC, which phosphorylates serines 395 and 405 of lamin B, followed by the breakdown of the nuclear envelope at mitosis.

Another PI-PLC product, inositol 1,4,5-trisphosphate (IP_3) increases the calcium content in nuclei. Rat liver nuclei possess IP_3 receptor and endogenous PKC type II. TPA-treatment of isolated rat liver nuclei activates the PKC that phosphoryles IP_3 receptor, resulting in increased potency of IP_3 in releasing calcium from the nuclei (*37*).

(2) Nuclear PI 3-kinase and signaling. PI 3-kinase, an enzyme that converts PI into 3-phosphorylated PI (D3-PI), is present in the nuclei of various types of cells, such as neurons, hepatocytes, osteosarcoma cells (Saos) and promyelocytic leukemia cells (HL-60) (*1, 2, 7*). Nuclear PI 3-kinase has been analyzed mainly with respect to cell differentiation. ATRA treatment of HL-60 for 4 days induces an increase of nuclear matrix-bound PI 3-kinase along with granulocytic differentiation, while no changes are seen in the cytoplasmic PI 3-kinase activity (*28, 29, 38, 39*). In nuclei of differentiating HL-60, a complex composed of three proteins, tyrosine-phosphorylated Vav/PLCγ/PI 3-kinase, is formed (*28, 29*). Vav, a proto-oncogene product, is present in both cytoplasm and nuclei and is phosphorylated at the tyrosine during ATRA-induced differentiation (see PI section). Since Vav has an NLS and preferentially localizes in the nucleus, the tyrosine-phosphorylated Vav may be directly involved in the nuclear localization of both PLCγ and PI 3-kinase, which have no NLS (*29*).

Monocytic differentiation of HL-60 by vitamin D3 induces the progressive translocation of PI 3-kinase into nucleus until 4 days of treatment (*25*). Simultaneously, PKC-ξ is translocated into the nucleus, which is blocked by Wortmannin, an inhibitor of PI 3-kinase (*25*). PI 3-kinase activity appears to be involved in the regulation of PKC-ξ translocation.

Nerve growth factor (NGF) induces rapid and transient increases in both protein mass and activity of nuclear PI 3-kinase in PC 12 cell differentiation (*40*), resulting in increased levels of nuclear PtdIns(3,4,5)P_3 (*40, 41*) and leading to the nuclear translocation of PKC-ζ (*40*).

Interleukin 1 causes a rapid and transient translocation of PI 3-kinase from the cytoplasm to the nucleus in human Saos-2 cells (*42*). Rapid generation of PtdIns(3,4,5)P_3 in the nucleus is detected in H_2O_2-treated 293 T cells (*41, 43*) and platelet-derived growth factor (PDGF)–treated NIH 3T3 cells (*41*).

Partial hepatectomy also activates PI 3-kinase C2β in nuclei of hepatocytes in residual rat liver at 22 h (~S phase) through a calpain-mediated proteolysis (*44*).

PIKE (phosphoinositide kinase enhancer), a novel nuclear GTPase composed of 753 amino acids, activates the catalytic subunit (p110) of nuclear PI 3-kinase by interacting with its regulatory subunit (p85) (*45*). Protein 4.1N blocks the activation of PI 3-kinase by preventing the PIKE association. Recently, Ye *et al.* have reported that PLCγ1 stimulates PIKE GTPase as a physiological guanine nucleotide exchange factor through its src homology 3 domain (SH3 domain) (*46*). In this connection, Ras protein activates cytoplasmic PI 3-kinase by binding to p110 catalytic subunit (*7*).

What is the function of D3-PI in the nucleus? It might modulate the activity of PtdIns(3,4)P_2-dependent protein kinase, Akt/PKB, which is translocated into the nucleus (*47*). Usually, PI-PLC cannot hydrolyze D3-PI. PTEN, which is known as phosphatase and tensin homolog located at human chromosome 10, reveals 3′-phosphatase activity for D3-PI. It is present in both the nucleus and the cytoplasm and converts PtdIns(3,4)P_2 to PtdIns(4)P, and PtdIns (3,4,5)P_3 to PtdIns(4,5)P_2 (*48, 49*). In a significant number of human cancers, PTEN is mutated and inactivated so that PI 3-kinase signaling is constitutively activated (*49*). As a result, a high level of PtdIns(3,4,5)P_3 accumulates. Thus, nuclear PTEN must play a role in the termination of nuclear PI 3-kinase signaling.

PtdIns(3,4,5)P_3-binding protein (PIP_3BP) possesses a putative NLS and is present in nuclei (*41*). When cells are stimulated, PIP_3BP is exported from the nucleus to the cytoplasm, depending on the activation of nuclear PI 3-kinase that produce PtdIns(3,4,5)P_3. PtdIns(3,4,5)P_3 may function in the nucleus through the association with PIP_3BP.

PI 3-kinase is also connected with nucleic acid metabolism. PI 3-kinase C2α which has an NLS, associates with nuclear speckles containing the pre-mRNA processing factors. Phosphorylation of PI 3-kinase C2α coincides with the inhibition of RNA polymerase II-dependent transcription and the enlargement and rounding up of the nuclear speckles (*50*).

Choline phospholipid metabolism in the cell nucleus

Choline phospholipids, phosphatidylcholine (PC) and sphingomyelin (SM), are present not only in the nuclear membrane, but also in chromatin and the nuclear matrix (*51, 52*). PC-metabolic enzymes found so far in the nucleus are: CTP:phosphocholine cytidyltransferase (CT) (*53, 54*); PC-specific phospholipase D (PC-PLD) (*2, 3*); PC:ceramide phosphocholinetransferase (tentative PC-PLC) (*55, 56*), phosphatidate phosphohydrolase (*57*). Among PC-metabolic enzymes, only CT possesses an NLS (*54*). Upon oleate-treatment of Hela cells, CT is dephosphorylated and translocated to the nuclear envelope (*53*). PC, as well as PI, may be implicated as the source of DAG production. Besides the well-known PI-PLC pathway for DAG generation, another pathway is the PC breakdown by PC-specific phospholipase C (PC-PLC), tentative PC-PLC or PC-PLD in cooperation with phosphatidate phosphohydrolase. D609, a PC-PLC-specific inhibitor, is routinely used to inhibit DAG production *via* the PC-PLC pathway. However, it has been shown that D609 also dramatically inhibits tentative PC-PLC (*58*), which also produces DAG. Therefore, tentative PC-PLC system could be the third source of DAG. The major role of the PC-PLC pathway in the production of DAG, established using D609, might need re-examination.

SM-metabolic enzymes found in the nucleus are Mg^{2+}-dependent, neutral sphingomyelinase (nSMase) (*59–63*), ceramidase (*62*), sphingosine kinase (SPHK) (*64*) and sphingomyelin synthase (SMS) (*55, 56*) which is also designated as tentative PC-PLC.

The stimuli promoting choline phospholipid-metabolic enzymes in the nucleus are summarized in Table II.

(1) Nuclear PC-PLD and signaling. Banno *et al.* have found a transient increase in ADP-ribosylation factor (ARF)–dependent nuclear PLD activity in the S-phase of the regenerating rat hepatocytes after partial hepatectomy (*65*). This enzyme is localized in the nuclear envelope. In contrast to the ARF-depndent PLD, the nuclear oleate-dependent PLD does not fluctuate during liver regeneration. Endogenous stimulators of nuclear PLD, *e.g.*, ARF, Rho A, and PKC-δ, also increase in nuclei of regenerating hepatocytes. These results might indicate that this nuclear PLD is involved in promoting cell proliferation.

When α-thrombin stimulates IIC9 cells to proliferate, the nuclear PC-PLD1 activity increases, accompanied by the translocation of RhoA to the nucleus (*66, 67*). RhoA GTPase may stimulate the nuclear PLD1 activity. Furthermore, α-thrombin selectively increases PKC-α in IIC9 cell nuclei, in addition to increasing the level of DAG (*68*). The α-thrombin–induced nuclear DAG may be derived from nuclear PC, since the fatty acid compositions of these two lipids are similar to each other but differ from the fatty acid profile of PI (*67, 69*). Interestingly, ethanol suppresses the production of phosphatidic acid but does not affect the increment of nuclear DAG in α-thrombin-treated IIC9 cells (*70*). This result indicates that the increased nuclear PLD is responsible for phosphatidic acid production but not for DAG generation. Instead, the tentative PC-PLC (or SMS) may play a role in the nuclear DAG generation. Nuclei of quiescent IIC9 cells possess DAG kinase-θ and -δ. In response to α-thrombin, only DAG kinase-θ is specifically activated (*71*), and this may regulate the nuclear DAG level that is generated in the PC-cycle.

Nuclear PC-PLD in Madin-Darby canine kidney (MDCK)-D1 cells is activated by either TPA or ATP (*57*). The activation is, thus, downstream of PKC and is regulated by Rho A. Phosphatidate phosphohydrolase is also detected in nuclei of MDCK-D1 cells (*57*).

Treating HL60 cells with camptothecin, an inhibitor of topoisomerase I, results in an increased level of nuclear DAG through the activation of nuclear PC-PLD, which is insensitive to ARF activation (*72*). Increased DAG activates nuclear PKC-α, which phosphorylates lamin B, indicating the involvement of nuclear PLD activation in the apoptotic signaling pathway. PC-PLD2, which is insensitive to Rho A activation, is found in the nucleus of human renal cancer cells and might be implicated in the tumorigenesis and/or promotion of cancer development (*73*).

(2) Nuclear nSMase and signaling. nSMase was first found in the nuclear matrix of rat ascites hepatoma AH 7974 cells (*59*), and recently 50% or more of nuclear nSMase was reported to be nSMase1, which possesses a nuclear export signal but not an NLS (*74*). nSMase also exists in nuclei of rat liver cells, associated with the nuclear envelope (*60, 61*), nuclear matrix (*51, 60*) and chromatin (*61*). In resting liver cell nuclei, nSMase is predominantly associated with the nuclear envelope, whereas in regenerating liver cells, it is translocated to the nuclear matrix (*60*). The level of nuclear nSMase transiently increases 18 h after partial hepatectomy, accompanied by increases of ceramide and sphingosine at the expense of SM in the nucleus (*60*). Chromatin-associated nSMase is different from

TABLE II. **Response of nuclear lipid-metabolic enzymes to extracellular stimuli.**

Enzyme	Cell stimulus	Cell or tissue stimulated	Responses of nuclear enzyme: Activity	Responses of nuclear enzyme: Mass	Mass of substrate or product in nucleus	Cell response	References
Phosphatidylcholine-metabolic enzymes							
CTP:phosphocholine cytidyltransferase	Oleate	HeLa		↑[a)] (15 min)			53
PLD (ARF-dependent)	Partial hepatectomy	Rat liver	↑ (S phase)			Liver regeneration	65
PLD (ATP-activated)	ATP	MDCK-D1	↑ (10-15 min)		↑ ↑ (10~15 min) PA DAG		57
PLD 1	α-thrombin	IIC9	↑ (15 min)			Proliferation	70, 67
PLD	Camptothecin	Hl-60	↑ (30-45 min)		↑ (30~45 min) PA	Apoptosis	72
Sphingolipid-metabolic enzymes							
nSMase	Ionizing radiation	TF-1	↑		↓[b)] ↑ (~8 min) (~8 min) SM Cer	Apoptosis	63
	Portal vein branch ligation	Rat liver	↑ (60 min)		↑ (60~90 min) Cer	Apoptosis	62
	ATRA	HL-60			↑ (72 h) Cer	Granulocytic differentiation	75
	Partial hepatectomy	Rat liver	↑ (61,62)[c)] (S phase)	↑ (60) (S phase)	↓ ↑ ↑ SM Cer Sph (60)	Liver regeneration	60 61 62
Ceramidase	Portal vein branch ligation	Rat liver	↑ (60 min)		↑ (60~90 min) Sph	Apoptosis	62
SPHK-1	PDGF	Swiss 3T3	↑ (24 h)			Proliferation	64
Deacylation enzymes							
Neutral PLA2 (Ca^{2+}-dependent)	Partial hepatectomy	Rat liver	↑ (S~G2~M)			Liver regeneration	*[d)]
Ca^{2+}-independent PLAs2	Retinoic acid	LA-N-1	↑ (10 h)			Differentiation	88
cPLA2	Angiotensin II	Vascular smooth muscle cells	↑	↑ (7 min)		Cell growth stimulation	91 92
	IgE/antigen	RBL-2H3.1	↑	↑ (5 min)			92
	Histamine	Endothelial cells		↑ (10 min)		Inflammatory vasodilatation PGI2 and PGE2 release	93

a) Increase b) decrease c) Reference number d) Unpublished data

nuclear envelope nSMase in the enzymological characteristics, and its activity also increases transiently 18 h after partial hepatectomy (*61*).

Using the portal vein branch ligation system of rat liver, Tsugane *et al.* observed the activation of both nSMase and ceramidase, with concomitant increases of their reaction products, ceramide and sphingosine, in the hepatocyte nuclei of ligated lobes prior the onset of apoptosis (*62*). The activation of enzymes was not detected in the proliferating hepatocyte nuclei of non-ligated lobes, nor in the plasma membranes of ligated and non-ligated lobes. These facts demonstrate that nuclear nSMase may play a role in apoptotic signaling. Jaffrezou *et al.* clearly showed the involvement of nuclear nSMase in radiation-induced apoptosis (*63*). nSMase activation, ceramide generation and apoptotic features were observed in response to ionizing radiation only in the nucleus of a radio-sensitive TF-1 cell clone, not in nucleus-free lysates and cytoplast, nor in the nucleus of a radio-resistant TF-1 cell clone.

The differentiation of HL-60 cells induced by ATRA is paralleled by the increases of ceramide and catalytically active PKC-ζ in the nucleus (*75*). PKC-ζ possesses a cysteine-rich domain that is hypothesized to play a role in the binding of ceramide to this kinase (*76*).

SPHK-1 was recently found in Swiss 3T3 cell nuclei either in the nuclear envelope or nucleoplasm. PDGF induces the rapid stimulation of cytosolic sphingosine kinase (in 10 min), and a large increase in sphingosine kinase activity in the nucleoplasm follows around 12–24 h after stimulation (*64*). The intracellular ratio between ceramide/sphingosine and sphingosine-1-phosphate may determine whether a cell will die or survive. Nuclear SPHK-1, therefore, must be a key enzyme in preventing the onset of apoptosis induced by the nuclear nSMase pathway. In this connection, sphingosine-1-phosphate stimulates DNA binding activity of AP-1 in quiescent Swiss 3T3 cells (*77*).

Acylation and deacylation cycle in the cell nucleus

An active acylation and deacylation cycle occurs in the nucleus, and the fatty acid composition of nuclear phospholipids is rapidly changed and affected profoundly by dietary fat (*78, 79*). In response to proliferation stimuli, 18:1 transiently increases at the expense of 20:4 and 22:6 in *sn*-2 position of all phospholipids in liver cell nuclei (*80*). Fatty acyl changes are more prominent at the *sn*-2 position than the *sn*-1 position in nuclear phospholipids of proliferating cells. The rapid turnover of 2-acyl chains of nuclear phospholipids has been reported by Neufeld *et al.*: [^{3}H]-arachidonic acid is rapidly incorporated into the nuclear membrane, then distributed into other subcellular fractions (*81*). According to Capriotti *et al.*, upon stimulation by bradykinin, the most recently-incorporated pool of arachidonate in the nuclear membrane was preferentially released in mouse fibrosarcoma cells (*82*). The active turnover of fatty acyl residues in nuclear lipids may be due to the action of acyltransferase (*83–86*) and phospholipase A_2 (PLA_2) (*51, 87, 88*) in nuclei.

$PLAs_2$ whose activities in the nucleus are increased by extracellular stimuli are shown in Table II.

(1) Nuclear PLA_2 and signaling. A novel nuclear PLA_2 associates with nuclear matrix of rat ascites hepatoma cells (*87*). This enzyme hydrolyzes PC and phosphatidylethanolamine (PE) with the same efficiency at neutral pH. Interestingly, the Ca^{2+} concentration for a maximal activation is lowered by the addition of an acidic phospholipid such as PI. Activity of this neutral PLA_2 is very low in other organella. A similar PLA_2 has also been found in rat liver nuclear matrix (*51*). Following partial hepatectomy, the neutral PLA_2 activity in liver nucleus increases at the S phase (T-Koizumi *et al.*, unpublished data).

LA-N-1 neuroblastoma cells contain two kinds of Ca^{2+}-independent $PLAsA_2$ that hydrolyze PE and ethanolamine plasmalogen, which differ from each other in their molecular mass, substrate specificity, and kinetic properties (*88*). They are present in cytosol, cytoplasmic membranes and the nucleus. Retinoic acid markedly stimulates $PLAsA_2$ activities in LA-N-1 cells committed to differentiation. Activities in the nucleus are stimulated first, then cytosolic activities, while membrane activities are not changed. It is speculated that nuclear enzymes may be involved in the cessation of mitosis and/or in extension of neurites.

Besides the roles in lipid metabolism, PLA_2 could be a growth factor. Exogenously added pancreatic PLA_2 (PLA_2-1) stimulates the proliferation of U_{III} cells, a stromal cell line derived from normal rat uterus (*89*). Endogenous PLA_2-1 is located in the nuclei of proliferating U_{III} cells, but in the cytoplasm in resting cells. Exogenously added PLA_2-1 is internalized and transported into the nucleus. Nuclei of U_{III} cells possess a specific cooperative binding site for PLA_2-1, suggesting that pancreatic PLA_2 interacts directly with nuclear components to exert its effects.

$cPLA_2$ is a ubiquitous enzyme that specifically hydrolyzes phospholipids containing arachidonic acid in the *sn*-2 position. Calcium binds to the C2 domain of $cPLA_2$ and induces translocation of the enzyme primarily to the nuclear envelope (*90–93*). Interestingly, other proteins implicated in eicosanoid biosynthesis, such as cyclooxygenase 1,2,5-lipoxygenase and 5-lipoxygenase activating protein, also localize at, or translocate to, the perinuclear region (*90*). A high level of arachidonic acid has been detected at the *sn*-2 position of nuclear phospholipids (*80*). Arachidonic acid, the product of PLA_2 action, may exert a direct effect on replication or transcription through the arachidonic acid responsive elements (*94, 95*).

TABLE III. **Effects of lipids on the *in vitro* activities of nuclear proteins.**

Protein	Lipid	Effect	Reference
Nuclear factor-4α (HNF-4α)	Long chain fatty acids	Modulation of transcriptional activity of HNF-4α	*102*
DNA polymerase α, β	Long chain fatty acids	Inhibition of enzyme activity	*103, 104, 105*
DNA primase	Sphingosine	Inhibition of enzyme activity	*106, 107*
AP-1	Sphingosine 1-phosphate	Stimulation of DNA binding activity of AP-1	*77*
Histones	$PtdIns(4,5)P_2$ Car PG	Reversal of the inhibitions of DNA replication and transcription caused by histones	*96, 97*
DNA polymerase α	Car PtdIns(4)P	Inhibition of enzyme activity	*98, 99*
DNA polymerase β	Car	Inhibition of enzyme activity	*99*
DNA polymerase γ	Car PtdIns PS PA	Inhibition of enzyme activity	*99*
DNA polymerase δ	PtdIns(4)P	Inhibition of enzyme activity	*98*
DNA polymerase ε	Car PtdIns PtdIns(4)P PS PA	Inhibition of enzyme activity	*98*
Topoisomerase I	Car PtdIns PG	Inhibition of enzyme activity	*100*
Terminaltransferase	Car	Inhibition of enzyme activity	*99*
DnaA	Car PG	Removal of ADP from DnaA	*97, 101*
SV40 T antigen	Car	Inhibition of the binding of SV40 T antigen to the replication origin	*97*

Concluding remarks

Nuclei contain key enzymes for lipid signaling. Their activities and/or amounts respond to extracellular stimuli. Nuclear lipid signaling may be independent of that at plasma membrane, although the molecular scenario is similar.

Some lipid signals from the plasma membrane ultimately reach the nucleus and regulate the nuclear function. In this case, however, generated lipids and their metabolites may not act directly on the nuclear factors. Nuclear lipid signaling has the advantage over the plasma membrane signaling that lipids or their metabolites may directly interact with nuclear factors. In this context, Table III lists a series of experimental findings mainly based on the *in vitro* effects of different lipids on the functional proteins in the nucleus. Remarkably, acidic phospholipids such as cardiolipin (Car) and PI strongly affect the activities of nuclear enzymes (*96–101*).

DNA and acidic phospholipid are known to share a common motif in their molecule (*97*). On the other hand, a number of PtdIns(4,5)P_2-binding nuclear proteins, such as lamin B, histones, DNA polymerases, RNA polymerases, topoisomerases, helicases and transcription factors, AP-1, AP-2, and Jun, possess a PtdIns-binding consensus sequence (*1*). These facts suggest that DNA-binding proteins may interact not only with DNA but also with acidic phospholipids in functioning domains in nuclear structures, such as replication foci or transcription factories. Thus it is likely that activities of DNA-binding proteins are masked by the associated acidic phospholipids but will be reactivated by the breakdown of these lipids. The breakdown might produce bioactive lipid molecules as second messengers that may, in turn, affect the activities of DNA-binding proteins. In fact, in the S phase, when the DNA synthesis machinery is activated, the amounts of nuclear phospholipids decrease significantly (*1*). Yu *et al.* has reported that PtdIns(4,5)P_2 binds with histone H1 and reduces its binding to DNA (*96*). As a result, PtdIns(4,5)P_2 cancels the inhibition of RNA polymerase II by histone H1 and promotes transcription. When histone H1 is phosphorylated by PKC, it loses the ability to bind to PtdIns(4,5)P_2, resulting in the termination of transcription. Besides acidic phospholipids, fatty acids and SM metabolites may also regulate functions of various nuclear proteins and enzymes *in vitro* (*102–107*). It is suggested that SM metabolism, as well as the acylation/deacylation cycle, may be closely related to replication and/or transcription in the nucleus.

At present, however, several questions remain unanswered. (i) How are signals transferred from the cell surface to nucleus to activate lipid signaling? (ii) What is the specificity of nuclear signaling compared to that on the cell membrane? (iii) What is the relationship between lipid signaling and lipids as structural component of the nucleus? Involvement of the cytoskeleton in IGF I–dependent activation of nuclear PLC β1 (*108*), as well as the internalization/translocation into nucleus of growth factors, hormones, cytokins, or their receptors harboring functional nuclear localization sequences (*109, 110*), may provide clues to solve these questions.

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