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Višnjić, D., Banfić, H. (2007) *Nuclear phospholipid signaling: phosphatidylinositol-specific phospholipase C and phosphoinositide 3-kinase.* Pflügers Archiv : European Journal of Physiology, [Epub ahead of print].

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Nuclear phospholipid signaling: phosphatidylinositol-specific phospholipase C and

phosphoinositide 3-kinase

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Abstract

Over the last 20 years, numerous studies have demonstrated the existence of nuclear phosphoinositide signaling distinct from the one at the plasma membrane. The activation of phosphatidylinositol-specific phospholipase C (PI-PLC) and phosphoinositide 3-kinase (PI3K), the generation of diacylglycerol (DAG) and the accumulation of the 3-phosphorylated phosphoinositides have been documented in the nuclei of different cell types. In this review, we summarize some recent studies of the subnuclear localization, mechanisms of activation and the possible physiological roles of the nuclear PI-PLC and PI-3 kinases in the regulation of cell cycle, survival and differentiation.

1. Introduction and historical overview

Over the last 50 years, since Hokin and Hokin (30) first showed an increased phospholipid turnover in the cholinergically stimulated pancreatic tissue, inositol phospholipid cycle has been one of the most thoroughly investigated signaling mechanisms operating at the cell membrane. Phosphatidylinositol (PtdIns), precursor of phosphorylated derivatives or phosphoinositides, accounts for less than 15% of the total cell phospholipids. Phosphoinositides are formed by reversible phosphorylation of PtdIns at one or a combination of positions at the inositol ring (3', 4' or 5') by the action of several different kinases and phosphatases. Among seven phosphorylated products, phosphoinositide $PtdIns(4,5)P_2$ is the best known as it serves as a substrate for the classical receptor-activated signaling enzymes. Activated phosphatidylinositol - specific phospholipase C (PI-PLC) is responsible for the hydrolysis of $PtdIns(4,5)P_2$ into $Ins(1,4,5)P_3$ and DAG; $Ins(1,4,5)P_3$ is released to mobilize calcium from intracellular stores, and DAG activates protein kinase C (PKC). The receptor-mediated activation of phosphoinositide 3-kinase (PI3K) phosphorylates $PtdIns(4,5)P_2$ on 3' position of the inositol ring and generates $PtdIns(3,4,5)P_3$ that further activates several downstream signaling molecules. Receptor-mediated hydrolysis and phosphorylation of $PtdIns(4,5)P_2$ at the cell membrane regulates diverse cellular functions, such as cell proliferation, survival, vesicle trafficking and gene transcription. In addition, $PtdIns(4,5)P_2$ transduces signals directly, and regulates membrane traffic and cytoskeleton by binding to specific proteins containing phosphoinositide-binding modules and recruiting cytoskeletal and signaling proteins to the membrane (19, 24, 72).

In 1965, Rose and Frenster first pointed to the possible role that phosholipids may play in the nuclei as they detected that both the quantity and the intensity of phospholipid metabolism was much higher in active than repressed chromatin (62). In 1983, Smith and

Wells observed increased incorporation of ^{32}P into a mixture of phospholipids containing PtdIns(4)P and PtdIns(4,5)P$_2$ in nuclear envelopes isolated from rat liver nuclei (65). As the major route of PtdIns(4,5)P$_2$ synthesis involves the activation of type I PIP kinase that phosphorylates PtdIns(4)P on 5' position, these results suggested the existence of both PtdIns 4-kinase and type I PIP kinase in rat liver nuclei. In 1987, Cocco *et al.*, showed that highly purified nuclei from Friend cells, washed free of nuclear membrane by detergent, can incorporate radiolabeled phosphate into phospholipids, depending on the differentiative state of the cells (14). The further evidences that the nuclear event occurs autonomously were provided by experiments performed on starved Swiss 3T3 cells stimulated with two different agonists; while bombesin induced a PtdIns(4,5)P$_2$ hydrolysis and DAG generation at the cell membrane with no changes in the nuclear compartment, IGF-I had no effects on the cell membrane but decreased the level of nuclear PtdIns(4,5)P$_2$, increased the level of nuclear DAG and stimulated the translocation of PKC to the nuclei (20). These were the initial studies in the field that has expanded over the last 20 years, involving various signaling enzymes, substrates and signaling molecules operating in the nuclei. Although still much less explored and defined than the classical phospholipid signaling, the existence of a separate nuclear phospholipid cycle that occurs independently from the one at the plasma membrane is now universally recognized (reviewed in 15, 28, 33). In this review, we will try to summarize some recent studies of the potential location, the mechanism of activation and the possible physiological roles of PtdIns(4,5)P$_2$ hydrolysis and phosphorylation mediated by nuclear PI-PLC and PI-3 kinases.

2. The basic structure of nucleus – „nuclear" and "endonuclear"

As obvious from the preceding short historical introduction, the term „nuclear signaling" has been used to define findings in various nuclear structures including nuclear envelopes, total nuclei, and nuclei treated with detergents to remove nuclear membranes. As suggested in a recent review article (32), the term „endonuclear" should be used to specify phospholipid that colocalizes with nuclear matrix following demonstrated nuclear envelope removal, and a term „nuclear" or „perinuclear" where the extent of any nuclear envelope or peripherally associated endoplasmic reticulum removal is unknown. To better understand the difference between the two, it is useful to recapitulate briefly the basic structure of nucleus **(Fig. 1).** The nucleus is separated from the cytoplasm by the nuclear envelope consisting of two concentric membranes; the outer and inner nuclear membrane. Both membranes are phospholipid bilayers; the outer nuclear membrane is an extension of the endoplasmic reticulum containing ribosomes, and the inner membrane contains specific proteins that bind nuclear lamina and chromatin. Nuclear lamina is a protein meshwork that is attached to the inner nuclear membrane, providing structural support to the nucleus and interacting directly with chromatin. In chromatin, DNA is wrapped around the histone forming the basic unit or nucleosome, and the structure of chromatin determines the transcriptional activity, DNA replication in replication factories and DNA repair. While the transcription of genes occurs throughout the nucleus, the splicing is restricted to several morphologically distinct structures called nuclear speckles. What is left when membrane-depleted nuclei are treated with DNase, RNase and high salt buffers to remove DNA, RNA, histones and other proteins is a residual network of fibers that is called nuclear matrix and which is supposed to perform the same function as cytoskeleleton in the cytoplasm (32, 35). Therefore, it is not unexpected that some inositol lipids or enzymes can be found in nuclei containing envelopes as it is known that

inositol lipids are usual components of phospholipid bilayers of various cellular membranes. However, it is still less clear what is the form and structure of the inositol lipids that are still present in nuclei even after complete removal of all membranes by the use of strong detergents **and several possibilities, including the formation of a crystalline array type of structure provided by highly-saturated and more abundant phosphatidylcholine, have been proposed** (34). The metabolism of these lipids is what is usually meant by term „endonuclear" signaling (32). **As shown in Fig. 1, "endonuclear" PtdIns(4,5)P$_2$ has been reported to localize to nuclear speckles (60, 76).**

3. Nuclear phospholipase C

Several different isoforms of phospholipase C are grouped into 6 families (-β,-δ,-γ,-ε,-ζ and -η) based on their structure and mechanisms of activation (61). Domain organization of PLC isoforms that are most **frequently** described in nuclei is shown in Fig. 2.

3.1. Nuclear phospholipase C-β$_1$

IGF-mediated PtdIns(4,5)P$_2$ hydrolysis and DAG generation in membrane-depleted nuclei of Swiss 3T3 cells pointed to the possible involvement of phospholipase C (20), and the activation of the nuclear PI-PLC-β$_1$ in response to IGF-I was later confirmed (46, 47). *In vivo* model of regenerating rat liver showed the similar increase in the level of the nuclear DAG 20 hours after partial hepatectomy (5). The early immunoanalysis of isolated rat liver nuclei for the presence of various PI-PLC isoforms demonstrated the nuclear presence of PI-PLC-β$_1$ (21). Therefore, among several different PI-PLC isoforms, PLC-β$_1$ became the most intensively investigated as a major PLC isoform involved in nuclear signaling.

PI-PLC-β is actually a whole family of enzymes consisting of four principal members ($-\beta_1$, $-\beta_2$, $-\beta_3$, and $-\beta_4$) that all contain a region at their C-terminal domain responsible for the nuclear localization (61). In addition, there are two splice variants of the PI-PLC-β_1 isoform, PI-PLC-β_{1a} and PI-PLC-β_{1b}, and several studies demonstrate that b splicing variant is the one that is predominantly nuclear (4, 25, 40, 47). The „classical" PI-PLC-β, i.e. the one operating at the cell membrane is G-protein-regulated but there are no data confirming any role of the nuclear G protein in the activation of the nuclear enzyme. However, several studies suggested the involvement and nuclear translocation of p42/p44 mitogen-activated protein kinase (MAPK) in agonist-mediated activation of the nuclear PI-PLC-β (47, 48). In a detailed study, Xu *et al.* demonstrated MAPK-mediated phosphorylation of the nuclear PI-PLC-β_1 at Ser 982 residue in IGF-treated Swiss 3T3 cells; the phosphorylation was inhibited by MEK inhibitor, PD98059, and mimicked by recombinant PI-PLC-β_1 and activated MAPK *in vitro* (77). Although the Ser982 phosphorylation was prerequisite for the PI-PLC activation, as shown in mutants carrying Ser982Gly, it was not sufficient alone, and several other components of mechanism involved in the activation of the nuclear PI-PLC still remain to be determined (Fig. 3). However, PD98059-sensitive activation and serine phosphorylation of the nuclear PI-PLC-β_1, including the b splicing variant, have been documented in response to several agonists in various cell systems (16, 40, 42, 75).

3.2. Other nuclear phospholipase C isoforms

In addition to PI-PLC-β_1, the nuclear localization of both PI-PLC-β_2 and $-\beta_3$ was confirmed in nuclei of HL-60 cells, and the level of the enzymes was found to increase during the differentiation in the presence of ATRA and vitamin D_3 (7, 54). In a model of ATRA-differentiated HL-60 cells, the level of another PI-PLC isoform, PI-PLC-γ_1, was found to

progressively increase in nuclei during the differentiation (7). At the cell membrane, PI-PLC-γ_1 is activated by receptor or nonreceptor tyrosine kinases. In the nuclei, the similar mechanism was found to operate as the increase in the PI-PLC-γ_1 activity measured in nuclei at 6 and 20 h after partial hepatectomy was associated with tyrosine phosphorylation of the enzyme (16). As PI-PLC-γ_1 contains no functional nuclear localization signal (NLS), the mechanism of the nuclear localization is supposed to depend on the association with other NLS-containing proteins; the protein Vav is one of the possible candidates as it was shown to associate with both PI-PLC-γ_1 and PI3K in immunoprecipitates of ATRA-treated HL-60 cells (8).

Among all known PI-PLC isoforms, nucleocytoplasmic shuttling is the best documented for PI-PLC-δ_1 – a member of PI-PLC subfamily delta that is the only one present in all eukaryotes (including Plc1p encoded by *PLC1* in yeasts) (79). The accumulation of both endogenous enzyme and GFP-PLC-δ_1 construct was observed in leptomycin B-treated MDCK cells and further sequence analysis revealed a nuclear export signal (NES) and a putative NLS-like signal (58, 80). Again, the mechanism of the nuclear PI-PLC-δ_1 activation is not well understood, but the activation of PI-PLC-δ_1 is not completely elucidated even at the cell membrane (61). At both locations, the mechanism seems to depend on binding to PtdIns(4,5)P$_2$ by PH domain (a property that has been extensively used to track the cellular PtdIns(4,5)P$_2$ by GST or GFP-constructs fused to PH-domain of PI-PLC-δ_1) and an increase in the level of Ca^{++} (59, 61, 76).

3.3. The subnuclear localization of nuclear phospholipase C

The sublocalization of different PI-PLC isoforms in nuclei has been investigated by using immunocytochemistry, biochemical fractionation of cells, and imaging of cells

expressing tagged proteins (6, 26). The majority of PI-PLC-β_1 is probably not located in the nuclear envelope as the activity of PI-PLC-β_1 is present in both whole nuclei (21, 40) and nuclei treated with different concentrations of detergents in order to remove the nuclear membrane (21, 25, 48, 68, 75, 77). In rat liver nuclei, fractionation experiments indicated that both PI-PLC-β_1 and PI-PLC-γ_1 persisted in nuclear matrix and lamina, obtained after nuclease digestion and extraction with high salt and detergent (6). Our recent quantitative analysis of nuclear PI-PLC isoforms during compensatory liver growth showed that PI-PLC-β_1 and PI-PLC-γ_1 account for 60% and 30% of the PI-PLC activity in membrane-depleted nuclei, respectively, and that the rest of the activity was due to the presence of PI-PLC-δ_1. Furthermore, while PI-PLC-β_1 and -γ_1 were associated with nuclear matrix, the activity of PI-PLC-δ_1 was immunoprecipitated with chromatin fraction of the nuclei (16). Although all of these studies suggest that several different isoforms of PI-PLC are really "endonuclear" as they localize in different parts of the nuclei, the functional significance of the colocalization findings is presently unknown. However, several studies localized the major substrate for the action of PI-PLC, PtdIns(4,5)P_2 to nuclear speckles suggesting some involvement in the splicing reaction (60). Furthermore, colocalization of PI-PLC-δ (i.e. Plc1p) with chromatin has been described in yeasts models and suggested to have a role in kinetochore function (37).

3.4. The role of the nuclear PI-PLC in differentiation, mitogenesis and cell cycle progression

The possible effects of the nuclear PI-PLC activation on differentiation were first indicated by studies showing an increase in the level of the nuclear enzymes in C2C12 myoblasts (26). A functional role for the nuclear enzyme was demonstrated in studies using the PI-PLC-β_1 mutant that is confined to the cytoplasm by destroying NLS; the nuclear

enzyme was found to be necessary for both the expression of CD24 marker during erythroid differentiation of Friend cells (27) and myoblastic differentiation of C2C12 cells (26).

IGF-mediated increase in the level of nuclear DAG and the activity of PI-PLC that was described 15 years ago (20, 46) has been later proved to have an important role in the onset of DNA synthesis and proliferation of Swiss 3T3 cells. The mitogenic response of the cells to IGF was abolished by the ablation of the isoform through antisense RNA (44), and cells overexpressing PI-PLC-β_1 showed mostly nuclear localization of the enzyme and an increased percentage of cells incorporating BrdU (11). Friend erythroleukemia cells overexpressing a or b splice variants of PI-PLC-β_1 showed an increase in the level of cyclinD/cdk4, phosphorylation of retinoblastoma protein (Rb) on Ser-795, activation of E2F and growth in the absence of serum, and none of these effects were observed in cells transfected with a mutant lacking the NLS (25). In conclusion, these studies confirmed the role of the nuclear PI-PLC-β_1 in mitogen-driven proliferation.

An early increase in the PI-PLC activity in mitogen-stimulated cells that were previously serum-starved probably corresponds to G_0/G_1-transition phase of the cell cycle. A series of experiments performed in aphidicolin-synchronized HL-60 cells demonstrated an additional point of the cell cycle characterized by an increase in the nuclear PI-PLC activity. When aphidicolin-synchronized HL-60 cells were released from the block and allowed to progress synchronously through the cell cycle, a PI-PLC inhibitor-sensitive increase in the level of DAG was observed in nuclei 8 h after release from the block that corresponded to G_2/M phase of the cell cycle (68). In addition, the presence of the PI-PLC inhibitor delayed the progression of the cells through G_2/M phase and correlated with translocation of PKC-βII that was previously shown to phosphorylate lamins (29, 68, 71). Therefore, a certain physiological role of G_2/M-associated nuclear PI-PLC activation was suggested as the phosphorylation of lamins is known to precede nuclear envelope breakdown at the beginning

of the mitosis. A similar increase in the level of DAG and translocation of PKC-βII were detected during G_2/M phase in U937 cells separated into distinct phases of the cell cycle by centrifugal elutriation (17).

Ever since an increase in the level of nuclear DAG was observed in rat liver nuclei during proliferation, a model of compensatory liver growth after partial hepatectomy has been used as a useful *in vivo* model to study the activation of nuclear PI-PLC at different phases of the cell cycle. Early studies pointed to the possible activation somewhere at the beginning of the S-phase (5), although a more detailed analysis proved that the increase actually precedes the increase in the incorporation of radioactive thymidine (16). Our recent analysis of PI-PLC activity in membrane-depleted nuclei of regenerating rat liver demonstrated two separate peaks of the nuclear PI-PLC activity; the early peak that was associated with an increase in serine-phosphorylation of PI-PLC-β_1 occurred 6 h after partial hepatectomy, and the later increase occurring at 20 h was characterized by an increase in the level of both PI-PLC-β_1 and tyrosine-phosphorylated PI-PLC-γ_1 (16).

These studies prompted us to check for the possible two waves of the nuclear PI-PLC activity during G_1 phase in a model of HL-60 cells blocked in G_2/M phase by nocodazole and released to progress through G_1 phase of the cycle. Two peaks of the nuclear PI-PLC activity were detected at 1 and 8.5 h after nocodazole release and both peaks correlated with an increase in the serine-phosphorylation of the nuclear PI-PLC-β_1 splice variant b (40). In addition, two waves of nuclear PI-PLC-β_{1b} activity were observed in serum-starved and re-feed HL-60 cells confirming that cyclic increases in nuclear PI-PLC activity were not the consequence of the nocodazole-synchronization procedures but genuine cell-cycle-related events (42). Both peaks of PI-PLC-β_{1b} activity were inhibited by PI-PLC and MEK inhibitors, and the later increase in the nuclear activity was found to be equally important for the progression into the S phase (42). In summary, combining data obtained in

HL-60 cells synchronized by different procedures, we can conclude that there are at least three distinct peaks of MAPK-mediated PI-PLC-β_{1b} activation in nuclei of cells progressing through the cell cycle, as summarized in Fig. 4 (and reviewed in 41).

The nuclear translocation of other PI-PLC isoforms has been described to be linked to the cell cycle. PI-PLC-δ_1, the same isoform that was found to participate in the increase in the PLC-activity in rat liver nuclei at 20h after hepatectomy (16), accumulates in the nuclei of both quiescent and thymidine-treated NIH 3T3 cells arrested at G$_1$/S boundary (67). PI-PLC-ζ is transported into the nucleoplasm of the newly formed pronucleus in the fertilized egg and remains nuclear during the first interphase. As the zygote enters first mitosis, the pronuclear envelopes breakdown occurs and PLC-ζ is released back to the cytoplasm (36).

It is not clear how the nuclear activation of any PI-PLC isoform might interact with the cell cycle machinery to regulate progression through different phases of the cell cycle. At the cell membrane, the products of PLC-mediated PtdIns(4,5)P$_2$ hydrolysis activate PKC and mobilize calcium from intracellular stores. Although there are numerous reports showing the presence of different PKC isoforms in nuclei in parallel with an increase in the level of the nuclear DAG and the phosphorylation of several nuclear proteins, there are few data confirming physiological role of PKC that is specifically attracted to the nuclei because of the activation of the nuclear PI-PLC (17, 20, 29, 41, 50, 56, 71, 78). Some of the functionally important nuclear PKC-mediated phosphorylation events include phosphorylation of lamins during G$_2$/M phase of the cell cycle (17, 29, 71), and PKC-α-mediated phosphorylation and down-regulation of PI-PLC-β_1 in IGF-stimulated Swiss 3T3 cells (78). There are even less studies that may confirm the hypothesis that Ins(1,4,5)P$_3$, generated by the activation of the nuclear PI-PLC, plays exactly the same role in the regulation of the nuclear calcium homeostasis as the Ins(1,4,5)P$_3$ generated at the cell membrane plays in the regulation of the

cytosolic calcium (12). However, $Ins(1,4,5)P_3$ may serve as a precursor for the phosphorylation and generation of inositol phosphates that have been convincingly proved to regulate such as important nuclear events like mRNA export (83), transcription (57), telomere length (85) and RNA editing (43). In addition, the signaling role of the nuclear PI-PLC might rely not only on the production of new lipid second messengers but on the regulation of the level of the nuclear substrate, as it is known that $PtdIns(4,5)P_2$ itself influences many nuclear processes directly (35). In murine erythroleukemia cells, the activity of enzymes responsible for the synthesis of $PtdIns(4,5)P_2$ increases during the progression through G_1 into S phase (13). Recent studies performed by Divecha and coworkers (23, 38) suggest that Rb protein, a key regulator of G_1/S phase transition, may provide a link between nuclear phospholipid signaling and cell cycle regulation as they demonstrate that pRb interacts with PtdIns(4)P 5-kinase, regulates the levels of the nuclear $PtdIns(4,5)P_2$ (23), and decreases the level of the nuclear DAG by activating the nuclear DAG kinase (38). **Data obtained in synchronized NIH 3T3 cells suggest that relative level of nuclear phosphoinositides can influence compartmentalization of PLC enzymes and stimulate their nuclear translocation as it was demonstrated that PI-PLC-δ_1 translocation correlated with the increase in the level of the nuclear $PtdIns(4,5)P_2$ and depended on high affinity $PtdIns(4,5)P_2$ binding through PH domain (67).**

Another possible role for the nuclear accumulation of the PLC enzyme can be simply to prevent the effects of the cytoplasmic activation as it was suggested in a model of fertilized mouse eggs during their first cell cycle. PLC-ζ-induced Ca^{2+} oscillations are absent during the interphase and occur exclusively during mitosis which is probably due to the NLS-dependent sequestration of the enzyme in the nuclei during interphase thus preventing the generation of cytoplasmic $Ins(1,4,5)P_3$ and subsequent Ca^{2+} release (36).

Table 1 summarizes findings on accumulation, activity and possible roles of different PI-PLC isoforms detected in nuclei.

4. Nuclear phosphoinositide 3-kinases

Phosphatidyilinositol 3-kinases phosphorylate phosphoinositides at the 3' position of the inositol ring to generate $PtdIns(3)P$, $PtdIns(3,4)P_2$, $PtdIns(3,5)P_2$ and $PtdIns(3,4,5)P_3$ in response to various stimuli. Several different members of PI3K family are divided into three classes based on their sequence homology, substrate preference and mechanism of activation. Phosphorylation of $PtdIns(4,5)P_2$ is mediated by class I enzymes that are further subdivided into group A and B; class IA enzymes consist of a 110-kDa catalytic subunit and an adaptor protein which links the enzyme to tyrosine kinases, and class IB enzymes are composed of $p110\gamma$ catalytic subunit and a subunit regulated by G proteins. The principal second messenger generated upon the activation of class I PI3Ks is $PtdIns(3,4,5)P_3$, that is known to mediate such important cellular responses as mitogenesis, cell growth and insulin-regulated glucose homeostasis. Much less is known about physiological role and mode of regulation of class II enzymes which include three isoforms in mammals (PI3K-C2α , -β and -γ). All class II enzymes show preference for PtdIns and $PtdIns(4)P$ as substrate *in vitro* and their sequences contain Phox homology (PX) and C2 domains at their C-termini. The mammalian class III enzyme phosphorylates only PtdIns to produce $PtdIns(3)P$ and the enzyme is homologous to yeast Vps34 which regulates vesicle trafficking (reviewed in 24).

4.1. Nuclear class I phosphoinositide 3-kinases

Biochemical, immunoblotting and immunofluorescence data confirmed the presence of p85/p110 or class IA PI3K in nuclei of various cell types and point to the possible role of the nuclear PI3K in mitogenesis (reviewed in 49). Again, the mechanism of the activation of the nuclear PI3K seems to differ from the classical one at the cell membrane. The best characterized are models of the nuclear class I PI3K regulation in PC12 rat pheochromocitoma cells stimulated by nerve growth factor (NGF) (Fig. 5) and ATRA-differentiated HL-60 cells.

4.1.1. NGF-stimulated nuclear PI 3-kinase in PC 12 cells

In PC12 cells, NGF treatment led to an increase in the level of the nuclear PtdIns(3,4,5)P$_3$ that was temporally distinct from the one occurring at the cell membrane and that was associated with an increase in both the amount and the enzyme activity of PI3K immunoprecipitated from the nuclei (55, 70). In the yeast two-hybrid analysis using a domain of cytoskeleton protein 4.1N as bait, a brain-specific nuclear GTPase that activates PI3K was identified and named *p*hospho *i*nositide *k*inase *e*nchancer (PIKE). Experiments performed on dominant-negative PIKE (K413AS414N) retrovirus–infected PC12 cells confirmed that both NGF-induced nuclear PI3K activation and cyclin D1 expression were mediated by PIKE (81).

PIKE, as other GTPases, cycles between an active GTP-bound and an inactive GDP-complexed state. Therefore, proteins that promote release of GDP (guanine nucleotide exchange factors, GEF) act as positive regulators of GTPases. Subsequent in vitro binding assays identified PI-PLC-γ_1 as an upstream regulator of PIKE; SH3 domain of PI-PLC-γ_1 was found to interact with proline rich domains (PRD) in PIKE (82). As GEF activity of PI-PLC-γ_1 depended only on that PRD-SH3 interaction and did not require the phospholipase catalytic activity, the model of PI-PLC-γ_1/PIKE/nuclear PI3K activity provided one explanation for the

previous findings showing that mitogenic activity of PI-PLC-γ_1 was not dependent on its phospholipase activity, but requires only its SH3 domain (66).

Further experiments in the same model of NGF-treated PC12 cells identified some downstream targets of the nuclear PIKE/PI3K/PtdIns(3,4,5)P_3. The one of these nuclear binding targets for PtdIns(3,4,5)P_3 is nucleophosmin/B23, a major nuclear phosphoprotein that plays an important role in ribosome biogenesis; immunodepletion of B23 from nuclear extracts in PC12 cells promotes DNA fragmentation and abolishes NGF-mediated antiapoptotic effect (2). Another important nuclear target is protein kinase B/Akt that is known to translocate into the nucleus in response to various agonists (reviewed in 51) and that was shown to prevent apoptosis in PC12 cells (1). In PC12 cells, acinus and Ebp1 are some recently identified direct nuclear Akt targets that participate in prevention of DNA fragmentation and chromatin condensation during apoptosis (3, 31).

4.1.2. Nuclear PI 3-kinase in myeloid differentiation

In addition to providing the survival signal in PC12 cells, the nuclear PI3K class I activation and PtdIns(3,4,5)P_3 has an important role in myeloid differentiation. In the model of ATRA and vitamin D_3-differentiated HL-60 cells, the increase in the level of immunoreactive p85, enzyme activity and endogenous PtdIns(3,4,5)P_3 was detected in nuclei of cells differentiated towards granulocytes or monocytes, respectively (8, 54). The inhibition of PI3K activity by wortmanin and a decrease of p85 expression obtained by antisense transfection inhibited ATRA-mediated granulocytic differentiation (9). Similar to NGF-treated PC 12 cells, the activation of class I PI3K in the nuclei of ATRA-differentiated cells was found to be associated with a nuclear accumulation of PI-PLC-γ_1, but the mechanism of nuclear activation, as previously described for the nuclear PI-PLC-γ_1, involves an adapter

protein Vav (8). An increase in the nuclear PI3K activity in ATRA-differentiated cells depends on the association of SH2 domain of p85 with tyrosine phosphorylated Vav and occurs independently from GEF activity of adapter protein (10). In ATRA-differentiated HL-60 cells, possible downstream targets of the nuclear PI3K class I activation so far include PKC-ζ (55) and Akt (52).

4.2. Nuclear class II phosphoinositide 3-kinases

Immunochemical and biochemical studies localized p85 in ATRA-differentiated cells to nuclear matrices (45, 54). *In vivo* model of rat liver nuclei showed the presence of class I PI3K in nuclei (39) and the absence of PtdIns(3,4,5)P$_3$ in nuclei depleted of their membranes suggesting that a majority of the class I PI3K activity in rat liver nuclei is not present in the endonuclear compartment (63). However, the activation of class II PI 3-kinases and an increase in the level of the nuclear PtdIns(3)P were observed in membrane-depleted rat liver nuclei at 20 h after partial hepatectomy. The increase in kinase activity was measured in nuclear extracts after immunoprecipitation using antibody raised against PI3K-C2β (63). Class II PI3Ks are good candidates for compartmentalization within the cell nucleus as they have been reported to associate predominantly with membrane fractions of the whole cells. PI3K-C2α, another member of class II PI 3-kinases, was found to be associated with nuclear speckles and a putative NLS was identified within C2-like domain of the kinase (18). Further studies in rat liver nuclei confirmed that PI3K-C2β was mostly associated with nuclear matrices of hepatocytes during compensatory liver growth (64). Again, the subnuclear localization of PI3K-C2β depends on the cell type. In models of ATRA-differentiated and aphidicolin-synchronized HL-60 cells, the majority of PI3K-C2β activity was detected in the nuclear envelopes (73, 74). In contrast to the subnuclear localization, the mechanism of the

nuclear PI3K-C2β activation does seem to depend more on the physiological function of the enzyme. The same mechanism of calpain-mediated proteolysis and activation of PI3K-C2β has been described in both regenerating rat liver (63) and aphidicolin-synchronized HL-60 cells during G_2/M-phase of the cell cycle (74), and both events seems to be related to the progression of cells through the cell cycle. In contrast, the nuclei of HL-60 cells that were induced to differentiate in the presence of ATRA show a different mechanism of PI3K-C2β activation. Instead of calpain-mediated proteolysis, the activation of PI3K-C2β during differentiation process relies on the tyrosine phosphorylation of the enzyme (73).

Concluding remarks

In last 20 years, a considering progress has been made in understanding the mechanism of the nuclear PI-PLC and PI3K activation. The imaging of cells expressing tagged proteins, the use of mutants lacking NLS, and the overexpression of enzymes in the nuclei revealed some precise and specific evidence for the role of PI-PLC-β_{1b} and PIKE/class I PI3K/Akt in defined physiological processes. **The nuclear enzymes do not seem to recapitulate always their roles at the plasma membranes; e.g. the importance of the nuclear localization of PI-PLC-ζ in fertilized eggs seems to be simply the sequestration of the enzyme from the cytoplasm and the prevention of the effects of Ins(3,4,5)P$_3$ on the cytosolic calcium (36). Furthermore, the mitogenic role of the nuclear PI-PLC-γ_1 does not depend on the catalytic activity but relies on binding to the nuclear GTPase PIKE (82). The activation of the nuclear PKC attracted by an increase in the level of the nuclear DAG (17, 71, 78) as well as the activation of the nuclear Akt in response to the increase in the nuclear PtdIns(3,4,5)P$_3$ (52, 55) seems to prove the necessity of second messenger generating function of the nuclear PLCs and PI3K. However, there are no**

convincing proofs that the nuclear Ins(3,4,5)P₃ plays the same role in the mobilization of calcium as the one generated at the plasma membrane, and there are many more proofs that, at least in yeast model, the role of the nuclear Ins(3,4,5)P₃ is to serve as a precursor for the generation of the higher inositol phosphates (84). Yeast provided an ideal model system within which to study the possible role of the phosphoinositides and inositol phosphates in the regulation of nuclear processes. Several years ago, when a possible role for the nuclear PI-PLC in nuclear envelope assembly and cell cycle emerged, one of the hypotheses suggested that the nucleus may have been the site at which phosphoinositide signaling originally evolved and that the cycle was later duplicated in the plasma membrane for the signaling purpose (22). The lack of PtdIns(3,4,5)P₃ in rat liver nuclei depleted of membranes with a parallel increase in the level of PtdIns(3)P, the association of the PLC1 analogue PLC-δ_1 with chromatin, the lack of Ins(1,4,5)P₃ substrates that can be involved in nuclear calcium homeostasis suggest that there are many similarities between **signaling in** yeasts and endonuclear phospholipid signaling and that the evolutionary standpoint can be informative. **The absence of the classical receptor-mediated phospholipid signaling in yeasts, including the lack of Ins(1,4,5)P₃-receptor gene in yeasts genome, raises the possibility that the primordial role for the phospholipase C was production of the precursor for the inositol phosphates and the regulation of nuclear processes. The nuclear localization of the enzymes necessary for the synthesis of PtdIns(4,5)P₂ and the nucleocytoplasmic shuttling of the Plc in yeasts have been reported (84).** The most promising areas of future research in the field of the nuclear phospholipid signaling involve the investigation of the metabolism and possible nuclear function of higher inositol phosphates in mammalian cells, the elucidation of the physicochemical forms of phosphoinositides within the nucleus and the identification of nuclear-specific phosphoinositide binding domains that allows the response of nuclear proteins to changes in

the nuclear phosphoinositide profile and regulation of chromatin structure, transcription, and DNA repair (34, 35).

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Figure legends:

Figure 1. The basic structure of nucleus – "nuclear" and "endonuclear" PtdIns(4,5)P$_2$. PtdIns(4,5)P$_2$ is a component of a classic phospholipid bilayer and the nuclear envelope is composed of the outer and inner nuclear membranes. "Endonuclear" PtdIns(4,5)P$_2$, i.e. PtdIns(4,5)P$_2$ that is present in nuclei after removal of membranes by the use of detergents, was found to be associated with various structures, including interchromatin granule clusters or nuclear speckles. The physicochemical structure of "endonuclear" phospholipids is unknown.
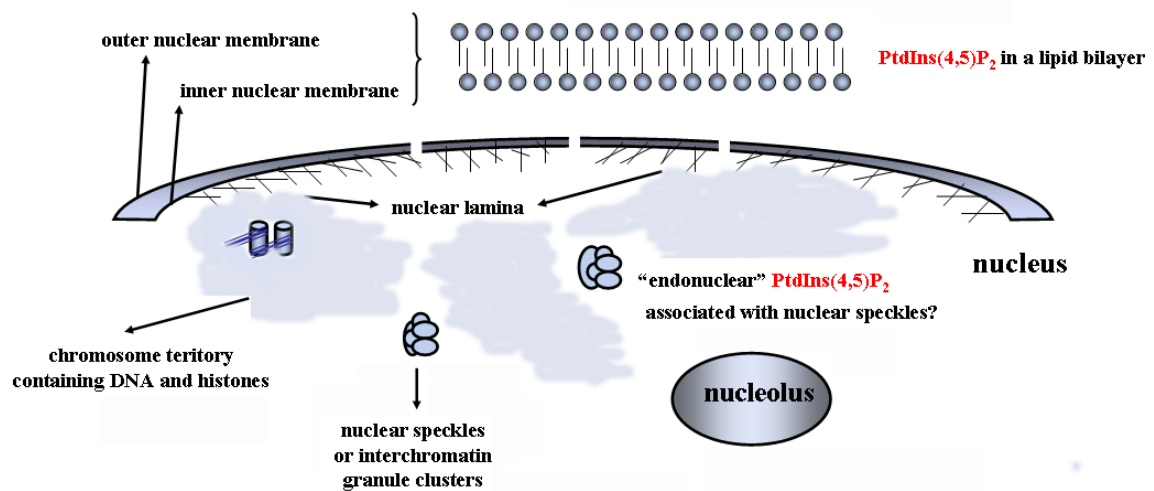
Figure 2. The structure of PI-PLC isoforms that are most frequently found in nuclei. PI-PLC-β, -γ and -δ isoforms contain catalytic domains X and Y, pleckstrin homology domain (PH), EF hands and C2 domain that binds calcium. **PI-PLC-ζ lacks PH domain.** PI-PLC-γ contains **additional** Src homology (SH) domains that enable the association of the isoform with ligand-activated tyrosine kinases. **A** region at C-terminal domain of PI-PLC-β **and a sequence between X and Y domain in PI-PLC-ζ were found to** provide a nuclear localization signal (NLS).
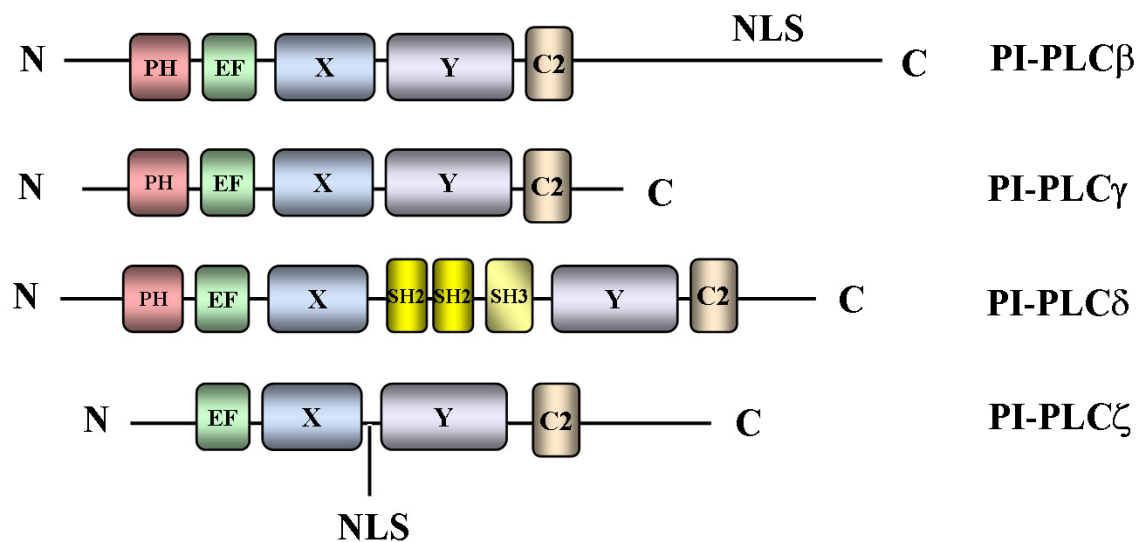
Figure 3. MAPK-mediated activation of the nuclear PI-PLC-β_{1b}. Several agonists (insulin, IGF, IL-2 and FBS) stimulate the activity of the nuclear PI-PLC-β_{1b} that is sensitive to the presence of MEK inhibitor PD98059. PI-PLC-β_{1b}-mediated hydrolysis of PtdIns(4,5)P$_2$ generates DAG and Ins(1,4,5)P$_3$. The nuclear DAG attracts several PKC isoforms to the nucleus; PKC-α phosphorylates PI-PLC-β_{1b} and inhibits its activity, PKC-βII mediates proliferative signal. The role of the nuclear Ins(1,4,5)P$_3$ may be the regulation of the nuclear calcium. In addition, Ins(1,4,5)P$_3$ may be further phosphorylated into higher inositol phosphates.
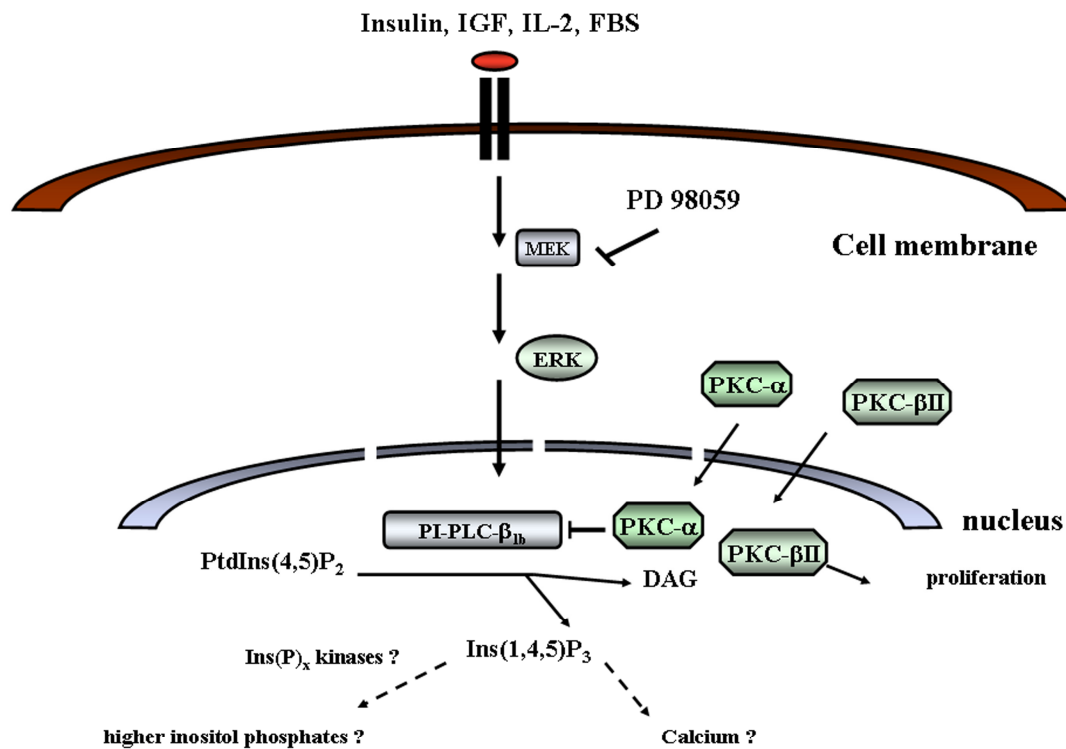
Figure 4. The activation of the nuclear PI-PLC during the progression through the cell cycle. The arrows indicate the phases of the cell cycle at which the increases in the activity of PI-PLC-β_{1b} were detected in nuclei of HL-60 cells.
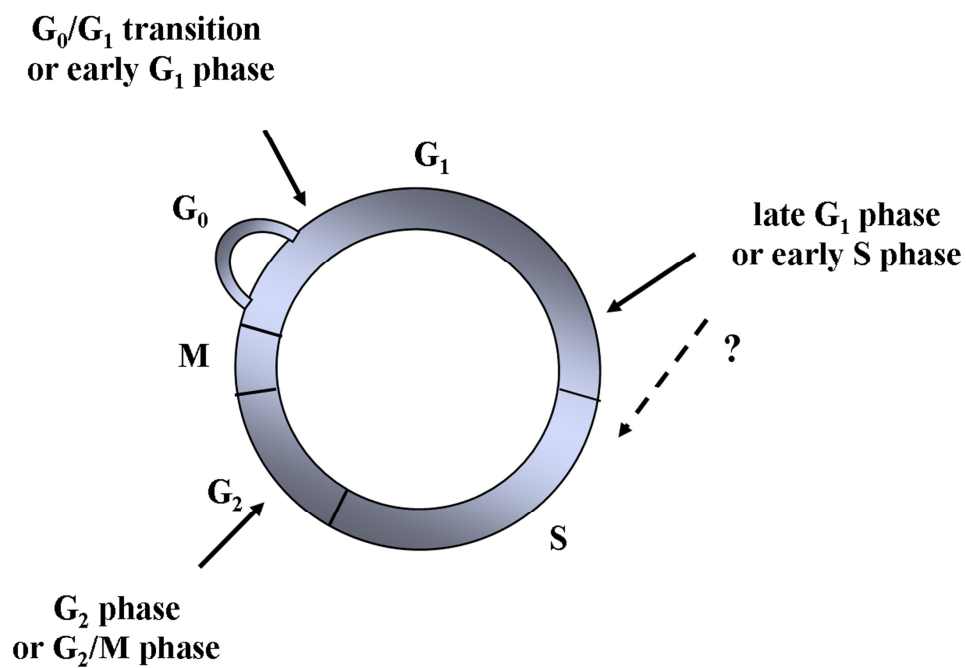
Figure 5. NGF-stimulated nuclear PI 3-kinase in PC 12 cells. Binding of NGF to its receptor stimulates translocation of both PI-PLC-γ and PI 3-kinase class I (p85α/p110) into the nucleus. A catalytically inactive PI-PLC-γ act as guanine exchange factor (GEF) and activates nuclear GTPase called PIKE (*p*hospho *i*nositide *k*inase *e*nchancer). PIKE stimulates the activity of the nuclear PI3K that phosphorylates PtdIns(4,5)P$_2$ into PtdIns(3,4,5)P$_3$. PtdIns(3,4,5)P$_3$ attracts PKC-ζ which translocates from cytoplasm to the nucleus and phosphorylates nucleolin. The complex of PtdIns(3,4,5)P$_3$ and nucleophosmin/B23 inhibits DNA fragmentation activity of caspase activating DNAse (CAD). Acinus is one of the recently identified targets of the activated nuclear Akt.
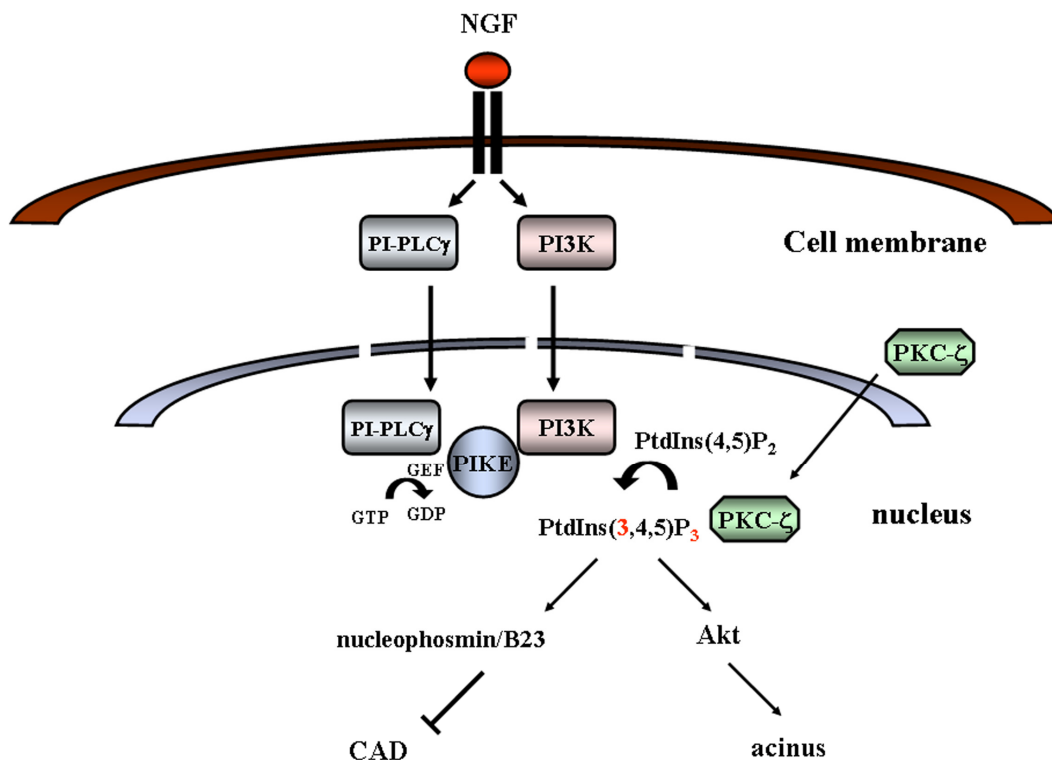
Table 1. Overwiew of different nuclear PLC isoform activation during cell cycle progression

Isoform	The phase of cell cycle	Model	The enzyme activity	The amount of enzyme	The interaction partner in the nucleus
PI-PLC-β1	G0/early G1	IGF, Swiss 3T3 cells [77, 47]	Increased (PLC assay) [77, 47]* MAPK-mediated phosphorylation at Ser982 [77]	No change [77, 47]	Immunoprecipitated with phospho-MAPK [77]
		IGF, HL-60 [56]	Increased (PLC assay) [56] Increased DAG [56]	No change [56]	ND [56]
−β1β		Insulin, NIH 3T3 [48]	Increased (PLC assay) [48] MEK inhibitor-sensitive serine phosphorylation [48]	No change [48]	ND [48]
		IL-2, NK cells [75]	Increased (PLC assay) [75] Increased DAG [75] MEK inhibitor-sensitive serine phosphorylation [75]	No change [75]	ND [75]
		FBS, HL-60 [42]	Increased (PLC assay) [42] MEK inhibitor-sensitive serine phosphorylation [42]	No change [42]	ND [42]
		Hepatectomy, liver [16]	Increased (PLC assay) [16] Serine phosphorylation [16]	No change [16]	ND [16]
	late G1/S	FBS, HL-60 [42] Nocodazole, HL-60 [40]	Increased (PLC assay) [40,42] MEK inhibitor-sensitive serine phosphorylation [40,42]	No change [40,42]	ND [40,42]
		Hepatectomy, liver [16]	Increased (PLC assay) [16] No phosphorylation [16]	Increased [16]	ND [16]
	G2/M	Nocodazole, HL-60 [40] Aphidicolin, HL-60 [68]	Increased (PLC assay) [40] Increased DAG [68] MEK inhibitor-sensitive serine phosphorylation [40]	No change [40]	ND [40,68]
PI-PLC-γ1	G0/early G1	Hepatectomy, liver [16,53]	Increased (PLC assay) [16,53] Tyrosine phosphorylation [16,53]	No change [16,53]	ND [16,53]
		NGF, PC12 [82]	ND [82]	Increased [82]	PIKE GTPase (PLCγ acts as GEF) [82]
		ATRA, HL-60 [8]	ND [8]	Increased [8]	Immunoprecipitated with Vav [8]
	late G1/S	Hepatectomy, liver [16,53]	Increased (PLC assay) [16,53] Tyrosine phosphorylation [16,53]	No change [16,53]	ND [16,53]
PI-PLC-δ1	G0/early G1	Reduced serum, NIH 3T3 [67]	ND [67]	Increased [67]	Phosphoinositides [67]
	Late G1/S	Thymidine block, NIH3T3 [67, 79]	ND [67, 79]	Increased [67, 79]	ND [67, 79]
		Hepatectomy, liver [16]	Increased (PLC assay) [16]	Increased [16]	ND [16]
PI-PLC-ζ	interphase	Fertilized eggs [36]	ND [36]	Increased [36]	ND [36]

ND- not determined
*- reference number