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Nucleocytoplasmic Shuttling of Phospholipase C-δ_1: A Link to Ca^{2+}

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Abstract Phosphoinositides (PIs) and proteins involved in the PI signaling pathway are distributed in the nucleus as well as at the plasma membrane and in the cytoplasm, although their nuclear localization mechanisms have not been clarified in detail. Generally, proteins that shuttle between the cytoplasm and nucleus contain nuclear localization signal (NLS) and nuclear export signal (NES) sequences for nuclear import and export, respectively. They bind to specific carrier proteins of the importin/exportin family and are transported to and from the nucleus. Thus there is a steady state shuttling of the cargo molecules to and from the nucleus, and the shift in equilibrium determines their nuclear or cytoplasmic localization. Our previous studies have shown that phospholipase C (PLC)-δ_1, regarded as having cytoplasmic- or plasma membrane-bound localization, accumulates in the nucleus when its NES sequence is disrupted. In addition, a cluster of positively charged residues on the surface of the catalytic barrel is important for nuclear import. In quiescent cells, the shuttling equilibrium seems to be shifted to the nuclear export of PLCδ_1. In this review, recent findings regarding the molecular machineries and mechanisms of the nucleocytoplasmic shuttling of PLCδ_1 will be discussed. It is important to know when and how they are regulated. A shift in the equilibrium in a certain stage of the cell cycle or by external stimuli is possible and resulting changes in the intra-nuclear environments (or architectures) may alter proliferation and differentiation patterns. Evidences support the idea that an increase in the levels of intracellular Ca^{2+} shifts the equilibrium to the nuclear import of PLCδ_1. A myriad of external stimuli have also been reported to change the nuclear PI metabolism following accelerated accumulation in the nucleus of other phospholipases such as phospholipase A_2 and phospholipase D in addition to PLC isoforms such as PLCβ_1 and PLCγ_1. The consequence of the nuclear accumulation of PLC is also discussed. J. Cell. Biochem. 97: 233–243, 2006.

Key words: phospholipase C; importin β; intracellular Ca^{2+}; nuclear export signal; nuclear localization signal; nucleocytoplasmic shuttling

The phosphoinositide (PI) cycle is an important signaling pathway for cell proliferation and differentiation. In our classical view, lipid metabolites are generated at the plasma membrane in a receptor-dependent manner. Although the presence of PI and metabolites in isolated nuclei has been reported since the 1960s, these findings were thought to be a result of contamination with other organelles or the cytoplasm [D'Santos et al., 1998; Irvine, 2003]. Thanks to the availability of antibodies against PI-synthesizing and PI-metabolizing enzymes and new PI lipid probes such as the pleckstrin homology (PH) domain, we now know that both lipid modifying enzymes and lipid substrates are present in the nucleus. For example, the presence of PtdIns(4,5)P_2 in the nucleus is confirmed in living cells or on fixed cells by using a specific PH domain probe or anti-PtdIns(4,5)P_2 antibodies [Watt et al., 2002; Yagisawa et al., 2002]. It is becoming clear that these molecules may be important for various nuclear events ranging from chromatin

Abbreviations used: DG, diacylglycerol; DGK, DG kinase; LMB, leptomycin B, NES, nuclear export signal; NLS, nuclear localization signal; NPC, nuclear pore complex; PH, pleckstrin homology; PI, phosphoinositide; PKC, protein kinase C; PLC, phospholipase C.

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remodeling [Rando et al., 2002] to RNA processing [Osborne et al., 2001] and export [York et al., 1999]. There are some updated reviews on nuclear lipid signaling [Irvine, 2003; Martelli et al., 2003; Jones and Divecha, 2004; Manzoli et al., 2005].

PHOSPHOLIPASE C AND NUCLEAR PHOSPHOLIPASE C

At present, the mammalian PI-specific phospholipase C (PLC) superfamily has been identified as being composed of six major groups, PLCβ, PLCγ, PLCδ, PLCϵ, PLCζ, and PLCη. These enzymes catalyze the hydrolysis of PtdIns(4,5)P_2 to Ins(1,4,5)P_3 and diacylglycerol (DG) in response to the activation of a plethora of cell surface receptors. The isoforms contain X and Y domains, which form the catalytic core, and various regulatory domains such as the PH domain that binds PtdIns(4,5)P_2 and tethers the entire molecule to membranes. The isoforms are thought to serve as regulatory molecules reducing the amount of PtdIns(4,5)P_2 at the plasma membrane as well as producing the second messengers that activate both Ca^{2+} and protein kinase C (PKC) signaling. At present, at least 13 isoforms with enzymatic activity have been identified in the mammalian PLC family. In addition, a few proteins that show very close homology to PLC but do not have the activity, such as p130 PRIP (PLC-related catalytically inactive protein), exist. Both active and inactive PLC homologs show varied tissue distribution.

PLCβ_1 and PLCγ_1, which both show cytoplasmic localization in unstimulated cells, go into the nucleus by activation of growth factor receptors [Bertagnolo et al., 1995]. Not all but some mitogenic signal such as insulin-like growth factor-1 on cultured fibroblasts causes nuclear translocation of PLCβ_1 and its activation in the nucleus [Divecha et al., 1991]. Highly transformed and proliferating cells accumulate PLCγ_1 in the nuclei [Diakonova et al., 1997]. As for δ isoforms, the plasma membrane localization of PLCδ_1 through its PH domain in many cell types has been reported [Yagisawa et al., 2002], while the continuous presence of PLCδ_4 in the nucleus has been observed [Liu et al., 1996]. Cell cycle-dependent nuclear translocation of PLCζ, which causes fertilization-like Ca^{2+} oscillations when injected into eggs, has been reported during early embryonic development (up to blastocysts) of mice [Larman et al., 2004; Sone et al., 2005]. Other enzymes related to the PI synthesis and metabolism, such as Type II PI*P* kinase (PtdIns(5)*P* 4-kinase) β [Ciruela et al., 2000] and DGKθ [Bregoli et al., 2001] were also found in the nucleus. Some of them are thought to shuttle between the cytoplasm and the nucleus.

NUCLEOCYTOPLASMIC SHUTTLING: A GENERAL VIEW

Generally, proteins that shuttle between the cytoplasm and the nucleus have a nuclear localization signal (NLS) sequence and a nuclear export signal (NES) sequence [Dingwall and Laskey, 1991; Fukuda et al., 1997]. NLS sequences usually consist of a cluster (or clusters) of basic amino acids. A cargo protein then binds to an adaptor protein called importin α via its NLS sequence. Importins are soluble carrier proteins that shuttle cargo proteins between the cytoplasm and the nucleus [Gorlich and Mattaj, 1996]. There are two subgroups, the importin α family and the importin β family. As mentioned above, importin α serves as an adaptor for a cargo protein containing a NLS sequence and also binds to importin β to form a ternary complex. The complex is then transferred to the internal face of the nuclear pore, recognized by a certain machinery in the nuclear pore complex (NPC) on the nuclear envelope by still-unknown mechanisms, and transported further into the nucleoplasm. The ternary complex then dissociates, and the carrier (importin β) and adaptor (importin α) are transported back by a small G protein called Ran [Gorlich and Kutay, 1999; Yoneda, 2000]. The cargo molecule, depending on its function, plays its role in the nucleus and then binds to another importin β-like molecule, exportin. Exportin recognizes NES on the cargo protein and the complex is then exported from the nucleus by binding with the GTP-bound form of Ran. Conversion of GTP-Ran to the GDP-bound form in the cytoplasm releases the cargo molecule from the complex.

There is a mechanism that does not require the presence of importin α for nuclear import. In this case, the cargo molecule directly binds to importin β, is recognized by the NPC and transported into the nucleus. This system has been described for several proteins, including SREBP (sterol regulatory element-binding protein)-2 and cyclin B1 [Yoneda, 2000].

INTRACELLULAR TRANSLOCATION OF PLCδ1

Because of the presence of the PH domain that shows a high affinity (K_d of 10–100 nM) for PtdIns(4,5)P_2, PLCδ1 is generally distributed at the inner leaflet of the plasma membrane and in the cytoplasm of various cell types. Although there are only a few reported agonists whose receptors directly activate PLCδ1, a myriad of external stimuli that modulate the levels of PtdIns(4,5)P_2 can dissociate PLCδ1 from the plasma membrane. Loss of PtdIns(4,5)P_2 by activation of any of the PLC isoforms or of PtdIns(4,5)P_2 phosphatases or sequesterization by PtdIns(4,5)P_2—binding proteins can facilitate the dissociation of PLCδ1. The dissociation can be readily observed by elevation of internal Ca^{2+} levels, since, of all the isoforms, PLCδ1 shows the highest sensitivity to Ca^{2+}. It is unlikely, however, that activation of PI 3-kinases that phosphorylates PtdIns(4,5)P_2 to produce PtdIns(3,4,5)P_3 reduces the membrane-attached PLCδ1. In some cell types, translocation of PLCδ1 to perinuclear regions was observed after dissociation from the plasma membrane [Fujii et al., 1999].

Nuclear Accumulation of PLCδ1 Proves the Presence of Nucleocytoplasmic Shuttling

To clarify whether PLCδ1 actually goes in to the nucleus, leptomycin B (LMB), an inhibitor of nuclear export, was used. LMB is an anti-fungal antibiotic and an inhibitor of the cell cycle in mammalian and fission yeast cells [Yoshida et al., 1990]. It covalently binds to cysteine residues in the structural core of exportin-1 (or CRM1: chromosome region maintenance-1), a receptor for canonical leucine-rich NES, blocking the nuclear export performed by this carrier molecule, causing accumulation of cargo proteins that shuttle between the nucleus and the cytoplasm in the nucleus. Endogenous PLCδ1 or ectopically expressed PLCδ1 accumulates in the nucleus within several hours after LMB treatment in NRK cells or in MDCK cells, respectively [Yamaga et al., 1999]. This indicates that although PLCδ1 is generally localized at the plasma membrane and in the cytoplasm in quiescent cells, it shuttles between the nucleus and cytoplasm.

When an N-terminal deletion mutant lacking the PH domain and half of the EF hand domain is expressed in MDCK cells, the mutant is not localized at the plasma membrane but is found in the cytoplasm and in the nucleus, even in the absence of LMB [Okada et al., 2002]. This indicates that about 200 amino acid residues at the N-terminus of PLCδ1 are necessary for nuclear export and the rest is sufficient for nuclear import. We finally identified a sequence of residues 164–177 in PLCδ1 that functions as an NES [Yamaga et al., 1999].

There is no apparent classical NLS sequence in PLCδ1. Strategic deletion studies revealed that a basic amino acid-rich region covering the C-terminus X domain and the XY-linker is necessary for the nuclear import of PLCδ1. Two lysine residues (K432 and K434) in the region are important for nuclear import, since a deletion mutant lacking the region or a site-directed mutant of the lysine residues does not accumulate in the nucleus, even in the presence of LMB. Thus the NLS-like region was identified in PLCδ1 [Okada et al., 2002].

As shown in Figure 1, eukaryotic PLC isoforms generally posses the NLS-like region in the vicinity of both ends of the XY-linker; two clusters of basic amino acids in the C-terminus X domain and a cluster of basic residues in the C-terminus of the linker domain, although it is not as apparent as the former ones. These regions would be structurally related and form docking sites for carrier proteins of nuclear import. Some PLC isoforms have canonical leucine-rich NES sequences in their EF-hand motifs. As far as rat δ isoforms are concerned, PLCδ3 has canonical leucine-rich NES as apparent as PLCδ1, but PLCδ4 does not (Fig. 1). This could explain, at least in part, why there is a different affinity in the nuclear localization among these isoforms. Rat PLCβ1, PLCγ1, PLCε, and PLCζ1 also have leucine-rich NES-like sequence in their EF-hand motifs (not shown).

According to the 3D structure [Essen et al., 1996], the NES sequence of PLCδ1 resides in close proximity to the EF-hand/C2 domain interface, whereas the NLS-like region is located at the opposite surface to the catalytic pocket on the TIM barrel structure consisting of the X and Y domains [Yagisawa, 2003].

An Increase in Intracellular Ca^{2+} Levels Promotes the Nuclear Accumulation of PLCδ1

In addition to the LMB treatment, we were able to see the accumulation of both endogenous PLCδ1 and ectopically expressed PLCδ1 after treatment of serum-starved cells with ionomycin,

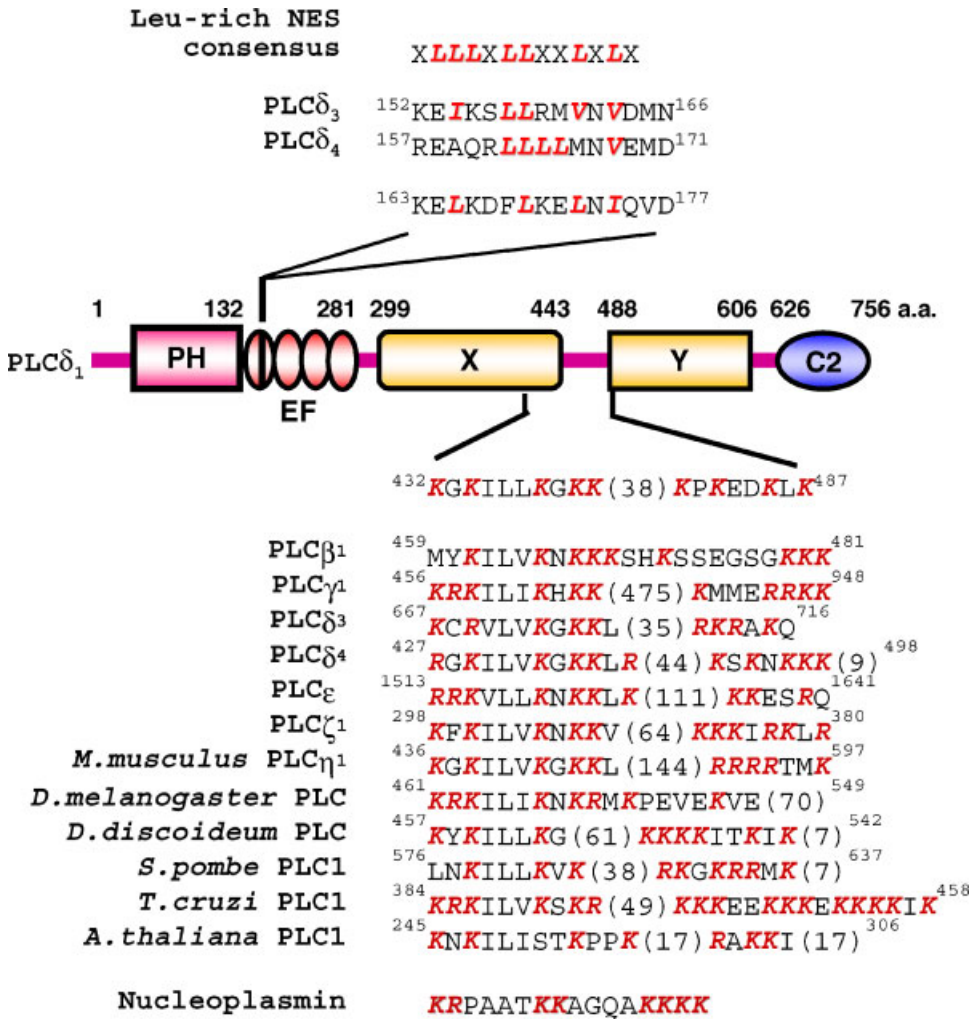

Fig. 1. Regions of PLCδ$_1$ important for the nucleocytoplasmic shuttling. Schematic representation of the rat PLCδ$_1$ structure is shown with the NES sequence of PLCδ$_1$. The corresponding regions in other δ isoforms and a consensus leucine-rich NES sequence, where ''X'' denotes any amino acid and bold letters indicate important hydrophobic residues, are also shown. PH, EF, X, Y, and C2 in the PLCδ$_1$ structure indicate the PH domain, the EF-hand domain, the catalytic X and Y domain, and the C2 domain, respectively. Polybasic regions corresponding to the C-terminal of the X domain and XY-linker region is necessary for the nuclear localization of PLCδ$_1$. Corresponding regions of various PLCs are also displayed for comparison. Sequences of PLC isoforms without names of species are those of rat. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

a Ca^{2+} ionophore [Okada et al., 2005]. Treatment of MDCK cells with thapsigargin, an inhibitor of the ER Ca^{2+}-pump and releaser of intracellular Ca^{2+}, also facilitates the nuclear import of PLCδ$_1$ [Okada, Naito, Yagisawa, unpublished]. These results suggest that stimuli that raise the intracellular Ca^{2+} concentration would facilitate the nuclear import of PLCδ$_1$. In fact, a portion (10%–20%) of serum-starved MDCK cells accumulated PLCδ$_1$ within 2 min after the treatment of cells with ATP (100 μM), suggesting that the activation of purinergic receptors causes the nuclear import of PLCδ$_1$. Similar results were obtained with bradykinin (1 μM)-treated cells [Okada, Naito, Yagisawa, unpublished].

Possible Mechanisms for the Ca^{2+}-Dependent Nuclear Accumulation of PLCδ$_1$

Given the nucleocytoplasmic shuttling of PLCδ$_1$ similar to other proteins that shuttle between the nucleus and the cytoplasm, an "in-and-out equilibrium" of the molecule via the nuclear envelope exists. A shift in the equilibrium of nucleocytoplasmic shuttling by Ca^{2+} may be caused by the following mechanisms: (1) increase in the import rate or decrease in the export rate resulting from Ca^{2+}-dependent

structural changes in the NPC (Fig. 2A), (2) inhibition of nuclear export resulting from decreased carrier–cargo complex formation in the presence of Ca^{2+} (Fig. 2B), or (3) facilitation of nuclear import resulting from increased carrier–cargo interaction in the presence of Ca^{2+} (Fig. 2C).

Although this is controversial, nearly half of the articles on nuclear Ca^{2+} measurements identify a Ca^{2+} gradient between the nucleus and cytoplasm. The Ca^{2+} gradients described are mostly transient. Ca^{2+} released from the nuclear envelope enters the nucleoplasm either directly through Ins(1,4,5)P_3 receptors or ryanodine receptors in the inner nuclear membrane or through the NPCs when released through these receptors in the outer nuclear membrane. Nuclear Ca^{2+} signaling could

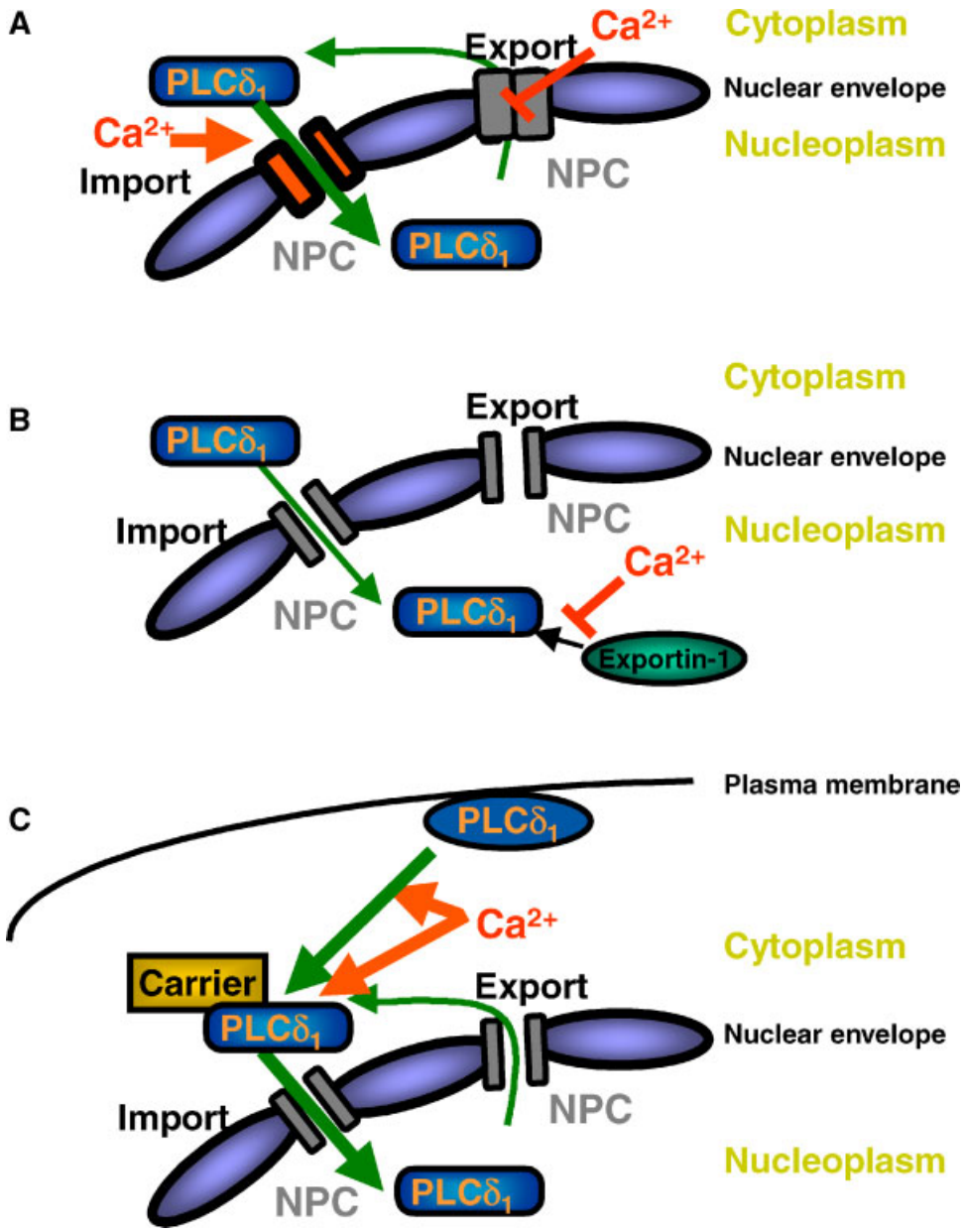

Fig. 2. Possible mechanisms for the Ca^{2+}-dependent regulation of nuclear transport of PLCδ$_1$. Ca^{2+} facilitates specific nuclear import by altering the structure of the NPC or it abrogates nuclear export by altering the structure of the NPC (**A**). Ca^{2+} down-regulates formation of the nuclear export complex (**B**). Ca^{2+} facilitates formation of the cargo-nuclear import carrier complex by: (i) changing the structure of PLCδ$_1$ to bind the carrier protein such as importins and/or (ii) increasing availability of PLCδ$_1$ in the cytoplasm by dissociation of PLCδ$_1$ from the plasma membrane after Ca^{2+}-induced PtdIns(4,5)P_2 hydrolysis by any PLC isoforms (**C**). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

therefore be spatially and temporally distinguishable from that which occurs in the cytoplasm. The NPC is a supra-macromolecular complex (about 125 MDa) consisting of 50–100 different proteins and forms a pore of approximately 120 nm in diameter on the nuclear envelope. There have been a few reports concerning changes in the superstructure of the NPC or in components of the NPC by Ca^{2+} [Moore-Nichols et al., 2002; Erickson et al., 2004]. Although there is a report indicating that nuclear transport is independent of lumenal Ca^{2+} stores [Strubing and Clapham, 1999], there is a possibility that spatially limited changes in Ca^{2+} levels regulate the NPC permeability [Perez-Terzic et al., 1997]. Thus nuclear or even cytoplasmic Ca^{2+} signaling may control the NPC structure, resulting in a shift in the steady-state equilibrium between nuclear import and export of specific cargo–carrier complexes.

Second, Ca^{2+} may downregulate the formation of the nuclear export complex, the PLCδ_1–exprotin-1 complex. Although the overall structure of PLCδ_1 does not change, the structure of the NES region in the presence of Ca^{2+}, especially the C-terminus of the NES differs from that in the absence of Ca^{2+} [Essen et al., 1996]. For example, the orientation of the side chains of Val176 and Asp177 is different. The structural alteration of NES could reduce the interaction between PLCδ_1 and exprotin-1, resulting in downregulation of the nuclear export.

Third, formation of the nuclear import complex can be upregulated by Ca^{2+}. To clarify the molecular mechanisms for the nuclear import of PLCδ_1, we examined the interaction between PLCδ_1 and importins by in vitro pull-down assay using GST-fused importins [Okada et al., 2005]. There was no apparent interaction between PLCδ_1 and importin α such as Rch1, Qip1, and NPI1 in Ca^{2+}-free buffer. Importin β_1 did not bind PLCδ_1 in the same buffer either. In the presence of sub-micromolar levels of Ca^{2+}, however, importin β_1 was found to be a direct binding partner of PLCδ_1 [Okada et al., 2005]. Therefore, it was concluded that Ca^{2+} promotes formation of the PLCδ_1–importin β_1 complex at least in vitro.

Ca^{2+}-SENSING MECHANISM

Since the importin β_1 structure is thought to be unchanged in the presence or in the absence of Ca^{2+}, it is likely that PLCδ_1 senses the levels of Ca^{2+}. We therefore sought the domain responsible for Ca^{2+} sensing in PLCδ_1. X-ray crystallographic studies so far have shown that PLCδ_1 has three Ca^{2+}-binding sites [Essen et al., 1996]; one is located in the catalytic domain and is essential for PtdIns(4,5)P_2 hydrolysis, while the other two are located in the C2 domain. To explore which Ca^{2+}-binding site is important for the interaction with importin β_1, we carried out in vitro binding assay using mutants of PLCδ_1 with diminished Ca^{2+}-binding activity [Okada et al., 2005]. In the presence of Ca^{2+}, the D653A or D706N mutant that has an amino acid substitution in the C2 domain bound to importin β_1, whereas the E341A mutant that has a substitution in the catalytic domain showed markedly reduced binding. These results suggest that Ca^{2+}-binding to the catalytic domain causes a structural change in PLCδ_1 to expose the positively charged cluster that can be recognized by importin β_1, which then carries the cargo molecule to the NPC.

Our model was further supported by pull-down assay using GST-importin β_1 or by coimmunoprecipitation assay using an anti-importin β_1 antibody, together with lysates from MDCK cells expressing GFP-PLCδ_1 [Okada et al., 2005]. Moreover, ionomycin treatment did not cause nuclear accumulation of GFP-PLCδ_1 E341A, confirming that Ca^{2+}-binding to the catalytic domain is essential for the nuclear import of PLCδ_1 [Okada et al., 2005].

STRUCTURE OF IMPORTIN β_1

Since importin β interacts with various nuclear proteins that shuttles through the NPC and is regulated by Ran, structural basis for the protein–protein interactions is important. The 3D structures of importin β_1 have been presented in several different ways: (i) a portion of isolated molecule [Lee et al., 2000], (ii) a complex with the importin β_1-binding (IBB) domain of importin α [Cingolani et al., 1999], (iii) a complex with the NLS-containing region of parathyroid hormone-related protein (PTHrP) [Cingolani et al., 2002], (iv) a complex with a FxFG repeat sequence of nucleoporin, a NPC component [Bayliss et al., 2000], (v) a complex with the GTP-bound form of Ran [Vetter et al., 1999], and (vi) a complex with

the NLS containing region of SREBP-2 [Lee et al., 2003]. Importin β_1 consists of 19 repeated helices called HEAT motifs, and the binding regions for the interacting molecules do not overlap. Moreover, apart from the SREBP-2-importin β_1, these interactions require short stretches of HEAT motifs and do not accompany drastic conformational changes (such as a bent) of the molecule. Interaction between importin β with SREBP-2, however, is somewhat different. Symmetrically located long two helices (heat7 and heat17) of importin β bind to the basic helix-loop-helix-leucine zipper (bHLH-Zip) structure of SREBP-2 (a dimer) causing a twist of the whole importin molecule [Lee et al., 2003].

It may be important to explore the structural basis of the importin β_1–PLCδ_1 interaction further, since the NLS-like regions of PLCδ_1 are shared by almost all eukaryotic PLCs (Fig. 1) and seems to be similar to the bHLH-Zip domain of SREBP-2 both in size and charge distribution. Another focus of future studies would be the Ca^{2+} effect on the structures of importins (and exportins), since little information is available on this issue as yet.

PHYSIOLOGICAL RELEVANCE

Cell Cycle

We have shown that equilibrium breakage of nuclear import and export of PLCδ_1 occurs after receptor activation by external stimuli that leads to an increase in intracellular Ca^{2+} levels. It is possible therefore that PLCδ_1 accumulates in the nucleus in certain phases of the cell cycle that accompany local charges in Ca^{2+}.

Levels of nuclear PIs decrease during the S phase of the cell cycle in HeLa cells [York and Majerus, 1994]. Sun et al. [1997] claimed that nuclear levels of DG fluctuate during the cell cycle because of the activation of PLCs in the nucleus [Sun et al., 1997]. They demonstrate that in synchronized human promyelocytic (HL60) leukemia cells there is a transient increase in nuclear DG levels to threefold higher than in G_1 phase at the G_2/M boundary and that the increase is blocked by PI-specific PLC inhibitors but not by inhibitors for phosphatidylcholine-specific PLC or phospholipase D. Thus nuclear PLC may have a role in the G_2/M transition, activating PKC (PKCβ_2) required for mitosis.

In yeasts, *PLC1*, an ortholog of PLCδ_1 and only PLC gene in the organism is essential for normal cell growth [Yoko-o et al., 1993]. $InsP_6$ generation from Ins(1,4,5)P_3, which is produced by the action of *PLC1*, is essential for mRNA export form the nucleus [York et al., 1999; Odom et al., 2000]. It has been shown that *PLC1* protein is in the nucleus and is localized to centromeric loci at the G_2/M checkpoint [DeLillo et al., 2003].

Stalling et al. [2005] has recently indicated that PLCδ_1 localizes in the nucleus during the G_1/S boundary and the G_0 phases of the cell cycle using NIH-3T3 fibroblasts. The authors demonstrated that a reduction in PtdIns(4,5)P_2 levels at the plasma membrane and an increase in its levels in the nucleus caused nuclear accumulation of PLCδ_1 during these phases. Nevertheless, the nuclear localization of a mutant that lacks the PtdIns(4,5)P_2-binding ability of the PH domain is still evident during G_1/S and in G_0, but to a lesser extent compared with that of wild-type, suggesting that other factors affect the translocation of PLCδ_1 into the nucleus as well as the PtdIns(4,5)P_2 balance. An increase in intracellular Ca^{2+} levels, therefore, constitutes a partial cue for the nuclear accumulation of PLCδ_1. The author's model explains that the cell cycle-linked elevation of PtdIns(4,5)P_2 in the nucleus sequesters PLCδ_1 in the nucleus, but how it could be achieved (by activation of nuclear lipid kinases, or, by inhibition of nuclear lipid phosphatases or PLCs) is unclear.

Compartmentalization of PIs can influence the localization and function of proteins that have specific PI-binding domains. The mammalian SWI/SNIF-like chromatin–remodeling complex, Brahma associated factors (BAF), contains actin and actin-related proteins. PtdIns(4,5)P_2 induces association of the complex with chromatin in T cells during antigen stimulation [Zhao et al., 1998] and likely has a role in pre-mRNA splicing [Osborne et al., 2001]. PtdIns(4,5)P_2 regulates the binding of Brg1, at least one component in the complex, with actin in vitro, changing the association mode of the complex [Rando et al., 2002]. Moreover, many chromatin-regulating proteins contain a plant homeodomain (PHD) finger motif that binds to phosphatidylinositol phosphates. Nuclear levels of PtdIns(5)*P* regulate ING2, one of the PHD containing protein and a regulator of histone acetylation, to activate p53 and p53-dependent apoptotic pathways [Gozani et al., 2003]. Furthermore, Tub, which binds

PtdIns(4,5)P_2 via its Tubby domain, translocates from the plasma membrane to the nucleus to exert its transcription factor function [Santagata et al., 2001].

Finally, as far as the dynamics of the nuclear Ins(1,4,5)P_3 in response to extracellular stimuli (membrane receptor agonists) is concerned, it shows synchronous fluctuation with cytoplasmic Ins(1,4,5)P_3 dynamics [Sato et al., 2005]. Whether nuclear compartmentalization of PLC during the cell cycle affects Ins(1,4,5)P_3-mediated nuclear Ca^{2+} dynamics or it simply modifies the availability of PtdIns(4,5)P_2 or other binding partners of PLC has received increasing attention.

Stress Responses

PI in the cerebral cortex decreases dramatically during cerebral ischemic damage [Sun and Hsu, 1996]. Activation of PLC is sometimes involved in continuous Ca^{2+} uptake during neuroexcitotoxicity [Bazan et al., 2002]. Taken the nuclear accumulation of PLCδ_1 by Ca^{2+}-influx together, it is possible to speculate that PLCδ_1 plays a particular role during neuronal cell death.

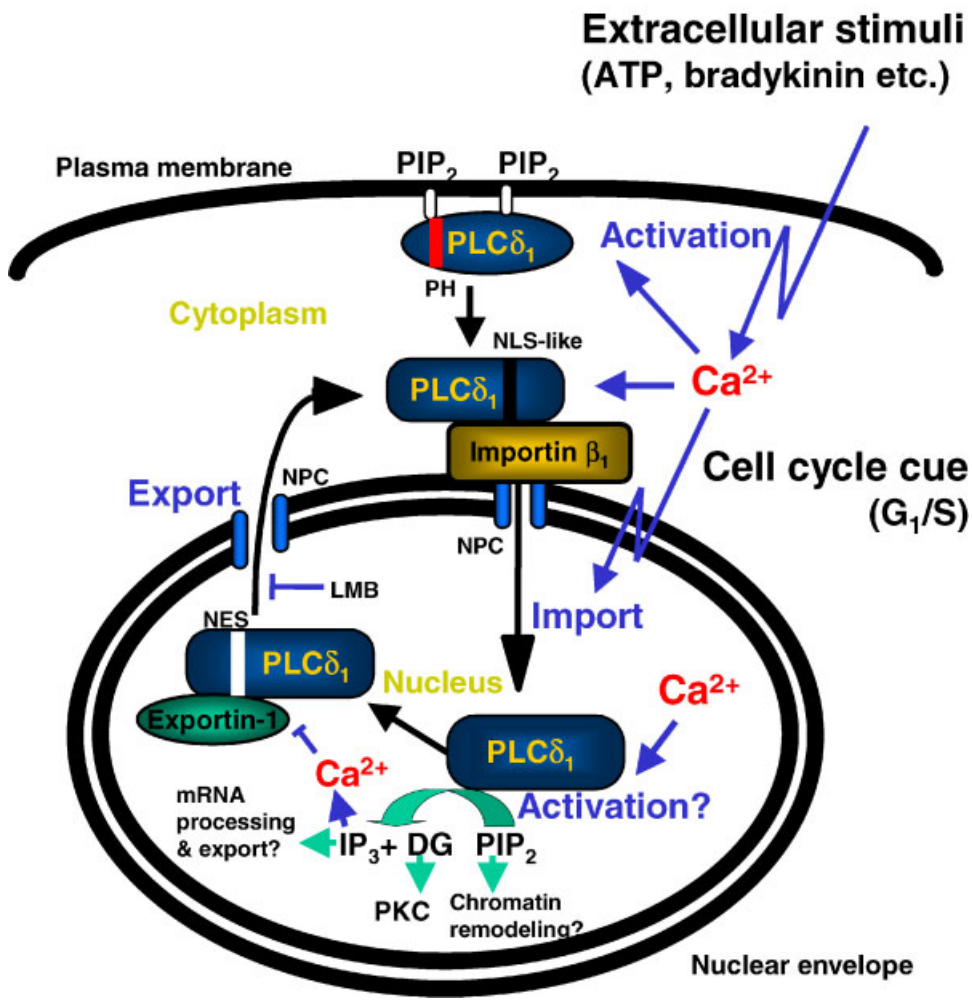

Fig. 3. A model for the intracellular translocation of PLCδ_1. PLCδ_1 undergoes nuclear cytoplasmic shuttling. Elevation in the levels of intracellular Ca^{2+} by external stimuli or by cell cycle transition reduces the plasma membrane-bound PLCδ_1 and facilitates the formation of PLCδ_1–imortin β_1 complex. The complex is imported to the nucleus through the NPC. PLCδ_1 may be activated in the nucleus, hydrolyzing nuclear PtdIns(4,5)P_2 to generate Ins(1,4,5)P_3 and DG, and therefore mobilizes Ca^{2+} from stores in the nuclear envelope and activates nuclear PKC. Ca^{2+} may also block formation of the PLCδ_1–exportin-1 complex by changing the structure of the NES sequence. Roles of PLC in the nucleus are uncertain, but could be related to nuclear functions such as chromatin remodeling, regulation of the nuclear matrix and transcription. Sequestration or loss of nuclear PtdIns(4,5)P_2 may affect chromatin remodeling complexes and the availability of inositol phosphates that regulate processing and export of mRNA. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

An aberrant accumulation of PLCδ1 with a loss of its specific activity was observed in neurofibrillary tangles (NFT), the neurites surrounding senile plaque cores and neuropil threads in the brains of patients with Alzheimer's disease [Shimohama et al., 1995b]. Furthermore, in glutamate neurotoxicity, Ca^{2+} influx-dependent formation of NO leads to similar modifications of PLCδ1 to those seen in Alzheimer's disease [Shimohama et al., 1995a]. Nuclear PLCδ1 can be activated by Ca^{2+} and produces Ins(1,4,5)P_3 to constitute a positive feedback loop for Ca^{2+} signaling locally. The nuclear localization of PLCδ1 may confer further mechanisms that facilitate cell death, or, conversely, that counteract cell damage.

Cellular stress induces the nuclear accumulation of many proteins, including importins themselves. Recently, Miyamoto et al. [2004] reported that importin α accumulates reversibly in the nucleus in response to cellular stress, including UV irradiation, oxidative stress, and heat shock [Miyamoto et al., 2004]. Although these stress conditions are not always associated directly with an increase in intracellular Ca^{2+} levels, their findings show that the classical nuclear import pathway is downregulated via the removal of importin α from the cytoplasm in response to the stresses. Since PLCδ1 utilizes the mechanisms independent of importin α, the nuclear import cannot be blocked by these stresses. It should be emphasized again, therefore, that the nuclear retention of PLCδ1 is a step necessary for progression of or protection from the stress-induced cytotoxicity.

SUMMARY AND FUTURE DIRECTIONS

PLCδ1 interacts directly with importin β1 in vitro and in vivo in the presence of Ca^{2+}. Binding of Ca^{2+} to the catalytic domain may cause structural changes to increase the interaction with importin β1. The nuclear import of PLCδ1 through the NPC is thus facilitated by the increase in intracellular Ca^{2+} levels. Both external stimuli and a certain stage of the cell cycle associated with increased intracellular Ca^{2+} cause the nuclear accumulation of PLCδ1 to exert some function. A model is summarized in Figure 3.

PLCδ1 is expressed abundantly in most tissues [Lee et al., 1999]. Importin β1 is also expressed ubiquitously, whereas importin α shows a unique expression pattern in various tissues [Kamei et al., 1999]. Importin β1, therefore, could be a general carrier for PLCδ1 in various tissues. The nuclear functions of PLCδ1 are still unclear; the enzyme could produce classical second messengers from nuclear PtdIns(4,5)P_2 or the function may be totally different from that in the cytoplasm or at the plasma membrane. They may be related to those of other nuclear PLC isoforms found in the nucleus such as PLCβ1 and PLCγ1.

Most enigmatic is the phenotype of homozygous PLCδ1 knockout mice. The knockout mice show a slight phenotypic difference from the wild-type, showing a defect in normal development of skin but overall cell division and proliferation are similar [Nakamura et al., 2003]. The results suggest functional redundancy among PLC isoforms during the development of mice. To get insights into the role of PLCδ1 in the nucleus and cell function as a whole, it would be useful to perform knockin experiments using various PLCδ1 mutants and embryonic fibroblastic (MEF) cells of the PLCδ1 null mice to see whether any alterations in proliferation or differentiation patterns can be observed.

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