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Invited Review

Lipid signaling and cell responses at the nuclear level

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Summary. The nucleus is known to be a site for an active lipid metabolism. Although phospholipids are present in the nuclear envelope, evidence suggests that they are also located further inside the nucleus. The function of these intranuclear lipids has escaped clarification for many years. Early experiments showed that they can interact with DNA double helix affecting its thermal stability and can influence RNA synthesis in isolated nuclei. However, in the last 10 years several investigations have suggested that they may be involved in signal transduction pathways at the nuclear level and a growing body of evidence supports this hypothesis.

Key words: Lipids, Nucleus, Signaling, Proliferation, Differentiation, Nuclear matrix

Abbreviations: DAG, diacylglycerol; DMSO, dimethyl sulfoxide; EPO, erythropoietin; HMBA, hexamethylene-bis-acetamide; IGF-I, insulin-like growth factor-I; IP_3, inositol 1,4,5-trisphosphate; IP_4, inositol 1,3,4,5-tetrakisphosphate; MAP kinase, mitogen-activated protein kinase; MEL, murine erythroleukemia; NGF, nerve growth factor; PA, phosphatidic acid; PC, phosphatidylcholine; PDGF, platelet-derived growth factor; PE, phosphatidylethanolamine; PKA, protein kinase A; PKB, protein kinase B; PKC, protein kinase C; PI, phosphatidylinositol; PIP, phosphatidylinositol 4-phosphate; PIP_2, phosphatidylinositol 4,5-bisphosphate; PIP_3, phosphatidylinositol 3,4,5-trisphosphate; PLA_2, phospholipase A_2; PLC, phospholipase C; PLD, phospholipase D; PS, phosphatidylserine; PI-TP, phosphatidylinositol transfer protein

Introduction

For a long time lipids have only been seen as structural blocks of cell membranes. However, it is now clear that they can be considered precursors of bioactive molecules that are generated in cells following stimulation of cell-surface receptors and function as second messengers. Therefore, lipids play a very important role in signal transduction events, i.e. in the communication of a signal initiated by an extracellular agonist to the cell interior (Divecha and Irvine, 1995a). Signal transduction pathways must, therefore, include mechanisms for the initiation of signals at the plasma membrane, a mechanism by which these signals traverse the cytoplasm and induce, finally, a nuclear response (Liscovitch and Cantley, 1994).

It is becoming evident that not only phosphoinositide hydrolysis is an important cellular response to agonists but also other lipids such as PC and sphingomyelin have been shown to generate intracellular messengers (Exton, 1994).

As far as phosphoinositides are concerned, many surface receptors activate PI-PLC isozymes to hydrolyze a minor membrane phospholipid, PIP_2, to give both DAG and IP_3, which act as intracellular messengers, mediating the activation of PKC and intracellular Ca^{++} release, respectively (Rhee and Bae, 1997). Although the known functions of PIP_2 occur at the plasma membrane, there have been several indications that these lipids may also exist at other cellular sites and therefore have previously unrecognized functions. Following up an early report by Smith and Wells (1983), which described the ability of nuclear envelopes to synthesize PIP and PIP_2, it was demonstrated that demembranated nuclei of MEL cells synthesize PIP and PIP_2 in vitro and this nuclear polyphosphoinositide synthesis becomes more pronounced if the cells are differentiated erythrocytes (Cocco et al., 1987a) by DMSO (Friedman and Schildkraut, 1977). Subsequently, several authors have confirmed and extended this observation. Furthermore, it has been shown that other nuclear phospholipid species are likely to be involved in signal transduction pathways. Prior to this finding, nuclear phospholipids have generally been regarded as minor chromatin components capable of regulating DNA stability, RNA synthesis, and gene expression (e.g. Manzoli et al., 1974; Capitani et al., 1986; Maraldi et al., 1992a).

In this article, we shall attempt to review the

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possible biological actions of lipid second messengers identified so far at the nuclear level. In particular, we will mainly focus on nuclear polyphosphoinositide metabolism changes which have been described to occur during cell growth and differentiation. However, we will also examine modifications in the metabolism of messengers derived from other molecules, such as PC, sphingomyelin, and arachidonic acid. Besides the biochemical evidence, we also intend to summarize the morphological data which have provided an important insight about the subnuclear localization of lipids and the enzymes involved in their metabolism.

Nuclear polyphosphoinositides and cell proliferation

The first report concerning the activation of a nuclear phosphoinositide metabolism following mitogenic stimuli was published in 1988 by Cocco et al., and showed results obtained with Swiss 3T3 cells which possess a wide variety of receptors for different growth factors (Rozengurt, 1986). It was demonstrated that in vitro incorporation of [γ-^{32}P]ATP into nuclear PIP and PIP_2 was decreased after 2 min of mitogenic stimulation with IGF-I. At 30 min after stimulation, no changes could be detected. In contrast, no variations were measured at any time in whole cell lysates or in nuclear fraction prepared from cells stimulated with bombesin, another powerful mitogenic factor for 3T3 fibroblasts. It is worth emphasizing that IGF-I acts through a classical tyrosine kinase receptor but does not activate the inositol lipid cycle at the plasma membrane.

These observations were subsequently corroborated by experiments in which Swiss 3T3 cells were labeled in vivo with high levels of [^{3}H]myoinositol and radioactivity in nuclear inositol lipids was measured. Treatment of cells for 2 min with mitogenic concentrations of IGF-I caused a marked decrease in nuclear PIP and PIP_2. Again, no similar changes were measurable in cell lysates (Cocco et al., 1989). Activation of nuclear inositide metabolism by IGF-I was related to the subsequent translocation to the nucleus of PKC (Martelli et al., 1991a). Radiolabeling of inositides in the nucleus by [^{3}H]myoinositol occurs at a very low level and all radiolabeling experiments are subject to difficulties in interpretation unless equilibrium labeling can be demonstrated unequivocally. For these reasons, Divecha et al. (1991) set up mass assays of picomole sensitivity to measure PIP, PIP_2, and DAG level in isolated nuclei. By this approach they detected in membrane-stripped nuclei isolated from IGF-I-stimulated 3T3 cells a decrease in PIP and PIP_2, a concomitant increase in DAG, and a translocation of PKC to the nucleus. No changes in PIP, PIP_2, and DAG were seen in whole cell homogenates or in nuclei in which the envelope was still present. These authors hypothesized about a possible role played by nuclear DAG to serve as chemoattractant for translocation of PKC to the nuclear compartment. A direct link between nuclear polyphosphoinositide metabolism, PKC activation, and early events leading to cell division in response to the mitogenic stimulus determined by IGF-I in quiescent 3T3 fibroblasts was demonstrated by Martelli et al. (1991b). In fact, IGF-I unresponsive cells did not show a decrease in nuclear PIP_2 or nuclear translocation of PKC and did not enter S-phase.

A major breakthrough came in 1992, when Martelli et al. demonstrated the existence in nuclei of 3T3 mouse fibroblasts of an entirely separate signaling system both in synthesizing PIP_2 and in its breakdown caused by the presence of the ß1 isozyme of phosphoinositide-specific PLC. On the other hand, in these cells the γ1 isoform was confined to the cytoplasm and IGF-I treatment stimulated exclusively the activity of nuclear PLC-ß1. More recently, by inducing both the inhibition of PLC-ß1 expression by antisense RNA and its overexpression, it has been shown that this nuclear PLC isoform is essential for the onset of DNA synthesis following IGF-I (but not PDGF) stimulation of quiescent 3T3 cells (Manzoli et al., 1997). Moreover, the inducible overexpression of PLC-ß1 showed increased levels of this enzyme in the nucleus in cells which entered S-phase, thus strengthening the importance of this PLC in activating the onset of DNA replication mediated by IGF-I (Billi et al., 1997).

We have very recently reported that in Swiss 3T3 cells the IGF-I-dependent increase in nuclear DAG production can be inhibited by the specific PI-PLC inhibitor 1-*O*-octadeyl-2-*O*-methyl-*sn*-glycero-3-phosphocholine or by neomycin sulfate but not by the purported PC-PLC specific inhibitor D609, or inhibitors of PLD-mediated DAG generation. Treatment of cells with 1-*O*-octadeyl-2-*O*-methyl-*sn*-glycero-3-phosphocholine or neomycin sulfate inhibited translocation of PKC-α to the nucleus. Moreover, exposure of cells to 1-*O*-octadeyl-2-*O*-methyl-*sn*-glycero-3-phosphocholine, but not to D609, dramatically reduced the number of cells entering S-phase upon stimulation with IGF-I. Therefore it is conceivable that the only phospholipase responsible for generation of nuclear DAG after IGF-I stimulation of 3T3 cells is PI-PLC since when this activity is inhibited neither DAG rise is seen nor PKC-α translocation to the nucleus occurs. Furthermore, this PI-PLC activity appears to be essential for the G_0/G_1 to S-phase transition (Neri et al., 1998).

Compensatory hepatic growth is an informative and well characterized model for natural cell proliferation. Therefore, it has been extensively studied to analyze changes in nuclear polyphosphoinositide metabolism. After partial hepatectomy increase in nuclear inositol phosphates was detected by Kuriki et al. (1992) who found that PLC activity in isolated nuclei increased 2.5-fold at 20-22 hr during the S-phase. It has been shown that also in rat liver PLC-ß1 is specifically localized in the nucleus (Divecha et al., 1993), and nuclear DAG is increased during liver regeneration, peaking at around 20 hr following hepatectomy (Banfic et al., 1993). The occurrence of PLC-ß1 in rat liver nuclei has also been reported by Bertagnolo et al. (1995) who, however,

Table 1. Polyphosphoinositides and related enzymes identified at the nuclear level.

TREATMENT OR CELL CONDITION	TISSUE OR CELL TYPES	MOLECULES/ENZYMES INVOLVED	CELL RESPONSE	REFERENCE
IGF-I	Swiss 3T3	PIP, PIP_2	Proliferation	Cocco et al. 1988, 1989
	Swiss 3T3	PIP, PIP_2, DAG	Proliferation	Divecha et al. 1991
	Swiss 3T3	PIP_2	Proliferation	Martelli et al. 1991b
	Swiss 3T3	PIP_2/ PLCß1	Proliferation	Martelli et al. 1992
	Swiss 3T3	PLCß1	Proliferation	Manzoli et al. 1997
	Swiss 3T3	PLCß1	Proliferation	Billi et al. 1997
	Swiss 3T3	DAG	Proliferation	Neri et al. 1998
	Rat embryo fibroblasts	PKB/Akt	Proliferation	Andjelkovic et al. 1997
Partial hepatectomy	Liver	PI-PLC	Proliferation	Kuriki et al. 1992
	Liver	DAG	Proliferation	Banfic et al. 1993
	Liver	PLCδ4	Proliferation	Asano et al. 1994, Liu et al. 1996
	Liver	PLCγ1	Proliferation	Neri et al. 1997
Prolactin	Liver	PIP, PIP_2	Proliferation	Santi et al. 1992
Hydrocortisone	Liver	PLCß1/PLCγ1	Transcription	Santi et al. 1994
Aphidicolin	HeLa	PI, PIP, PIP_2	Proliferation	York and Majerus 1994
(Cell synchronization)	HL-60	PIP_2, DAG	Proliferation	Sun et al. 1997
DMSO	MEL	PIP_2	Differentiation	Cocco et al. 1987a
	MEL	DAG	Differentiation	Trubiani et al. 1990
	MEL	PLCß1	Differentiation	Martelli et al. 1994
	MEL	PIP, PIP_2	Differentiation	Martelli et al. 1995
	MEL	DAG/PLCß1	Differentiation	Divecha et al. 1995b
	MEL	PI-TP, PI, PLC 1	Differentiation	Rubbini et al. 1997
HMBA	MEL	PI, PIP, PIP_2	Differentiation	Capitani et al. 1991
Tiazofurin	MEL	PIP, PIP_2	Differentiation	Billi et al. 1993
	MEL	PLCß1	Differentiation	Manzoli et al. 1995
Interferon α (IFN)	Daudi	PIP_2, DAG	Growth arrest	Cataldi et al. 1990
	Daudi	DAG/PI-PLC	Growth arrest	Cataldi et al. 1993a
	Daudi	PLC 1	Growth arrest	Cataldi et al. 1994a, 1995a
	Daudi IFN resistant	PIP_2/ PLC 1	Transformation	Cataldi et al. 1993b
Phorbol Esters	KM-3	PIP_2	Differentiation	Trubiani et al. 1993
All-*trans* retinoic acid	HL-60	PLCß2,γ1,γ 2	Differentiation	Bertagnolo et al. 1997
NGF	PC12	PLCδ1	Differentiation	Neri et al. 1993
		PI 3-kinase	Differentiation	Neri et al. 1994
Interleukin 1α	Saos-2	PIP, PIP_2/PLCß1	Differentiation	Marmiroli et al. 1994
	Saos-2 MR	PIP_2/PLCß1	Transformation	Zini et al. 1997
Sperm maturation	Germinal cells	PIP_2/PLCß1	Differentiation	Caramelli et al. 1996
Tumor growth	A431, HeLa, MH22A	PLCγ1	Transformation	Diakonova et al. 1997
Ionizing radiation	MEL	PI, PIP, PIP_2	DNA repair	Rana et al. 1994
EPO	B6Sut.EP	DAG	Differentiation	Mallia et al. 1997

described the presence in this cell compartment of PLC-γ1, but not of -δ1. By means of immunostaining and confocal microscopy analysis, nuclear PLC-γ1 showed a marked decrease at 3 and 16 hr after partial hepatectomy but a clear increase at 22 hr, whereas the ß1 isozyme was not modified (Neri et al., 1997). These modifications were paralleled by changes in the phosphodiesterase activity on PIP_2 that reached the highest value at 22 hr. Nevertheless, Asano et al. (1994) and Liu et al. (1996) discovered and characterized a novel PLC in rat ascite hepatoma cells, member of the δ family and named PLC-δ4, that is dominantly present in the nucleus. Nuclear PLC-δ4 dramatically increases at the transition from G_1 to S-phase during liver regeneration. Interestingly, this isoform is also present in the nucleus of mouse 3T3 fibroblasts and is more abundantly expressed during the S-phase.

Changes in nuclear inositol lipid metabolism have also been observed in isolated rat liver nuclei incubated with prolactin, a known hepatic mitogen (Santi et al., 1992). PLC-ß1 and -γ1 activities strongly increased in rat liver nuclei prepared from animals with high hydrocortisone blood levels, suggesting that inositol lipid metabolism may play a role in the nuclear modifications accompanying steroid hormone induction of transcriptional activity (Santi et al., 1994).

Additional information on a possible role played by nuclear inositol lipids during the different phases of the

cell cycle has been obtained from investigations performed using synchronized cell populations. In HeLa cells labeled with [^{3}H]myoinositol, the levels of inositol lipids were measured at various times during the S-phase. While the levels of cytoplasmic PI, PIP, and PIP_2 did not vary throughout the cell cycle, in isolated nuclei the levels of inositol lipids decreased by over 50% during the S-phase and returned to original levels after it (York and Majerus, 1994). In aphidicolin-synchronized HL-60 human promyelocytic leukemia cells, the levels of nuclear DAG fluctuate during the cell cycle. In particular, DAG levels in G_2/M-phase are 3-fold higher than in G_1-phase and this results in translocation and activation of PKC-ßII at the nuclear level with a subsequent phosphorylation of its mitotic nuclear envelope substrate lamin B (Sun et al., 1997). Experiments with the specific PI-PLC inhibitor, 1-*O*-octadeyl-2-*O*-methyl-*sn*-glycerol-3-phosphocholine, indicated that this enzyme is responsible for the generation of DAG from PIP_2.

It could also be reminded that inositol polyphosphate 1-phosphatase was detected within the nucleus of several cell lines (Madin-Darby canine kindey cells, HeLa, and COS-7 cells) by indirect immunofluorescent staining (York et al., 1994). The enzyme hydrolyzes the 1-phosphate from inositol 1,4-bisphosphate and inositol 1,3,4-trisphosphate. Since in cells transiently over-expressing this enzyme DNA synthesis was reduced 50% in comparison to controls, the authors proposed that it degrades stimulatory inositol phosphate(s) and thereby inhibits genome duplication in agreement with a previous suggestion by Sylvia et al. (1988). In this connection, it is worth recalling that both PI and PIP_2 have been shown to activate DNA polymerase α in vitro (Sylvia et al., 1986a,b).

Nuclear polyphosphoinositides and cell differentiation

Following the original report by Cocco et al. (1987a) showing increased phosphorylation levels of PIP_2 in nuclei isolated from MEL cells induced to differentiation with DMSO, several papers concerning changes in nuclear inositol lipids observed in this cell system have been published. An early report by Trubiani et al. (1990) demonstrated that the levels of newly synthesized DAG decrease in nuclei isolated from MEL cells terminally differentiated in the erythroid pathway. Since these changes were not seen in whole cell lysates, the results were consistent with the hypothesis that nuclear lipids and namely the products of polyphosphoinositide hydrolysis, such as DAG, are involved in the regulation of the processes leading to the differentiation. Using an alternative differentiating agent, HMBA, it was shown that in MEL cells nuclear polyphosphoinositides underwent dramatic changes which were not seen in the cytoplasmic fraction (Capitani et al., 1991). Further studies showed that when MEL cells undergo terminal erythroid differentiation in the presence of DMSO the nuclear PLC-ß1 isoform was down-regulated as demonstrated by immunochemical and immunocytochemical analysis (Martelli et al., 1994). In contrast, the amount of nuclear PLC-γ1 and its activity were unaffected by erythroid differentiation. In addition, when PI, PIP, and PIP_2 were labeled with high levels of [^{3}H]myoinositol and measured in isolated nuclei of DMSO-differentiated cells, an increase in PIP and PIP_2 was seen (Martelli et al., 1995). A decrease in nuclear DAG and PLC-ß1 activity have also been reported by Divecha et al. (1995b). Therefore, the differentiation process toward erythrocytes in MEL cells is accompanied by a reduction in the amount of these messengers in the nucleus. Furthermore, the presence of a nuclear PLC-ß1, the activity and expression of which are modulated during differentiation of MEL cells, implicates a role for nuclear phosphoinositide signaling in the processes of cell determination and indicates nuclear PLC-ß1 as a key enzyme of the cycle in relation to the erythroid differentiative commitment of these cells.

Erythroid differentiation in MEL cells can also be induced by the anti-tumor drug tiazofurin, an inhibitor of IMP-dehydrogenase (Olah et al., 1988). Changes in both synthesis and breakdown of PIP and PIP_2, due to the inhibition of the nuclear PLC activity, were detected in the nucleus, indicating nuclear inositol lipid cycle as a target of tiazofurin (Billi et al., 1993). Further studies have demonstrated that the nuclear PLC-ß1 isoform is down-regulated by tiazofurin, as analyzed by immunoblotting (Manzoli et al., 1995).

An additional enzyme involved in polyphosphoinositide metabolism found in isolated MEL cell nuclei is phosphatidylinositol-transfer protein, an enzyme characterized by its ability to transfer and exchange phospholipids between membranes. It has been suggested that this enzyme delivers PI to lipid kinases to yield PIP and PIP_2, and at least two isoforms of phosphatidylinositol-transfer protein, referred to as α and ß, have been identified (Wirtz, 1991; Thomas et al., 1993; Cockroft, 1997). In differentiated MEL cells, the amount of nuclear phosphatidylinositol-transfer protein α decreases in comparison to nuclei prepared from control cells (Rubbini et al., 1997), thus suggesting a direct relationship between the traffic of PI, its kinases and nuclear PLC-ß1. Indeed, when nuclear PLC-ß1 is down-regulated phosphatidylinositol-transfer protein is scarcely present in the nucleus suggesting that the requirement for the substrate is linked to the state of activity of the PLC-coupled signaling system.

EPO is a hormone, as a well as a hematopoietic growth factor, that specifically regulates differentiation and proliferation of erythroid progenitor cells. Even though the EPO receptor has no intrinsic kinase activity, it triggers the activation of protein kinases via PLA_2, PLC, and PLD. A cascade of serine/threonine kinases, which include Raf-1, MAP kinase, and PKC is then activated. In the murine target cell line B6Sut.EP, EPO induces a rapid rise in the mass of nuclear DAG as well as translocation of PKC-ßII. These phenomena become

evident after 1 min of stimulation with EPO (Mallia et al., 1997).

Nuclear polyphosphoinositide cycle has also been studied in lymphoid and myeloid cell lines exposed to a variety of differentiating molecules. For example, brief treatment of Daudi lymphoma cells with interferon α (a cytokine with a growth inhibitory effect) resulted in an enhanced rate of PIP_2 phosphorylation and an increase in the DAG mass at the nuclear level (Cataldi et al., 1990). Such an increase in the nuclear DAG mass was the result of activation of PI-PLC (Cataldi et al., 1993a) which has been identified as the ß1 isoform (Cataldi et al., 1994a, 1995a). Cataldi et al. (1994b) have also shown that interferon treatment induces an increase in the exogenous PI nuclear uptake and processing in comparison to control. It is also worth mentioning that interferon can modulate nuclear polyphosphoinositide metabolism in MEL cells (Miscia et al., 1991). Finally, in human T lymphocytes, interferon ß increases the expression of PLC-γ1 at the nuclear level (Cataldi et al., 1995b).

In the lymphoblastoid cell line KM-3, phorbol esters promote differentiation that is accompanied by a decrease in the levels of terminal deoxynucleotidyl transferase and an increase in the phosphorylation levels of nuclear PIP_2 (Trubiani et al., 1993). The human promyelocytic cell line HL-60 undergoes differentiation into morphologically and functionally mature granulocyte-like cells in the presence of all-*trans*-retinoic acid (Collins, 1987). Upon this treatment, PLC-ß2 appears inside nuclei and is up-regulated until 72 hr (Bertagnolo et al., 1997). Also nuclear ß3 isozyme is increased until 72 hr and both the isoforms lower their intranuclear amount at 96 hr. In contrast, PLC-γ1 and -γ2 isozymes progressively increased in the nucleus during granulocytic differentiation even after 72 hr of treatment. PIP_2 and PIP hydrolysis paralleled the prevalence of the ß or γ subfamily, respectively. It was concluded that the nuclear translocation of PLC-ß2 candidates this isoform as a key enzyme in the granulocytic differentiative commitment of HL-60 cells.

The rat pheochromocytoma PC12 cell line differentiates into sympathetic neurons under NGF treatment (Levi Montalcini, 1987). In unstimulated cells PLC-ß1 was detected in the nucleus (Mazzoni et al., 1992; Neri et al., 1993) whereas PLC-δ1 was only cytoplasmic. PLC-γ1 was found in both cell compartments. Upon NGF treatment, the amount of intranuclear δ1 isozyme increased (Neri et al., 1993). Immunocytochemical analysis of NGF-treated PC12 cells also showed that PI 3-kinase translocates into the nucleus (Neri et al., 1994). This enzyme phosphorylates the D3 position of PIP_2 to produce PIP_3 (Carpenter and Cantley, 1996). PIP_3 has been shown to activate Ca^{++}-independent PKC isoforms (Nakanishi et al., 1993) such as PKC-ζ, the presence of which has been described at the nuclear level (Rosenberger et al., 1995; Zauli et al., 1996; Zhou et al., 1997). Alternatively, nuclear PIP_3 might be involved in the regulation of PLC-γ isozymes (see later and Bae et al., 1998).

The human osteosarcoma cell line Saos-2 is sensitive to interleukin 1α, a cytokine which promotes gene expression and bone resorption (Maraldi et al., 1993b). Treatment of cells with this molecule induces a rapid and transient increase in nuclear PLC activity (which is due to PLC-ß1 isozyme) as well as a decrease in nuclear PIP and PIP_2 levels (Marmiroli et al., 1994).

Sperm maturation can be considered a natural model of terminal cell differentiation (Skinner, 1991). In the maturing cell lineage of the seminiferous tubule PLC-ß1 isoform was found to be predominantly nuclear while PLC-γ1 was mainly cytoplasmic. The ß1 isozyme was almost absent in nuclei of late spermatids and absent in spermatozoa. It is interesting to note that PIP_2 accumulated in the nuclei of late spermatids and spermatozoa, indicating a decrease in PLC activity (Caramelli et al., 1996).

Nuclear polyphosphoinositides, cell transformation, drug resistance, and DNA repair

Diakonova et al. (1997) have demonstrated by fluorescent immunocytochemical staining that PLC-γ1 is present only in nuclei of highly transformed cell lines (A 431, HeLa, mouse hepatoma MH 22A, rat Zajdela ascitic hepatoma) and is not observed in nuclei of primary embryo skin or lung fibroblasts. Therefore, this distinctive cellular localization in normal and highly transformed cell lines may reflect differences in cell signaling systems and mitogenic cell transduction.

Daudi lymphoma cells that are resistant to interferon treatment did not show the well-characterized changes of nuclear polyphosphoinositide metabolism that were seen in interferon-responsive cells (Cataldi et al., 1993b). Moreover, an increase in both nuclear PLC-ß1 and PIP_2 was detected in multidrug-resistant Saos-2 cells in comparison to controls, thus hinting at the fact that elements of the nuclear signal transduction system may be related to a particular phenotype (Zini et al., 1997). The involvement of nuclear inositol lipids in processes related to DNA repair which follows ionizing radiation exposure was also demonstrated by Rana et al. (1994).

In Table 1 we summarized polyphosphoinositide-related molecules and enzymes involved at the nuclear level in the cellular responses described above.

Other nuclear lipids involved in signaling events

Even though much of the current research has focused on polyphosphoinositides, a growing body of evidence indicates that other nuclear lipids are involved in signaling events. Besides inositol lipids, nuclear DAG may be generated by PC hydrolysis. Usually, DAG produced by PIP_2 hydrolysis are seen immediately after agonist stimulation, but sustained presence of DAG generally reflects involvement of other lipids (Nishizuka, 1992). Nuclear PC was found to increase after interferon α treatment of Daudi lymphoma cells

Table 2. Other lipids and related enzymes identified at the nuclear level.

TREATMENT OR CELL CONDITION	TISSUE OR CELL TYPE	MOLECULES/ENZYMES INVOLVED	CELL RESPONSE	REFERENCE
Interferon α	Daudi	PC	Growth arrest	Miscia et al. 1990
α-thrombin	IIC9	DAG/PKC	Proliferation	Leach et al. 1992
	IIC9	PC, DAG	Proliferation	Jarpe 1994
	IIC9	PLD, RhoA	Proliferation	Baldassare et al. 1997
ATP	MDCK	DAG/PLD	-	Balboa et al. 1995
Phorbol esters	MDCK	DAG/PLD	-	Balboa and Insel 1995
Angiotensin II	Vasc. smooth muscle c.	PLA_2	Proliferation	Freeman et al. 1998
All-*trans* retinoic acid	HL-60	Arachidonic acid	Differentiation	Matsumoto et al. 1994
Vitamin D3	HL-60	Arachidonic acid	Differentiation	Matsumoto et al. 1994
Partial hepatectomy	Liver	Oleic, arachidonic. acid	Proliferation	Ishihara et al. 1991
	Liver	Docasahexaenoic acid	Proliferation	Ishihara et al. 1991
	Liver	Sphingomyelinase	Proliferation	Ales
enko and Chatterjee 1995				
		Sphingomyelinase	Proliferation	Albi and Viola Magni 1997
A23187	Macrophages	5-Lipoxygenase	Cell motility	Woods et al. 1995
	Neutrophils	5-Lipoxygenase	Cell motility	Brock et al. 1997
~~G_2 phase of cell cycle~~	~~HL-60~~	~~Phosphatidylglycerol~~	~~Proliferation~~	~~Murray et al. 1998~~

(Miscia et al., 1990) and an enzyme involved in PC biosynthesis, CTP:phosphocholine cytidyltransferase, has been identified by immunofluorescent staining in the nucleus of several cell lines (HepG2, NIH 3T3, L-cells) as well as in rat liver tissue (Wang et al., 1993). Cell fractionation experiments provided evidence that over 95% of the enzyme is located in the nucleus of L-cells. In IIC9 fibroblasts, PC hydrolysis is responsible for the generation of nuclear DAG at all times following stimulation with α-thrombin (Jarpe et al., 1994). In this experimental model, production of DAG is followed by translocation to the nucleus of PKC-α (Leach et al., 1992). PC is the major substrate of PLD, which, however, in some organisms and tissues, can act on PE and PI. Activation of PLD results in the production of PA, which, in turn, is acted upon by PA phosphohydrolase to produce DAG (Exton, 1997). A PLD-mediated pathway for generating DAG in nuclei from Madin-Darby canine kidney cells has been described and its activity was enhanced by either phorbol esters or ATP (Balboa et al., 1995). Further studies have shown that activation of nuclear PLD is mediated by the small GTP-binding protein RhoA (but not by RhoB) and is downstream of PKC, as demonstrated by the fact that PKC inhibitors could block PLD stimulation (Balboa and Insel, 1995). As expected, also in IIC9 cells α-thrombin stimulation of nuclear PLD activity, which follows translocation to the nucleus of RhoA, has recently been described (Baldassare et al., 1997). It should be emphasized that in the aforementioned investigations, PLD activity was detected in nuclei not exposed to detergents. Indeed, if IIC9 cell nuclei were prepared in the presence of detergents, neither DAG nor PKC activity could be detected, suggesting that these signal transduction events take place in the nuclear envelope (Raben et al., 1994). Therefore, these authors envisioned the envelope as the most important domain for cell signaling pathways operating at the nuclear level. However, Clark et al. (1997) have recently shown that HL-60 cell nuclei lacking the envelope still possess a PLD activity which is insensitive to the ADP-ribosylation factor, ARF. Therefore, the enzyme, other than at the nuclear periphery, must be present further inside the nucleus. Other investigators have demonstrated that acidic phospholipids (such as PIP_2, PS, and cardiolipin) inhibit nuclear PLD activity, hinting at possible cross-talks between different signal transduction pathways taking place in the nuclear compartment (Kanfer et al., 1996). However, it should be remembered that recent work by Pettitt et al. (1997) has demonstrated that DAG and phosphatidate generated by PLC and PLD, respectively, have distinct fatty acid compositions and functions since PLD-derived DAG does not activate PKC in porcine aortic endothelial cells.

The characterization of physiological activator(s) of nuclear PKC has also been tackled by Murray and Fields (1998) who identified in phosphatidylglycerol a lipid that can regulate the activity of the ßII isozyme at the nucleus.

PC (and PE) can also be hydrolyzed by PLA_2 resulting in the generation of arachidonic acid. The presence of PLA_2 at the nuclear level in rat ascite hepatoma cells was first demonstrated by Tamiya-Koizumi et al. (1989a). Also in this case acidic phospholipids (PIP and PIP_2) inhibited enzymic activity (Tamiya-Koizumi et al., 1989a). A nuclear PLA_2 activity has been identified in rat liver by other investigators (Neitcheva and Peeva, 1995). Interestingly, in sub-confluent bovine endothelial cells PLA_2 resides prominently in the nucleus, whereas in confluent cultures it becomes primarily cytoplasmic (Sierra-Honigmann et al., 1996). Nuclear localization was also

seen in HeLa and Madin-Darby canine kidney cells, but in this case no changes were detected upon reaching confluency. It is of interest that histamine caused a rapid and dose-dependent redistribution of PLA_2 from nucleus to nuclear envelope in subconfluent endothelial cells and from cytoplasm to nuclear envelope and plasma membrane (at intercellular junctions) in confluent cells (Sierra-Honigmann et al., 1996). Freeman et al. (1998) have recently reported that angiotensin II is capable of inducing a nuclear translocation of PLA_2 in vascular smooth muscle cells.

The metabolism of arachidonic acid has been intensely studied due to the fact that metabolites of this fatty acid, collectively known as eicosanoids, constitute an important and powerful class of second messengers. Almost all of arachidonic acid in unstimulated cells is found esterified to phospholipids. To release arachidonic acid from phospholipids two principal mechanisms exist: hydrolysis from intact phospholipids by means of PLA_2 (see above) or generation of DAG (usually by means of a PLC) followed by its hydrolysis. Early investigations by Neufeld et al. (1985) showed that after [^{3}H]arachidonate labeling, the nuclear membrane exhibited the highest specific activity. Moreover, it has been found that upon stimulation of a mouse fibrosarcoma cell line with bradykinin, arachidonate preferentially originated from precursors most recently incorporated into nuclear membrane phospholipids (Capriotti et al., 1988). Nuclei contain enzymes involved into arachidonic acid metabolism, such as 5-lipoxygenase that catalyzes conversion of arachidonic acid to leukotriene. Such an enzyme resides in the nuclear envelope of activated human lymphocytes (Woods et al., 1993), while it is located in the euchromatin in resting human alveolar macrophages and translocates to the nuclear envelope upon cell activation by the calcium ionophore A23187 (Woods et al., 1995). However, others have found that in neutrophils cytosolic 5-lipoxygenase is rapidly imported into the nucleus after stimulation with glycogen. Upon activation with A23187, the intranuclear 5-lipoxygenase translocated to the nuclear envelope (Brock et al., 1997). Rat liver cell nuclei were also found to contain another of the key enzymes of arachidonic acid metabolism, i.e. Δ-5 desaturase (Ves-Losada and Brenner, 1995). Differences in the nuclear arachidonic acid metabolism have been described following treatment of HL-60 cells with differentiating agents such as retinoic acid or vitamin D3 (Matsumoto et al., 1994). Recent data from Surette and Chilton (1998) have revealed that nuclei from monocyte-like human THP-1 cells contain 22% of total cellular arachidonate and the distribution of arachidonyl-phospholipids in nuclei is markedly different from that of cell membranes. Isolated nuclei contain 25-30% of the cellular activity of CoA-independent transacylase, the key enzyme responsible for arachidonate-phospholipid remodelling. Recent evidence by Catalan et al. (1997a) demonstrates that arachidonic acid may be involved in the regulation of nuclear Ca^{++} mobilization.

Currently, much interest surrounds sphingomyelin because several cell surface receptors have been shown to generate signals triggering the hydrolysis of this lipid to generate diffusible ceramides, which can regulate the activity of protein kinases and/or phosphatases (Testi, 1996). For example, it is now recognized that ceramide inactivates PKC-α (Lee et al., 1996). A Mg^{++}-dependent, neutral sphingomyelinase has been identified in nuclei of both rat ascites hepatoma cells (Tamiya-Koizumi, 1989b) and normal liver cells (Neitcheva and Peeva, 1995). The activity of nuclear sphingomyelinase has been reported to increase during liver regeneration (Alessenko and Chatterjee, 1995; Albi and Viola Magni, 1997).

Sphingolipids such as sphingosine and sphingosylphosphorylcholine have been shown to be present in nuclei and might be involved in regulating intranuclear Ca^{++} concentration (Catalan et al., 1997b).

Given the importance of fatty acid composition of DAG involved in the possible activation of PKC isoforms in the nucleus, studies on this issue would seem desirable. However, the available evidence is quite limited. The fatty acid compositions of nuclear phospholipids (PC, PE, PI, and PS) showed changes which are associated with liver regeneration. Indeed, at 28 hr post-hepatectomy oleic acid at the 2-position increased transiently at the expense of arachidonic acid and docasahexaenoic acid. In contrast, changes at the 1-position were quite limited (Ishihara et al., 1991).

The composition of phospholipid molecular species (PC and PE) of mouse liver nuclei was shown by Chapkin et al. (1992) to be influenced by dietary eicosapentaenoic and docohexaenoic acid ethyl esters which, therefore, could potentially regulate nuclear functions.

In Table 2 we summarized other nuclear lipids and related enzymes involved in the cellular responses described above.

Topographical localization of nuclear lipids

Although several of the papers listed in the previous sections reported data concerning nuclei not exposed to detergents (e.g. Jarpe et al., 1994; Balboa et al., 1995; Balboa and Insel 1995; Baldassare et al., 1997) many investigators have shown that demembranated nuclei retain a significant percentage of lipids and lipid-related enzymes (e. g. Cocco et al., 1987a,b; Divecha et al., 1991; Martelli et al., 1992; Previati et al., 1994; Vann et al., 1997). Actually, in some cases, changes in phosphoinositide metabolism have only been detected in nuclei stripped of their membrane (see for example Divecha et al., 1991; York and Majerus, 1994), indicating that phospholipids are present not only at the nuclear periphery but also further inside. To localize where intranuclear lipids are located, subfractionation techniques and morphological approaches have been widely utilized.

Cocco et al. (1987b) reported that phospholipids are

Table 3. Summary of topographical localization of nuclear lipids and related enzymes identified by morphological and biochemical investigations.

NUCLEAR DOMAIN	MOLECULES/ENZYMES INVOLVED	TISSUE OR CELL TYPE	REFERENCE
Nuclear matrix	Phospholipids	Liver	Cocco et al. 1987b
	Phospholipids	Liver	Miscia et al. 1988
	PLA_2	Rat hepatoma cells	Tamiya-Koizumi et al. 1989a
	PLA_2	Liver	Neitcheva and Peeva 1995
	Polyphosphoinositides	Liver	Capitani et al. 1989
	PIP kinase, DAG-kinase, PLC	Liver, NIH 3T3	Payrastre et al. 1992
	PLCß1	Swiss 3T3	Zini et al. 1993
	PLCß1/PLCδ1	Saos-2	Maraldi 1993a, Marmiroli et al. 1995
	PLCß1/ PLCδ1	PC12	Zini et al. 1994
	PLCß1/ PLCδ1	MEL	Zini et al. 1995
	PIP_2	MEL, Swiss 3T3	Mazzotti et al. 1995
	PI 3-kinase	Saos-2	Zini v1996b
Nuclear lamina	PI kinase	Liver, NIH 3T3	Payrastre et al. 1992
Nuclear interior	DAG kinase	Liver	Previati et al. 1994
	PIP, PIP_2 phosphomonoesterase	Liver	Vann et al. 1997
	PA hydrolase	Liver	Vann et al. 1997
	PI 3-kinase	Liver	Lu et al. 1998
	PLC ß1b	C6Bu-1	Bahk et al. 1998
Nuclear membrane	PIP_2	Liver	Tran et al. 1993
Chromatin	PIP_2	RL-34C (Calf thymus)	Yu et al. 1998
Nuclear matrix and RNA	Phospholipids	Liver	Zini et al. 1989
	PLC	Liver, Pancreas	Maraldi et al. 1992b
Replicating DNA	Phospholipids	Liver	Maraldi et al. 1993b
Inner nuclear membrane	IP_3 receptor	Liver	Malviya et al. 1990
	IP_4 receptor	Liver	Koppler et al. 1993, 1996
Outer nuclear membrane	IP_4 receptor	Liver	Koppler et al. 1993, 1996

components of the nuclear matrix and treatment with PLC reduced nuclear matrix-bound DNA polymerase α activity (Miscia et al., 1988). Moreover, PLA_2 activity is associated with the nuclear matrix (Tamiya-Koizumi, 1989a; Neitcheva and Peeva, 1995). The nuclear matrix (also called nuclear scaffold) is a proteinaceous entity that resists treatment of isolated nuclei with detergents, solutions of high ionic strength, and nucleases (Berezney et al., 1995; Martelli et al., 1996a). Also RNA is a main component of the nuclear matrix (Berezney et al., 1995). Many different functions, such as DNA replication and repair, RNA synthesis, processing and transport, protein phosphorylation, etc. are thought to occur in tight association with the matrix, which would act as a dynamic skeletal structure of the nucleus (Martelli et al., 1996a). Three distinct domains can be identified in the nuclear matrix at the ultrastructural level: a peripheral lamina, an internal fibrogranular network, and nucleolar remnants (Berezney et al., 1995). Capitani et al. (1989) first showed that enzymes of the polyphosphoinositide cycle are associated with the nuclear matrix in rat liver. Using nuclear subfractionation techniques allowing the separation of the outer lamina from the inner fibrogranular network, Payrastre et al. (1992) showed that phosphatidylinositol 4-kinase is exclusively located in the peripheral lamina of both rat liver and NIH 3T3 fibroblasts, while phosphatidylinositol(4) phosphate 5-kinase, DAG kinase, and PLC activities are found preferentially associated with the internal matrix. A very detailed analysis of metabolism and possible compartmentalization of inositol lipids in isolated rat liver nuclei was very recently performed by Vann et al. (1997). These authors have reported that in nuclei deprived of their membrane by treatment with 0.04% Triton X-100, 36% of DAG and 24% of PIP originally present in nuclei with an intact envelope were still detectable. The recovery of PIP_2 was higher (about 40%) demonstrating that this lipid has an intranuclear location, probably bound to nuclear proteins. The addition of exogenous substrate (DAG, PI, and PIP) to membrane-depleted nuclei resulted in reconstitution of the majority of lipid phosphorylation from [γ-^{32}P]ATP, suggesting a predominantly intranuclear location for the respective kinases. An intranuclear localization of DAG kinase in rat liver has previously been reported also by Previati et al. (1994). Vann et al. (1997) were also able to demonstrate the presence in rat liver nuclei of both phosphomonoesterase (acting on PIP and PIP_2) and PA hydrolase activity. Nevertheless, some of the radioactivity incorporated in vitro into these lipids from [γ-^{32}P]ATP was resistant to these enzymes suggesting the existence of multiple pools of intranuclear inositol lipids.

In several of the papers listed in the previous sections, immunofluorescent staining was employed to study the intranuclear location of lipid-related enzymes (e. g. Neri et al., 1993, 1997; York et al., 1994;

Bertagnolo et al., 1997; Diakonova et al., 1997). However, to more precisely map the exact location of these molecules, immunocytochemical investigations at the ultrastructural level have been widely utilized.

Immunoelectron microscopy studies have revealed that PLC-ß1 is present in the inner matrix isolated from Swiss 3T3 fibroblasts (Zini et al., 1993), Saos-2 cells (Maraldi et al., 1993a; Marmiroli et al., 1995), PC12 cells (Zini et al., 1994), and MEL cells (Zini et al., 1995). It should be noted that in some cases PLC-γ1 has also been found by immunogold to be located within the nucleus (Maraldi et al., 1993a; Zini et al., 1994, 1995). However, this isozyme is usually more abundantly present in the cytoplasm where it associates with cytoskeletal elements (Zini et al., 1993, 1994, 1995). Also PIP_2 has been immunochemically located at the ultrastructural level by means of a specific monoclonal antibody in the internal nuclear matrix (Mazzotti et al., 1995), while previous evidence, obtained by fluorescent immunostaining, had suggested that PIP_2 was mainly present at the nuclear membrane level (Tran et al., 1993). Since PIP_2 seems to be predominantly located inside the nucleus where no membranes exist, this phospholipid must be associated with some proteins, to form proteolipid complexes. It is well documented that PIP_2 binds to several polypeptides such as profilin, cofilin, gelsolin, gCap, α-actinin, vinculin, and filamin (Yu et al., 1998). Interestingly, several nuclear polypeptides, including lamin B, HMG proteins, DNA and RNA polymerases, nucleolin, DNA topoisomerase and helicase exhibit a PI-binding consensus sequence, that is K(X)n KXKK (where n=3-7) (Cocco et al., 1994). Some of these proteins (lamin B and DNA topoisomerase, for example) are abundant components of the nuclear matrix, thus suggesting that PIP_2 might be tightly bound to the nucleoskeleton. However, Yu et al. (1998) have recently identified histone H1 and H3 as additional nuclear PIP_2-binding proteins and the binding site for PIP_2 has been mapped to the carboxy-terminal 103 amino acids of H1. Moreover, these authors have been able to extract PIP_2 from purified histone H1 and H3 and have demonstrated that in vitro phosphorylation of H1 by PKC (but not by PKA or cdc2 kinase) decreases the amount of PIP_2 bound to the histone. In a Drosophila in vitro transcription system, PIP_2 binding to H1 counteracted the H1-mediated repression of basal transcription by RNA polymerase II. The effect of other inositol lipids (i.e. PIP and PIP_3) in the same system was much weaker, while PI and other acidic lipids had no effect. The authors suggested that PIP_2 binding to H1 may contribute to the regulation of transcription in eukaryotic cells, in agreement with previous suggestions by Capitani et al. (1986).

The ultrastructural approach has also revealed changes in the intranuclear amount of both PIP_2 and/or PLC-ß1 in cells exposed to mitogenic or differentiating stimuli (Maraldi et al., 1995; Zini et al., 1996a). At variance with the biochemical data by Payrastre et al. (1992), who measured enzyme activity, PI 3-kinase has been found to be matrix-associated by immunogold labeling in Saos-2 cells (Zini et al., 1996b). Recent data from Lu et al. (1998) confirmed that also rat liver nuclei contain PI 3-kinase.

A technique that has provided additional information about intranuclear lipids is labeling by means of PLC-colloidal gold particles (Maraldi et al., 1992b). Zini et al. (1989) suggested that nuclear phospholipids possibly mediate interaction between RNA and protein components of the nuclear matrix. This is in agreement with Albi et al. (1996) who showed by biochemical analysis that 48% of nuclear phospholipids are lost upon RNA removal.

PLC-colloidal gold labeling coupled with immunostaining for bromodeoxyuridine (a marker for the S-phase of the cell cycle) has also allowed the determination that S-phase nuclei contain a significantly lower amount of phospholipids in comparison to other phases of the cell cycle (Maraldi et al., 1993b).

In Table 3 we summarized the topographical localization of nuclear lipids and related enzymes identified by morphological and biochemical investigations

Concluding remarks

The existence of several nuclear signaling systems based on phospholipids is now universally accepted. However, several problems still remain unsettled. First of all, evidence points to the likelihood that nuclear lipids (or their second messengers) are involved in functions other than signaling, such as direct control of DNA replication and transcription. Moreover, cross-talks between different signaling systems are likely to take place at the nuclear level. For example, a Chinese hamster lung cell line defective in thrombin-induced signaling has been found to have low levels of nuclear PLC-ß1 (Fee et al., 1994). Interestingly, thrombin activation of PLD and PLA_2 is impaired in this cell line, perhaps because the deficiency in nuclear PLC-ß1 causes defective targeting of PKC-α to specific sites which may be required for activation of downstream phospholipases. Second, a problem that so far has only been partly tackled is a detailed study of PI turnover in isolated nuclei using phosphatidylinositol-transfer protein (Capitani et al., 1990). Indeed, this enzyme is likely to play an important role in the nucleus, also considering the fact that fluorescently-labeled phosphatidylinositol-transfer protein α, microinjected into foetal bovine heart endothelial cells, is mainly targeted to the nucleus (de Vries et al., 1996).

Another problem stems from the heterogeneity of PI-PLC isoforms that can be detected at the nuclear level. In this connection, it should be recalled that Kim et al. (1996) have demonstrated that particular regions of the COOH-terminal domain of PLC-ß isozymes, containing a high proportion of basic residues, are critical for permitting nuclear localization of this class of phospholipases. Indeed, transfection experiments of Rat

2 and Swiss 3T3 cells with PLC-ß1 mutants in the COOH terminus, have shown that substitution of the cluster of lysine residues 1056, 1063, and 1070 in the COOH-terminal domain almost completely abolishes nuclear localization of PLC-ß1. Members of the γ and δ subfamilies do not possess this long COOH-terminal sequence downstream of their catalytic domain, so that other sequences must be responsible for the nuclear localization capability. Nevertheless, it is now established that two forms of PLC-ß1 exist, which are generated by alternative splicing (Bahk et al., 1994). They are referred to as PLC-ß1a and PLC-ß1b, differing at their carboxy-terminal sequence of 43 amino acids that is present only in the PLC-ß1a form. It is interesting that in rat C6Bu-1 glioma cells PLC-ß1b was found by immunoblotting and immunocytochemistry to be predominantly located within the nucleus, while PLC-ß1a was located preferentially in the cytosol (Bahk et al., 1998), indicating that other subtle differences in the aminoacidic composition may have marked effects on the subcellular distribution of PLC isozymes.

At present, the most challenging issue is to understand how signals generated at the plasma membrane may regulate intranuclear signaling systems. It is difficult to explain how molecules such as prolactin (Santi et al., 1992) or HIV-Tat (Zauli et al., 1995) could directly activate inositol lipid cycle when incubated in vitro with purified nuclei. In these cases it is necessary to envision that these activators could interact with the kinases and/or phospholipases.

At the nuclear level the synthesis of both PIP and PIP_2 could be controlled by GTP-binding proteins, in analogy with plasma membranes (Martelli et al., 1996b). The presence of GTP-binding proteins at the nuclear level is well documented (e.g. Crouch, 1991; Saffitz et al., 1994). The small monomeric GTP-binding protein RhoA has been shown to activate PLD in the nucleus (Balboa and Insel, 1995). PLC-ß isozymes are regulated by members of the heterotrimeric GTP-binding protein family (Lee and Rhee, 1995; Rhee and Bae, 1997). Nevertheless, our unpublished data have shown that either GTP-γ-S or AlF_4 failed to stimulate PLC activity in Swiss 3T3 cell isolated nuclei. However, we have found that, after IGF-I stimulation of 3T3 cells, there is transient serine/threonine phosphorylation of PLC-ß1 which parallels enzyme activation (A.M. Martelli, unpublished data). In this connection, it should be mentioned that examination of PLC-ß1 sequence reveals the existence of a putative MAP kinase consensus sequence, P-S-S-P, at amino acids 980-983 (Bahk et al., 1994). Therefore, phosphorylation levels might regulate the activity of nuclear PLC-ß1 (Rhee and Bae, 1997). In any case, recent data from our laboratory have indicated that the existence of an intact cytoskeleton and activation of PI 3-kinase are essential for subsequent activation of PLC-ß1 in nuclei of Swiss 3T3 cells exposed to mitogenic concentrations of IGF-I (Martelli et al., submitted for publication). It might be that these events are located upstream of MAP kinase translocation to the nucleus (Kim and Kahn, 1997). Indeed, it is known that IGF-I activates MAP kinase in Swiss 3T3 cells (Hansonn and Thorén, 1995). A physiological target of PIP_3 is PKB/Akt (Meier et al., 1997). Three isoforms of PKB/Akt, referred to as α, ß, and γ, have been identified. Activation of PKBß is dependent on translocation to the plasma membrane followed by serine/threonine phosphorylation (Andjelkovic et al., 1997). Then, the activated enzyme translocates to the nucleus and these events can be elicited by several growth factors, including IGF-I (Meier et al., 1997). Thus, PKB/Akt might phosphorylate nuclear PLC-ß1. Activation of PLC-γ isoforms is regulated by either tyrosine residue phosphorylation or directly by several lipid-derived second messengers such as phosphatidic and arachidonic acid (Rhee and Bae, 1997). Recent evidence has indicated that PIP_3 is also capable of activating these isozymes by interacting with their Src homology 2 domains (Bae et al., 1998). Interestingly, the presence of PI 3-kinase at the nuclear level has been demonstrated (Neri et al., 1994; Zini et al., 1996b). Control of δ family of PLC has so far escaped clarification even though Ca^{++} could be directly involved (Rhee and Bae, 1997). In this sense, the presence of a phosphoinositide cycle at the nuclear level might be intimately related to the regulation of Ca^{++} in this organelle, a much debated issue which still awaits definitive resolution (see Santella and Carafoli, 1997, for an updated review on this problem). In any case, it should be reminded that the IP_3 receptor is located on the inner nuclear membrane, while the IP_4 receptor has been found to be present both in the inner and in the outer nuclear membrane. Interestingly, nuclear IP_3 receptor is a substrate for PKC (Malviya et al., 1990; Matter et al., 1993; Koppler et al., 1993, 1996; Malviya, 1994; Humbert et al., 1996).

In conclusion, nuclear signal transduction pathways are attracting more attention because they may provide many avenues by which nuclear functions are regulated. Identification of the plasma membrane to nucleus coupling mechanisms, regulative events and physiological roles of these pathways constitutes a new and intriguing area of research with a broad impact in our understanding of cell signaling phenomena.

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