

# Nuclear phospholipid signaling: phosphatidylinositol-specific phospholipase C and phosphoinositide 3-kinase

---

Višnjić, Dora; Banfić, Hrvoje

Source / Izvornik: **Pflügers Archiv : European Journal of Physiology, 2007, 455, 19 - 30**

Journal article, Accepted version

Rad u časopisu, Završna verzija rukopisa prihvaćena za objavljivanje (postprint)

<https://doi.org/10.1007/s00424-007-0288-1>

Permanent link / Trajna poveznica: <https://um.nsk.hr/um:nbn:hr:105:962711>

Rights / Prava: [In copyright](#) / [Zaštićeno autorskim pravom.](#)

Download date / Datum preuzimanja: **2025-02-08**



Repository / Repozitorij:

[Dr Med - University of Zagreb School of Medicine  
Digital Repository](#)





## Središnja medicinska knjižnica

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].

The original publication is available at [www.springerlink.com](http://www.springerlink.com)  
<http://www.springerlink.com/content/185k402537342587/>

<http://medlib.mef.hr/139>

University of Zagreb Medical School Repository

<http://medlib.mef.hr/>

Nuclear phospholipid signaling: phosphatidylinositol-specific phospholipase C and  
phosphoinositide 3-kinase

Dora Visnjic and Hrvoje Banfic\*

Department of Physiology and Croatian Institute for Brain Research, School of  
Medicine, University of Zagreb, Salata 3, 10 000 Zagreb, Croatia

\* Corresponding author: Prof. dr. Hrvoje Banfic, Zavod za fiziologiju, Medicinski fakultet,  
Sveuciliste u Zagrebu, Salata 3, POB 978, 10 001 Zagreb, Croatia.

Tel: 385-1-4590-260

Fax: 385-1-4590-207

E-mail: [hrvoje.banfic@zg.t-com.hr](mailto:hrvoje.banfic@zg.t-com.hr)

## **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.

**Keywords:** phospholipid, nuclei, phospholipase C, phosphoinositide 3-kinase

## 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<sub>2</sub> 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<sub>2</sub> into Ins(1,4,5)P<sub>3</sub> and DAG; Ins(1,4,5)P<sub>3</sub> 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<sub>2</sub> on 3' position of the inositol ring and generates PtdIns(3,4,5)P<sub>3</sub> that further activates several downstream signaling molecules. Receptor-mediated hydrolysis and phosphorylation of PtdIns(4,5)P<sub>2</sub> at the cell membrane regulates diverse cellular functions, such as cell proliferation, survival, vesicle trafficking and gene transcription. In addition, PtdIns(4,5)P<sub>2</sub> 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 phospholipids 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  $^{32}\text{P}$  into a mixture of phospholipids containing PtdIns(4)P and PtdIns(4,5) $\text{P}_2$  in nuclear envelopes isolated from rat liver nuclei (65). As the major route of PtdIns(4,5) $\text{P}_2$  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) $\text{P}_2$  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) $\text{P}_2$ , 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) $\text{P}_2$  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 cytoskeleton 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<sub>2</sub> 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 (- $\beta$ , - $\delta$ , - $\gamma$ , - $\epsilon$ , - $\zeta$  and - $\eta$ ) 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- $\beta_1$

IGF-mediated PtdIns(4,5)P<sub>2</sub> 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- $\beta_1$  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- $\beta_1$  (21). Therefore, among several different PI-PLC isoforms, PLC- $\beta_1$  became the most intensively investigated as a major PLC isoform involved in nuclear signaling.



PI-PLC- $\beta$  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- $\beta_1$  isoform, PI-PLC- $\beta_{1a}$  and PI-PLC- $\beta_{1b}$ , and several studies demonstrate that b splicing variant is the one that is predominantly nuclear (4, 25, 40, 47). The „classical“ PI-PLC- $\beta$ , 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- $\beta$  (47, 48). In a detailed study, Xu *et al.* demonstrated MAPK-mediated phosphorylation of the nuclear PI-PLC- $\beta_1$  at Ser 982 residue in IGF-treated Swiss 3T3 cells; the phosphorylation was inhibited by MEK inhibitor, PD98059, and mimicked by recombinant PI-PLC- $\beta_1$  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- $\beta_1$ , 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- $\beta_1$ , the nuclear localization of both PI-PLC- $\beta_2$  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<sub>3</sub> (7, 54). In a model of ATRA-differentiated HL-60 cells, the level of another PI-PLC isoform, PI-PLC- $\gamma_1$ , was found to

progressively increase in nuclei during the differentiation (7). At the cell membrane, PI-PLC- $\gamma_1$  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- $\gamma_1$  activity measured in nuclei at 6 and 20 h after partial hepatectomy was associated with tyrosine phosphorylation of the enzyme (16). As PI-PLC- $\gamma_1$  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- $\gamma_1$  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- $\delta_1$  – 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- $\delta_1$  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- $\delta_1$  activation is not well understood, but the activation of PI-PLC- $\delta_1$  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<sub>2</sub> by PH domain (a property that has been extensively used to track the cellular PtdIns(4,5)P<sub>2</sub> by GST or GFP-constructs fused to PH-domain of PI-PLC- $\delta_1$ ) and an increase in the level of Ca<sup>++</sup> (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- $\beta_1$  is probably not located in the nuclear envelope as the activity of PI-PLC- $\beta_1$  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- $\beta_1$  and PI-PLC- $\gamma_1$  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- $\beta_1$  and PI-PLC- $\gamma_1$  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- $\delta_1$ . Furthermore, while PI-PLC- $\beta_1$  and - $\gamma_1$  were associated with nuclear matrix, the activity of PI-PLC- $\delta_1$  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<sub>2</sub> to nuclear speckles suggesting some involvement in the splicing reaction (60). Furthermore, colocalization of PI-PLC- $\delta$  (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- $\beta_1$  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- $\beta_1$  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- $\beta_1$  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- $\beta_1$  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<sub>0</sub>/G<sub>1</sub>-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<sub>2</sub>/M phase of the cell cycle (68). In addition, the presence of the PI-PLC inhibitor delayed the progression of the cells through G<sub>2</sub>/M phase and correlated with translocation of PKC- $\beta$ II that was previously shown to phosphorylate lamins (29, 68, 71). Therefore, a certain physiological role of G<sub>2</sub>/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- $\beta$ II were detected during G<sub>2</sub>/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- $\beta$ <sub>1</sub> 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- $\beta$ <sub>1</sub> and tyrosine-phosphorylated PI-PLC- $\gamma$ <sub>1</sub> (16).

These studies prompted us to check for the possible two waves of the nuclear PI-PLC activity during G<sub>1</sub> phase in a model of HL-60 cells blocked in G<sub>2</sub>/M phase by nocodazole and released to progress through G<sub>1</sub> 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- $\beta$ <sub>1</sub> splice variant b (40). In addition, two waves of nuclear PI-PLC- $\beta$ <sub>1b</sub> 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- $\beta$ <sub>1b</sub> 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- $\beta_{1b}$  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- $\delta_1$ , 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<sub>1</sub>/S boundary (67). PI-PLC- $\zeta$  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- $\zeta$  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<sub>2</sub> 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<sub>2</sub>/M phase of the cell cycle (17, 29, 71), and PKC- $\alpha$ -mediated phosphorylation and down-regulation of PI-PLC- $\beta_1$  in IGF-stimulated Swiss 3T3 cells (78). There are even less studies that may confirm the hypothesis that Ins(1,4,5)P<sub>3</sub>, 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<sub>3</sub> generated at the cell membrane plays in the regulation of the

cytosolic calcium (12). However,  $\text{Ins}(1,4,5)\text{P}_3$  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  $\text{PtdIns}(4,5)\text{P}_2$  itself influences many nuclear processes directly (35). In murine erythroleukemia cells, the activity of enzymes responsible for the synthesis of  $\text{PtdIns}(4,5)\text{P}_2$  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  $\text{PtdIns}(4)\text{P}$  5-kinase, regulates the levels of the nuclear  $\text{PtdIns}(4,5)\text{P}_2$  (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- $\delta_1$  translocation correlated with the increase in the level of the nuclear  $\text{PtdIns}(4,5)\text{P}_2$  and depended on high affinity  $\text{PtdIns}(4,5)\text{P}_2$  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- $\zeta$ -induced  $\text{Ca}^{2+}$  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  $\text{Ins}(1,4,5)\text{P}_3$  and subsequent  $\text{Ca}^{2+}$  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**

Phosphatidylinositol 3-kinases phosphorylate phosphoinositides at the 3' position of the inositol ring to generate PtdIns(3)P, PtdIns(3,4)P<sub>2</sub>, PtdIns(3,5)P<sub>2</sub> and PtdIns(3,4,5)P<sub>3</sub> 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<sub>2</sub> 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<sub>3</sub>, 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-C2 $\alpha$ , - $\beta$  and - $\gamma$ ). 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 pheochromocytoma 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<sub>3</sub> 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 *phospho inositide kinase enhancer* (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- $\gamma_1$  as an upstream regulator of PIKE; SH3 domain of PI-PLC- $\gamma_1$  was found to interact with proline rich domains (PRD) in PIKE (82). As GEF activity of PI-PLC- $\gamma_1$  depended only on that PRD-SH3 interaction and did not require the phospholipase catalytic activity, the model of PI-PLC- $\gamma_1$ /PIKE/nuclear PI3K activity provided one explanation for the

previous findings showing that mitogenic activity of PI-PLC- $\gamma_1$  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<sub>3</sub>. The one of these nuclear binding targets for PtdIns(3,4,5)P<sub>3</sub> 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<sub>3</sub> has an important role in myeloid differentiation. In the model of ATRA and vitamin D<sub>3</sub>-differentiated HL-60 cells, the increase in the level of immunoreactive p85, enzyme activity and endogenous PtdIns(3,4,5)P<sub>3</sub> 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- $\gamma_1$ , but the mechanism of nuclear activation, as previously described for the nuclear PI-PLC- $\gamma_1$ , 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- $\zeta$  (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<sub>3</sub> 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-C2 $\beta$  (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-C2 $\alpha$ , 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-C2 $\beta$  was mostly associated with nuclear matrices of hepatocytes during compensatory liver growth (64). Again, the subnuclear localization of PI3K-C2 $\beta$  depends on the cell type. In models of ATRA-differentiated and aphidicolin-synchronized HL-60 cells, the majority of PI3K-C2 $\beta$  activity was detected in the nuclear envelopes (73, 74). In contrast to the subnuclear localization, the mechanism of the

nuclear PI3K-C2 $\beta$  activation does seem to depend more on the physiological function of the enzyme. The same mechanism of calpain-mediated proteolysis and activation of PI3K-C2 $\beta$  has been described in both regenerating rat liver (63) and aphidicolin-synchronized HL-60 cells during G<sub>2</sub>/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-C2 $\beta$  activation. Instead of calpain-mediated proteolysis, the activation of PI3K-C2 $\beta$  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- $\beta_{1b}$  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- $\zeta$  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<sub>3</sub> on the cytosolic calcium (36). Furthermore, the mitogenic role of the nuclear PI-PLC- $\gamma_1$  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<sub>3</sub> (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)P<sub>3</sub> 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)P<sub>3</sub> 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)P<sub>3</sub> 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- $\delta_1$  with chromatin, the lack of Ins(1,4,5)P<sub>3</sub> 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)P<sub>3</sub>-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)P<sub>2</sub> 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).

**Acknowledgments** – This work was by the Ministry of Science, Education and Sport of the Republic of Croatia, grants No. 108-1081347-1448 (to D.V.) and 108-1081347-0173 (to H. B.).

## References

- [1] Ahn JY, Rong R, Liu X, Ye K (2004) PIKE/nuclear PI 3-kinase signaling mediates the antiapoptotic actions of NGF in the nucleus. *EMBO J* 23: 3995-4006
- [2] Ahn JY, Liu X, Cheng D, Peng J, Chan PK, Wade PA, Ye K (2005) Nucleophosmin/B23, a nuclear PI(3,4,5)P(3) receptor, mediates the antiapoptotic actions of NGF by inhibiting CAD. *Mol Cell* 13: 435-445
- [3] Ahn JY, Liu X, Liu Z, Pereira L, Cheng D, Peng J, Wade PA, Hamburger AW, Ye K (2006) Nuclear Akt associates with PKC-phosphorylated Ebp1, preventing DNA fragmentation by inhibition of caspase-activated DNase. *EMBO J* 25: 2083-2095
- [4] Bahk YY, Song H, Baek SH, Park BY, Kim H, Ryu SH, Suh PG (1998) Localization of two forms of phospholipase C-beta1, a and b, in C6Bu-1 cells. *Biochim Biophys Acta* 1389: 76-80
- [5] Banfic H, Zizak M, Divecha N, Irvine RF (1993) Nuclear diacylglycerol is increased during cell proliferation in vivo. *Biochem J* 290: 633-636
- [6] Bertagnolo V, Mazzoni M, Ricci D, Carini C, Neri LM, Previati M, Capitani S (1995) Identification of PI-PLC beta 1, gamma 1, and delta 1 in rat liver: subcellular distribution and relationship to inositol lipid nuclear signalling. *Cell Signal* 7: 669-678
- [7] Bertagnolo V, Marchisio M, Capitani S, Neri LM (1997) Intranuclear translocation of phospholipase C beta2 during HL-60 myeloid differentiation. *Biochem Biophys Res Commun* 235: 831-837
- [8] Bertagnolo V, Marchisio M, Volinia S, Caramelli E, Capitani S (1998) Nuclear association of tyrosine-phosphorylated Vav to phospholipase C-gamma1 and phosphoinositide 3-kinase during granulocytic differentiation of HL-60 cells. *FEBS Lett* 441: 480-484

- [9] Bertagnolo V, Neri LM, Marchisio M, Mischiati C, Capitani S (1999) Phosphoinositide 3-kinase activity is essential for all-trans-retinoic acid-induced granulocytic differentiation of HL-60 cells. *Cancer Res* 59: 542-546
- [10] Bertagnolo V, Brugnoli F, Marchisio M, Celeghini C, Carini C, Capitani S (2004) Association of PI 3-K with tyrosine phosphorylated Vav is essential for its activity in neutrophil-like maturation of myeloid cells. *Cell Signal* 16: 423-433
- [11] Billi AM, Matteucci A, Faenza I, Manzoli L, Rubbini S, Gilmour RS, Rhee SG, Cocco L (1997) Control of expression of PLCbeta1 by LAC repressor system: relationship between nuclear PLCbeta1 overexpression and growth factor stimulation. *Biochem Biophys Res Commun* 241: 122-126
- [12] Bootman MD, Thomas D, Tovey SC, Berridge MJ, Lipp P (2000) Nuclear calcium signalling. *Cell Mol Life Sci* 57: 371-378
- [13] Clarke JH, Letcher AJ, D'Santos CS, Halstead JR, Irvine RF, Divecha N (2001) Inositol lipids are regulated during cell cycle progression in the nuclei of murine erythroleukemia cells. *Biochem J* 357: 905-910
- [14] Cocco L, Gilmour RS, Ognibene A, Letcher AJ, Manzoli FA, Irvine RF (1987) Synthesis of polyphosphoinositides in nuclei of Friend cells. Evidence for polyphosphoinositide metabolism inside the nucleus which changes with cell differentiation. *Biochem J* 248: 765-770
- [15] Cocco L, Faenza I, Fiume R, Billi AM, Gilmour RS, Manzoli FA (2006) Phosphoinositide-specific phospholipase C (PI-PLC)  $\beta_1$  and nuclear lipid-dependent signaling. *Biochim Biophys Acta* 1761: 509-521
- [16] Crljen V, Visnjic D, Banfic H (2004) Presence of different phospholipase C isoforms in the nucleus and their activation during compensatory liver growth. *FEBS Lett* 571: 35-42



- [17] Deacon EM, Pettitt TR, Webb P, Cross T, Chahal H, Wakelam MJ, Lord JM (2002) Generation of diacylglycerol molecular species through the cell cycle: a role for 1-stearoyl, 2-arachidonyl glycerol in the activation of nuclear protein kinase C-betaII at G<sub>2</sub>/M. *J Cell Sci* 115: 983-989
- [18] Didichenko S, Thelen M (2001) Phosphatidylinositol 3-kinase c2alpha contains a nuclear localization sequence and associates with nuclear speckles. *J Biol Chem* 276: 48135-48142
- [19] Di Paolo G, De Camilli P (2006) Phosphoinositides in cell regulation and membrane dynamics. *Nature* 443: 651-657
- [20] Divecha N, Banfic H, Irvine RF (1991) The polyphosphoinositide cycle exists in the nuclei of Swiss 3T3 cells under the control of a receptor (for IGF-I) in the plasma membrane, and stimulation of the cycle increases nuclear diacylglycerol and apparently induces translocation of protein kinase C to the nucleus. *EMBO J* 10: 3207-3214
- [21] Divecha N, Rhee SG, Letcher AJ, Irvine RF (1993) Phosphoinositide signalling enzymes in rat liver nuclei: phosphoinositidase C isoform beta1 is specifically, but not predominantly, located in the nucleus. *Biochem J* 289: 617-620
- [22] Divecha N, Banfic H, Irvine RF (1993) Inositides and the nucleus and inositides in the nucleus. *Cell* 74: 405-407
- [23] Divecha N, Roefs M, Los A, Halstead J, Bannister A, D'Santos C (2002) Type I PIPkinases interact with and are regulated by the retinoblastoma susceptibility gene product-pRb. *Curr Biol* 12: 582-587
- [24] Engelman JA, Luo J, Cantley LC (2006) The evolution of phosphatidylinositol 3-kinases as regulators of growth and metabolism. *Nat Rev Genet* 7: 606-619
- [25] Faenza I, Matteucci A, Manzoli L, Billi AM, Aluigi M, Peruzzi D, Vitale M, Castorina S, Suh PG, Cocco L (2000) A role for nuclear phospholipase Cbeta 1 in cell cycle control. *J Biol Chem* 275: 30520-30524

- [26] Faenza I, Bavelloni A, Fiume R, Lattanzi G, Maraldi NM, Gilmour RS, Martelli AM, Suh PG, Billi AM, Cocco L (2003) Up-regulation of nuclear PLCbeta1 in myogenic differentiation. *J Cell Physiol* 195: 446-452
- [27] Fiume R, Faenza I, Matteucci A, Astolfi A, Vitale M, Martelli AM, Cocco L (2005) Nuclear phospholipase C beta1 (PLCbeta1) affects CD24 expression in murine erythroleukemia cells. *J Biol Chem* 280: 24221-24226
- [28] Gonzales ML, Anderson RA (2006) Nuclear phosphoinositide kinases and inositol phospholipids. *J Cell Biochem* 97: 252-260
- [29] Goss VL, Hocevar BA, Thompson LJ, Stratton CA, Burns DJ, Fields AP (1994) Identification of nuclear beta II protein kinase C as a mitotic lamin kinase. *J Biol Chem* 269: 19074-19080
- [30] Hokin MR, Hokin LE (1953) Enzyme secretion and the incorporation of P<sup>32</sup> into phosphoinositides of pancreas slices. *J Biol Chem* 203: 967-977
- [31] Hu Y, Yao J, Liu Z, Liu X, Fu H, Ye K (2005) Akt phosphorylates acinus and inhibits its proteolytic cleavage, preventing chromatin condensation. *EMBO J* 24: 3543-3554
- [32] Hunt AN (2006) Dynamic lipidomics of the nucleus. *J Cell Biochem* 97: 244-251
- [33] Irvine RF (2003) Nuclear lipid signalling. *Nat Rev Mol Cell Biol* 4: 349-360
- [34] Irvine RF (2006) Nuclear inositide signalling – expansion, structures and clarification. *Biochim Biophys Acta* 1761: 505-508
- [35] Jones DR, Divecha N (2004) Linking lipids to chromatin. *Curr Opin Genet Dev* 14: 196-202
- [36] **Larman MG, Saunders CM; Carrol J, Lai FA, Swann K (2004) Cell cycle-dependent Ca<sup>2+</sup> oscillations in mouse embryos are regulated by nuclear targeting of PLCzeta. *J Cell Sci* 117: 2513-2521**

- [37] Lin H, Choi JH, Hasek J, DeLillo N, Lou W, Vancura A (2000) Phospholipase C is involved in kinetochore function in *Saccharomyces cerevisiae*, *Mol Cell Biol* 20: 3597-3607
- [38] Los A, Vinke FP, de Widt J, Topham MK, van Blitterswijk WJ, Divecha N (2006) The retinoblastoma family proteins bind to and activate diacylglycerol kinase  $\zeta$ . *J Biol Chem* 281: 858-866
- [39] Lu PJ, Hsu AL, Wang DS, Yan HY, Yin HL, Chen CS (1998) Phosphoinositide 3-kinase in rat liver nuclei. *Biochemistry* 37: 5738-5745
- [40] Lukinovic-Skudar V, Donlagic L, Banfic H, Visnjic D (2005) Nuclear phospholipase C-beta1b activation during G<sub>2</sub>/M and late G<sub>1</sub> phase in nocodazole-synchronized HL-60 cells. *Biochim Biophys Acta* 1733: 148-156
- [41] Lukinovic-Skudar V, Visnjic D, Crljen-Manestar V, Matkovic K, Banfic H (2006) Role of nuclear phospholipase C in regulation of cell cycle. *Period Biol* 108: 571-580
- [42] Lukinovic-Skudar V, Matkovic K, Banfic H, Visnjic D (2007) Two waves of the nuclear phospholipase C activity in serum-stimulated HL-60 cells during G<sub>1</sub> phase of the cell cycle. *Biochim Biophys Acta* 1771: 514-521
- [43] Macbeth MR, Schubert HL, Vandemark AP, Lingam AT, Hill CP, Bass BL (2005) Inositol hexakisphosphate is bound in the ADAR2 core and required for RNA editing. *Science* 309: 1534-1539
- [44] Manzoli L, Billi AM, Rubbini S, Bavelloni A, Faenza I, Gilmour RS, Rhee SG, Cocco L (1997) Essential role for nuclear phospholipase C beta1 in insulin-like growth factor I-induced mitogenesis. *Cancer Res* 57: 2137-2139
- [45] Marchisio M, Bertagnolo V, Colamussi ML, Capitani S, Neri LM (1998) Phosphatidylinositol 3-kinase in HL-60 nuclei is bound to the nuclear matrix and increases during granulocytic differentiation. *Biochem Biophys Res Commun* 253: 346-351

- [46] Martelli AM, Gilmour RS, Bertagnolo V, Neri LM, Manzoli L, Cocco L (1992) Nuclear localization and signalling activity of phosphoinositidase C beta in Swiss 3T3 cells. *Nature* 358: 242-245
- [47] Martelli AM, Cocco L, Bareggi R, Tabellini G, Rizzoli R, Ghibellini MD, Narducci P (1999) Insulin-like growth factor-1-dependent stimulation of nuclear phospholipase C- $\beta$ 1 activity in Swiss 3T3 cells requires an intact cytoskeleton and is paralleled by increased phosphorylation of the phospholipase. *J Cell Biochem* 72: 339-348
- [48] Martelli AM, Billi AM, Manzoli L, Faenza I, Aluigi M, Falconi M, De Pol A, Gilmour RS, Cocco L (2000) Insulin selectively stimulates nuclear phosphoinositide-specific phospholipase C (PI-PLC) beta1 activity through a mitogen-activated protein (MAP) kinase-dependent serine phosphorylation. *FEBS Lett* 486: 230-236
- [49] Martelli AM, Manzoli L, Cocco L (2004) Nuclear inositides: facts and perspectives. *Pharmacol Ther* 101: 47-64
- [50] Martelli AM, Evangelisti C, Nyakern M, Manzoli FA (2006) Nuclear protein kinase C. *Biochim Biophys Acta* 1761: 542-551
- [51] Martelli AM, Faenza I, Billi AM, Manzoli L, Evangelisti C, Fala F, Cocco L (2006) Intranuclear 3'-phosphoinositide metabolism and Akt signaling: new mechanisms for tumorigenesis and protection against apoptosis? *Cell Signal* 18: 1101-1107
- [52] Matkovic K, Brugnoli F, Bertagnolo V, Banfic H, Visnjic D (2006) The role of the nuclear Akt activation and Akt inhibitors in all-trans-retinoic acid-differentiated HL-60 cells. *Leukemia* 20: 941-951
- [53] Neri LM, Ricci D, Carini C, Marchisio M, Capitani S, Bertagnolo V (1997) Changes of nuclear PI-PLC gamma1 during rat liver regeneration. *Cell Signal* 9:353-362**
- [54] Neri LM, Marchisio M, Colamussi ML, Bertagnolo V (1999) Monocytic differentiation of HL-60 cells is characterized by the nuclear translocation of phosphatidylinositol 3-kinase

and of definite phosphatidylinositol-specific phospholipase C isoforms. *Biochem Biophys Res Commun* 259: 314-320

[55] Neri LM, Martelli AM, Borgatti P, Colamussi ML, Marchisio M, Capitani S (1999) Increase in nuclear phosphatidylinositol 3-kinase activity and phosphatidylinositol (3,4,5) trisphosphate synthesis precede PKC-zeta translocation to the nucleus of NGF-treated PC12 cells. *FASEB J* 13: 2299-2310

[56] Neri LM, Bortul R, Borgatti P, Tabellini G, Baldini G, Capitani S, Martelli AM (2002) Proliferating or differentiating stimuli act on different lipid-dependent signaling pathways in nuclei of human leukemia cells. *Mol Biol Cell* 13: 947-964

[57] Odom AR, Stahlberg A, Wenthe SR, York JD (2000) A role for nuclear inositol 1,4,5-trisphosphate kinase in transcriptional control. *Science* 287: 2026-2029

[58] Okada M, Fujii M, Yamaga M, Sugimoto H, Sadano H, Osumi T, Kamata H, Hirata H, Yagisawa H (2002) Carboxyl-terminal basic amino acids in the X domain are essential for the nuclear import of phospholipase C delta 1. *Genes Cells* 7: 985-996

[59] Okada M, Ishimoto T, Naito Y, Hirata H, Yagisawa H (2005) Phospholipase C delta (1) associates with importin beta1 and translocates into the nucleus in a Ca<sup>2+</sup>-dependent manner. *FEBS Lett* 579: 4949-4954

[60] Osborne SL, Thomas CL, Gschmeissner S, Schiavo G (2001) Nuclear PtdIns(4,5)P<sub>2</sub> assembles in a mitotically regulated particle involved in pre-mRNA splicing. *J Cell Sci* 114: 2501-2511

[61] Rhee SG (2001) Regulation of phosphoinositide-specific phospholipase C. *Annu Rev Biochem* 70: 281-312

[62] Rose HG, Frenster JH (1965) Composition and metabolism of lipids within repressed and active chromatin of interphase lymphocytes. *Biochim Biophys Acta* 106: 577-591

- [63] Sindic A, Aleksandrova A, Fields AP, Volinia S, Banfic H (2001) Presence and activation of nuclear phosphoinositide 3-kinase C2beta during compensatory liver growth. *J Biol Chem* 276: 17754-17761
- [64] Sindic A, Crljen V, Matkovic K, Lukinovic-Skudar V, Visnjic D, Banfic H (2006) Activation of phosphoinositide 3-kinase C2 beta in the nuclear matrix during compensatory liver growth. *Adv Enzyme Regul* 46: 280-287
- [65] Smith CD, Wells WW (1983) Phosphorylation of rat liver nuclear envelopes. II Characterization of in vitro lipid phosphorylation. *J Biol Chem* 258: 59368-9373
- [66] Smith MR, Liu YL, Matthews NT, Rhee SG, Sung WK, Kung HF (1994) Phospholipase C-gamma 1 can induce DNA synthesis by a mechanism independent of its lipase activity. *Proc Natl Acad Sci USA* 91: 6554-6558
- [67] **Stallings JD, Tall EG, Pentylala S, Rebecchi MJ (2005) Nuclear translocation of phospholipase C-delta1 is linked to the cell cycle and nuclear phosphatidylinositol 4,5-bisphosphate. *J Biol Chem* 280: 22060-222069**
- [68] Sun B, Murray NR, Fields AP (1997) A role for nuclear phosphatidylinositol-specific phospholipase C in the G<sub>2</sub>/M phase transition. *J Biol Chem* 272: 26313-26317
- [69] **Swann K, Saunders CM, Rogers NT, Lai FA (2006) PLCzeta: a sperm protein that triggers Ca<sup>2+</sup> oscillations and egg activation in mammals. *Semin Cell Dev Biol* 17:264-273**
- [70] Tanaka K, Horiguchi K, Yoshida T, Takeda M, Fujisawa H, Takeuchi K, Umeda M, Kato S, Ihara S, Nagata S, Fukui Y (1999) Evidence that a phosphatidylinositol 3,4,5-triphosphate-binding protein can function in nucleus. *J Biol Chem* 274: 3919-3922
- [71] Thompson LJ, Fields AP (1996) BetaII protein kinase C is required for the G<sub>2</sub>/M phase transition of cell cycle. *J Biol Chem* 271: 15045-15053

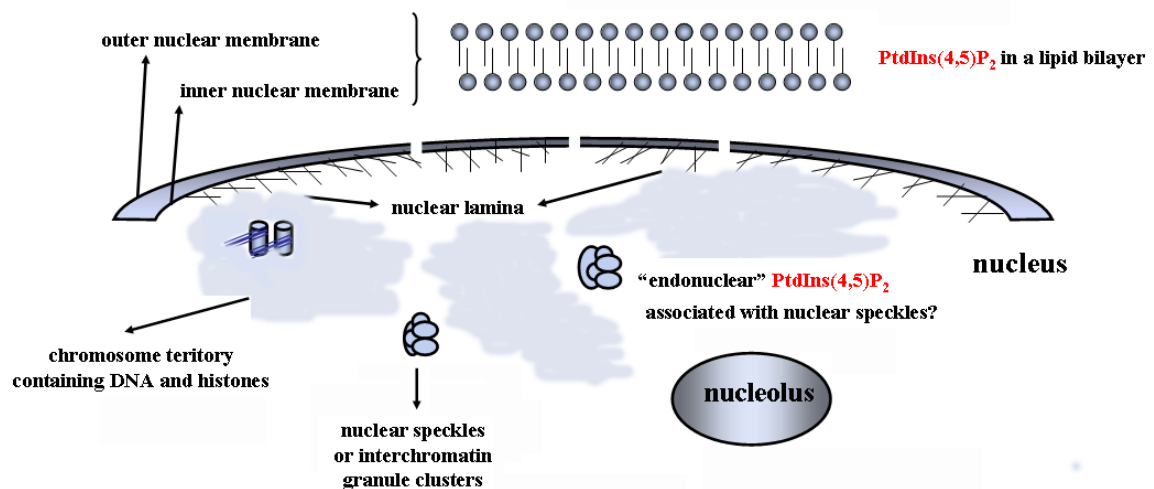
- [72] Toker A (2002) Phosphoinositides and signal transduction. *Cell Mol Life Sci* 59: 761-779
- [73] Visnjic D, Crljen V, Curic J, Batinic D, Volinia S, Banfic H (2002) The activation of nuclear phosphoinositide 3-kinase C2beta in all-trans-retinoic acid-differentiated HL-60 cells. *FEBS Lett* 529: 268-274
- [74] Visnjic D, Curic J, Crljen V, Batinic D, Volinia S, Banfic H (2003) Nuclear phosphoinositide 3-kinase C2beta activation during G2/M phase of the cell cycle in HL-60 cells. *Biochim Biophys Acta* 1631: 61-71
- [75] Vitale M, Matteucci A, Manzoli L, Rodella L, Mariani AR, Zauli G, Falconi M, Billi AM, Martelli AM, Gilmour RS, Cocco L (2001) Interleukin 2 activates nuclear phospholipase C beta by mitogen-activated protein kinase-dependent phosphorylation in human natural killer cells. *FASEB J* 15: 1789-1791
- [76] Watt SA, Kular G, Fleming IN, Downes CP, Lucocq JM (2002) Subcellular localization of phosphatidylinositol 4,5-bisphosphate using the pleckstrin homology domain of phospholipase C delta1. *Biochem J* 363: 657-666
- [77] Xu A, Suh PG, Marmy-Conus N, Pearson RB, Seok OY, Cocco L, Gilmour RS (2001) Phosphorylation of nuclear phospholipase C beta1 by extracellular signal-regulated kinase mediates the mitogenic action of insulin-like growth factor I. *Mol Cell Biol* 21: 2981-2990
- [78] Xu A, Wang Y, Xu LY, Gilmour RS (2001) Protein kinase C alpha-mediated negative feedback regulation is responsible for the termination of insulin-like growth factor I-induced activation of nuclear phospholipase C beta1 in Swiss 3T3 cells. *J Biol Chem* 276: 14980-14986
- [79] Yagisawa H, Okada M, Naito Y, Sasaki K, Yamaga M, Fujii M (2006) Coordinated intracellular translocation of phosphoinositide-specific phospholipase C- $\delta$  with the cell cycle. *Biochim Biophys Acta* 1761: 522-534

- [80] Yamaga M, Fujii M, Kamata H, Hirata H, Yagisawa H (1999) Phospholipase C-delta1 contains a functional nuclear export signal sequence. *J Biol Chem* 274: 28537-28541
- [81] Ye K, Hurt KJ, Wu FY, Fang M, Luo HR, Hong JJ, Blackshaw S, Ferris CD, Snyder SH (2000) Pike. A Nuclear GTPase that enhances PI3Kinase activity and is regulated by protein 4.1N. *Cell* 103: 919-930
- [82] Ye K, Aghdasi B, Luo HR, Moriarity JL, Wu FY, Hong JJ, Hurt KJ, Bae SS, Suh PG, Snyder SH (2002) Phospholipase C gamma 1 is a physiological guanine nucleotide exchange factor for the nuclear GTPase PIKE. *Nature* 415: 541-544
- [83] York JD, Odom AR, Murphy R, Ives EB, Wentz SR (1999) A phospholipase C-dependent inositol polyphosphate kinase pathway required for efficient messenger RNA export. *Science* 285: 96-100
- [84] York JD (2006) Regulation of nuclear processes by inositol polyphosphates. *Biochim Biophys Acta* 1761: 552-559**
- [85] York SJ, Armbruster BN, Greenwell P, Petes TD, York JD (2005) Inositol diphosphate signaling regulates telomere length. *J Biol Chem* 280: 4264-4266

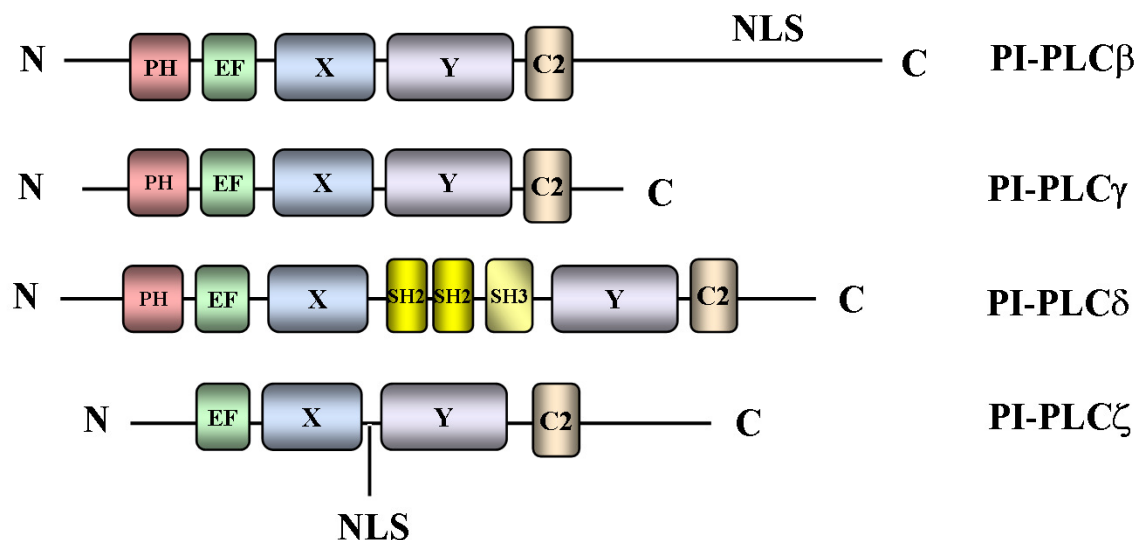


### Figure legends:

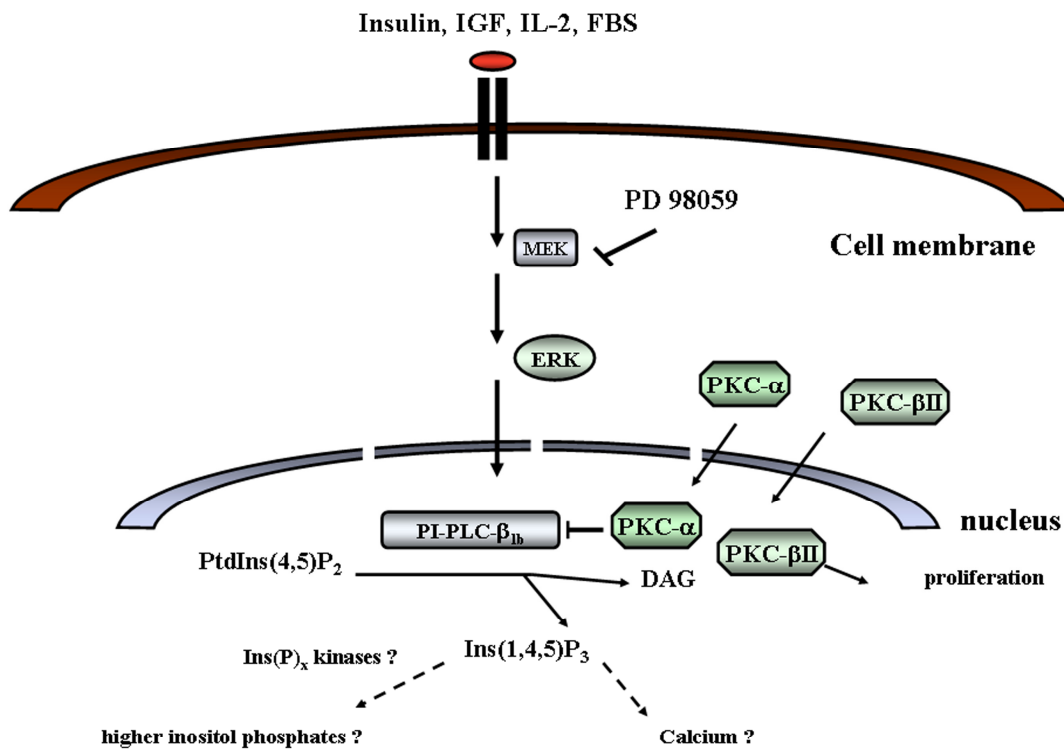
**Figure 1. The basic structure of nucleus – “nuclear” and “endonuclear” PtdIns(4,5)P<sub>2</sub>.** PtdIns(4,5)P<sub>2</sub> is a component of a classic phospholipid bilayer and the nuclear envelope is composed of the outer and inner nuclear membranes. “Endonuclear” PtdIns(4,5)P<sub>2</sub>, i.e. PtdIns(4,5)P<sub>2</sub> 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.



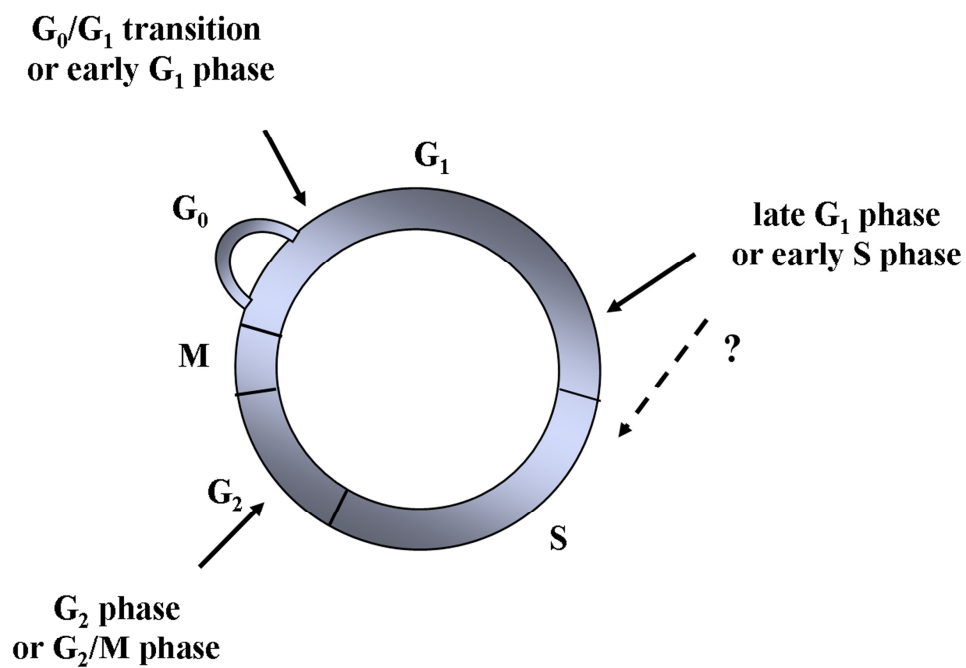
**Figure 2. The structure of PI-PLC isoforms that are most frequently found in nuclei. PI-PLC- $\beta$ , - $\gamma$  and - $\delta$  isoforms contain catalytic domains X and Y, pleckstrin homology domain (PH), EF hands and C2 domain that binds calcium. PI-PLC- $\zeta$  lacks PH domain. PI-PLC- $\gamma$  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- $\beta$  **and** a **sequence between X and Y domain in PI-PLC- $\zeta$**  were found to provide a nuclear localization signal (NLS).**



**Figure 3. MAPK-mediated activation of the nuclear PI-PLC- $\beta_{1b}$ .** Several agonists (insulin, IGF, IL-2 and FBS) stimulate the activity of the nuclear PI-PLC- $\beta_{1b}$  that is sensitive to the presence of MEK inhibitor PD98059. PI-PLC- $\beta_{1b}$ -mediated hydrolysis of PtdIns(4,5)P<sub>2</sub> generates DAG and Ins(1,4,5)P<sub>3</sub>. The nuclear DAG attracts several PKC isoforms to the nucleus; PKC- $\alpha$  phosphorylates PI-PLC- $\beta_{1b}$  and inhibits its activity, PKC- $\beta$ II mediates proliferative signal. The role of the nuclear Ins(1,4,5)P<sub>3</sub> may be the regulation of the nuclear calcium. In addition, Ins(1,4,5)P<sub>3</sub> 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- $\beta_{1b}$  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- $\gamma$  and PI 3-kinase class I (p85 $\alpha$ /p110) into the nucleus. A catalytically inactive PI-PLC- $\gamma$  act as guanine exchange factor (GEF) and activates nuclear GTPase called PIKE (*phospho inositide kinase enhancer*). PIKE stimulates the activity of the nuclear PI3K that phosphorylates PtdIns(4,5)P<sub>2</sub> into PtdIns(3,4,5)P<sub>3</sub>. PtdIns(3,4,5)P<sub>3</sub> attracts PKC- $\zeta$  which translocates from cytoplasm to the nucleus and phosphorylates nucleolin. The complex of PtdIns(3,4,5)P<sub>3</sub> 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.

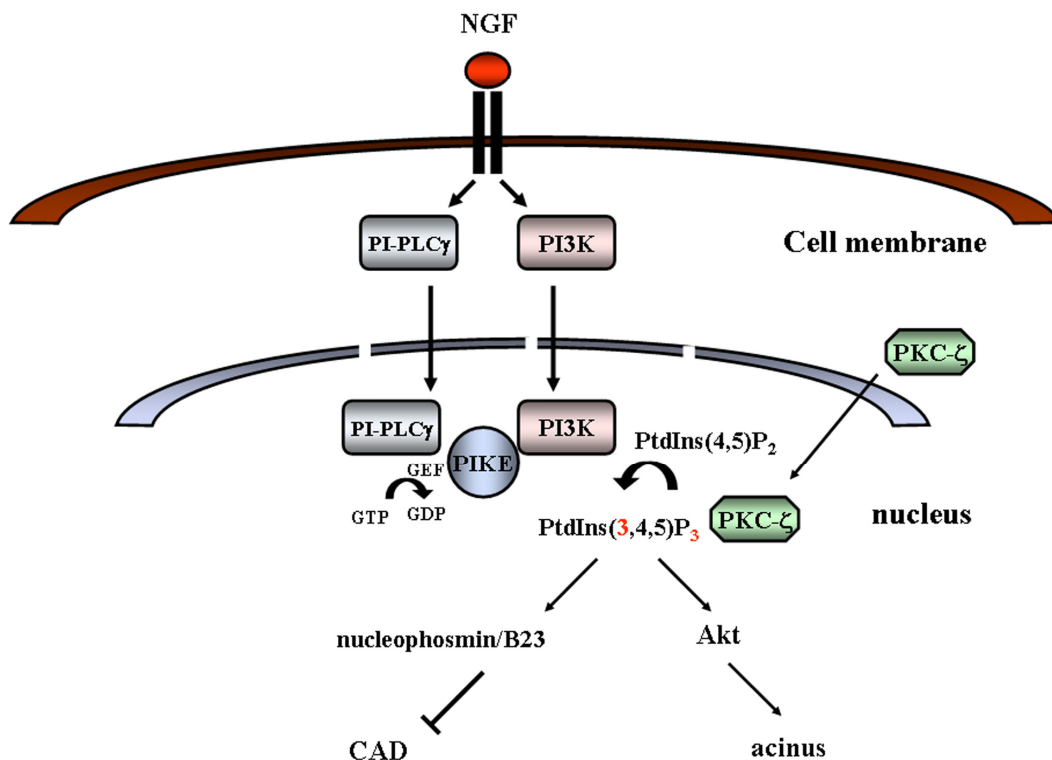


Table 1. Overview of different nuclear PLC isoform activation during cell cycle progression

Isoform	The phase of cell cycle	Model	The enzyme activity	The amount of enzyme	The interaction partner in the nucleus
PI-PLC- $\beta_1$	G <sub>0</sub> /early G <sub>1</sub>	IGF, Swiss 3T3 cells <sup>(77, 47)</sup>	Increased (PLC assay) <sup>(77, 47)*</sup> MAPK-mediated phosphorylation at Ser982 <sup>(77)</sup>	No change <sup>(77, 47)</sup>	Immunoprecipitated with phospho-MAPK <sup>(77)</sup>
		IGF, HL-60 <sup>(56)</sup>	Increased (PLC assay) <sup>(56)</sup> Increased DAG <sup>(56)</sup>	No change <sup>(56)</sup>	ND <sup>(56)</sup>
- $\beta_{1\beta}$		Insulin, NIH 3T3 <sup>(48)</sup>	Increased (PLC assay) <sup>(48)</sup> MEK inhibitor-sensitive serine phosphorylation <sup>(48)</sup>	No change <sup>(48)</sup>	ND <sup>(48)</sup>
		IL-2, NK cells <sup>(75)</sup>	Increased (PLC assay) <sup>(75)</sup> Increased DAG <sup>(75)</sup> MEK inhibitor-sensitive serine phosphorylation <sup>(75)</sup>	No change <sup>(75)</sup>	ND <sup>(75)</sup>
		FBS, HL-60 <sup>(42)</sup>	Increased (PLC assay) <sup>(42)</sup> MEK inhibitor-sensitive serine phosphorylation <sup>(42)</sup>	No change <sup>(42)</sup>	ND <sup>(42)</sup>
	late G <sub>1</sub> /S	Hepatectomy, liver <sup>(16)</sup>	Increased (PLC assay) <sup>(16)</sup> Serine phosphorylation <sup>(16)</sup>	No change <sup>(16)</sup>	ND <sup>(16)</sup>
		FBS, HL-60 <sup>(42)</sup> Nocodazole, HL-60 <sup>(40)</sup>	Increased (PLC assay) <sup>(40,42)</sup> MEK inhibitor-sensitive serine phosphorylation <sup>(40,42)</sup>	No change <sup>(40,42)</sup>	ND <sup>(40,42)</sup>
		Hepatectomy, liver <sup>(16)</sup>	Increased (PLC assay) <sup>(16)</sup> No phosphorylation <sup>(16)</sup>	Increased <sup>(16)</sup>	ND <sup>(16)</sup>
		Nocodazole, HL-60 <sup>(40)</sup> Aphidicolin, HL-60 <sup>(68)</sup>	Increased (PLC assay) <sup>(40)</sup> Increased DAG <sup>(68)</sup> MEK inhibitor-sensitive serine phosphorylation <sup>(40)</sup>	No change <sup>(40)</sup>	ND <sup>(40,68)</sup>
PI-PLC- $\gamma_1$	G <sub>0</sub> /early G <sub>1</sub>	Hepatectomy, liver <sup>(16,53)</sup>	Increased (PLC assay) <sup>(16,53)</sup> Tyrosine phosphorylation <sup>(16,53)</sup>	No change <sup>(16,53)</sup>	ND <sup>(16,53)</sup>
		NGF, PC12 <sup>(82)</sup>	ND <sup>(82)</sup>	Increased <sup>(82)</sup>	PIKE GTPase (PLC $\gamma$ acts as GEF) <sup>(82)</sup>
	late G <sub>1</sub> /S	ATRA, HL-60 <sup>(8)</sup>	ND <sup>(8)</sup>	Increased <sup>(8)</sup>	Immunoprecipitated with Vav <sup>(8)</sup>
		Hepatectomy, liver <sup>(16,53)</sup>	Increased (PLC assay) <sup>(16,53)</sup> Tyrosine phosphorylation <sup>(16,53)</sup>	No change <sup>(16,53)</sup>	ND <sup>(16,53)</sup>
		Reduced serum, NIH 3T3 <sup>(67)</sup>	ND <sup>(67)</sup>	Increased <sup>(67)</sup>	Phosphoinositides <sup>(67)</sup>
PI-PLC- $\delta_1$	Late G <sub>1</sub> /S	Thymidine block, NIH3T3 <sup>(67, 79)</sup>	ND <sup>(67, 79)</sup>	Increased <sup>(67, 79)</sup>	ND <sup>(67, 79)</sup>
		Hepatectomy, liver <sup>(16)</sup>	Increased (PLC assay) <sup>(16)</sup>	Increased <sup>(16)</sup>	ND <sup>(16)</sup>
PI-PLC- $\zeta$	interphase	Fertilized eggs <sup>(36)</sup>	ND <sup>(36)</sup>	Increased <sup>(36)</sup>	ND <sup>(36)</sup>

ND- not determined

\*- reference number