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PROSPECTS

# Nuclear Ptdlns(3,4,5)$P_3$ Signaling: An Ongoing Story

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**Abstract** Phosphatidylinositol 3,4,5-*tris*phosphate (Ptdlns(3,4,5)$P_3$) is linked to a variety of cellular functions, such as growth, cell survival, and differentiation. Ptdlns(3,4,5)$P_3$ is primarily synthesized by class I phosphoinositide 3-kinases and its hydrolysis by two 3-phosphoinositide 3-phosphatases, PTEN and SHIP proteins, leads to the production of two other second messengers, Ptdlns(4,5)$P_2$ and Ptdlns(3,4)$P_2$, respectively. Evidence accumulated over the last years strongly suggest that Ptdlns(3,4,5)$P_3$ is an important component of signaling pathway operating within the nucleus. Moreover, recent advances indicated that nuclear translocation of cell surface receptors could activate nuclear phosphoinositide 3-kinase suggesting a new mode of signal transduction. The aim of this review is intended to summarize the state of our knowledge on nuclear Ptdlns(3,4,5)$P_3$ and its metabolizing enzymes, and to highlight the emerging roles for intranuclear Ptdlns(3,4,5)$P_3$. J. Cell. Biochem. 98: 469–485, 2006. 

**Key words:** nucleus; Ptdlns(3,4,5)$P_3$; phosphoinositide 3-kinase; PTEN; SHIP2

In the late 80's, the evidence appeared that phosphorylated derivatives of phosphatidylinositol (Ptdlns), collectively called PI, are present inside cell nucleus [Smith and Wells, 1983; Cocco et al., 1987]. PI represent a group of seven known stereoisomers stem from the same precursor, Ptdlns, and are among the most versatile of regulatory molecules. This versatility arises from the degree and the position of phosphorylation of inositol head group. Out of five candidate phosphorylation positions, only the hydroxyls at position D-3, D-4, and D-5 are phosphorylated separately or in all possible combinations (Fig. 1A). Ptdlns(4,5)$P_2$ is the first component identified within the nucleus together with enzymes involved in the classical PI pathway: PI 4-kinase, PI4P 5-kinase, phospholipase C (PLC) and diacylglycerol kinase (Fig. 1B) [Payrastre et al., 1992]. An important result was that nucleus contains a Ptdlns(4,5)$P_2$-based signaling system that is distinct from that in the cytoplasm [Cocco et al., 1989; Divecha et al., 1991] and that is involved in cell growth, differentiation, and neoplastic transformation. The literature regarding this nuclear PI pathway is quite extensive and recent reviews updated classical PI metabolism and its role in nuclear function [Irvine, 2002; Cocco et al., 2004; Manzoli et al., 2005; Martelli et al., 2005].

Ptdlns, Ptdlns(4)P, and Ptdlns(4,5)$P_2$ can also be phosphorylated by a family of PI 3-kinases

Abbreviations used: Ptdlns, phosphatidylinositol; Ptdlns(4,5)$P_2$, phosphatidylinositol (4,5) *bis*phosphate; Ptdlns(3,4,5)$P_3$, phosphatidylinositol (3,4,5) *tris*phosphate PI3K, phosphoinositide 3-kinase; SH2, Src homology 2; SH3, Src homology 3; PIKE, phosphoinositide 3-kinase enhancer; PH, pleckstrin homology; PLC, phospholipase C; IRS-1, insulin receptor substrate-1; NGF, nerve growth factor; EGF, epidermal growth factor; FGF, fibroblast growth factor; PTEN, phosphatase and tensin homolog deleted on chromosome ten; MAGI, membrane-associated guanylate kinase with inverted orientation; CK2, caseine kinase 2; GSK3, glycogen synthase kinase 3; SHIP-2, SH2-containning domain inositol polyphosphate 5-phosphatase-2; PKC, protein kinase C; PDK1, phosphoinositide-dependant kinase; NES, nuclear export signal; NLS, nuclear localization signal.

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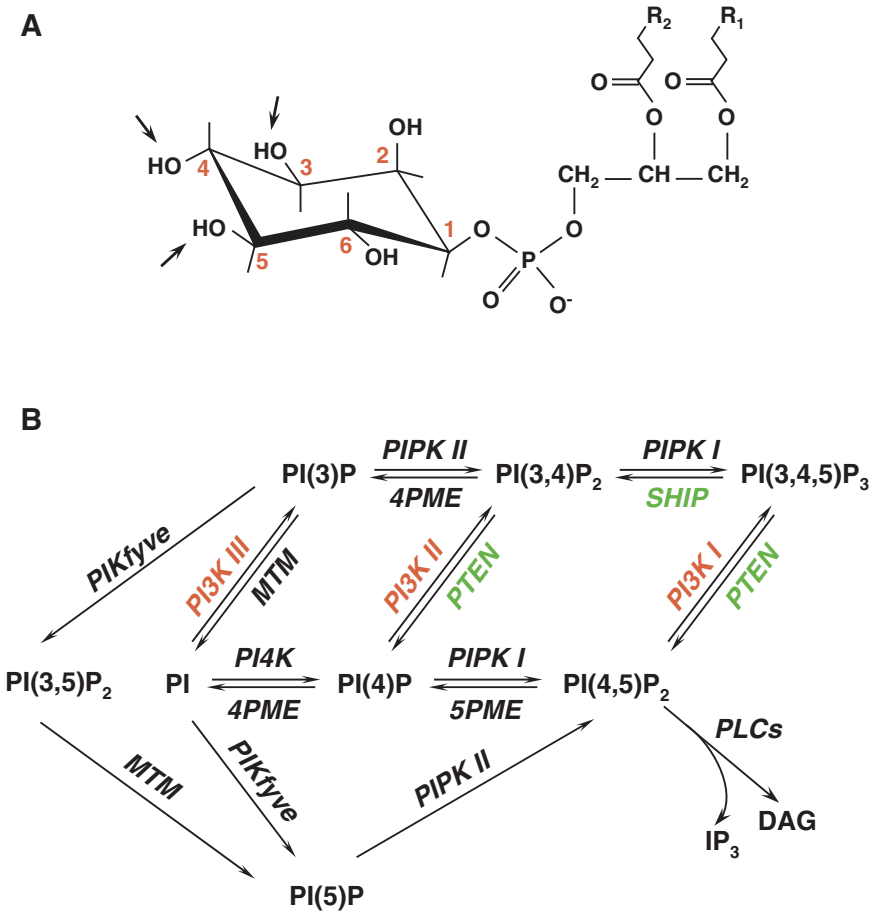


**Fig. 1.** Major pathways of phosphoinositide metabolism. **A**: Phosphatidylinositol (Ptdlns) is the basic building block of phosphorylated phosphoinositides. In mammalian cells Ptdlns have almost exclusively a 1-stearoyl (R1) and a 2-arachidonyl (R2) fatty acid composition. The myoinositol head group can be phosphorylated in the 3-, 4- and 5-positions to generate different phosphoinositides. **B**: The figure shows the main metabolic pathways involved in Ptdlns metabolism. The various kinases and phosphatases involved in the classical PI pathways are not depicted here. Phosphoinositide 3-kinases (gray) responsible for the synthesis of PI(3)P, PI(4,5)$P_2$, and PI(3,4,5)$P_3$, in particular PI3K I, and phosphatases responsible for PI(3,4,5)$P_3$ degradation are the focus of this review. Abbreviations used: PI3K I, II, or III, Class I, II, or III phosphoinositide 3-kinase; SHIP, Src homology 2-containing inositol-5-phosphatase; PTEN, phosphatase and tensin homolog deleted on chromosome ten; MTM, myotubularin; PME, phosphomonoesterase; PIK, phosphoinositide kinase; PI(3)P, Ptdlns(3)P; PI(4)P, Ptdlns(4)P; PI(3,4)$P_2$, Ptdlns(3,4)$P_2$; PI(3,5)$P_2$, Ptdlns(3,5)$P_2$; PI(4,5)$P_2$, Ptdlns(4,5)$P_2$; PI(3,4,5)$P_3$, Ptdlns(3,4,5)$P_3$; IP3, inositol 1,4,5 triphosphate; DAG, diacylglycerol. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

(PI3Ks) at the D-3 position of the inositol ring, thus generating 3-PI: Ptdlns(3)P, Ptdlns(3,4)$P_2$, and Ptdlns(3,4,5)$P_3$ [Traynor-Kaplan et al., 1988; Whitman et al., 1988]. Ptdlns(3)P is constitutively present in eukaryotic cells and its level is largely unaltered upon cellular activation. By contrast, Ptdlns(3,4)$P_2$ and Ptdlns(3,4,5)$P_3$ are rapidly and transiently produced in response to various agonist-mediated cell stimulation and act as second messengers. In the past two decades it has been established that Ptdlns(3,4,5)$P_3$ is linked to an incredibly diverse set of key cellular functions including carbohydrate metabolism, vesicular trafficking, cytoskeleton remodeling, and also migration, proliferation, differentiation, and survival. Thus, the emerging link between Ptdlns(3,4,5)$P_3$ and many diseases: inflammation, allergy, diabetes, cancer, and cardiovascular diseases has made it the focus of intense study. However, it is only in the past few years that evidence for the presence of Ptdlns(3,4,5)$P_3$ in the nucleus has become convincing. In this review, we will therefore focus on both PI3Ks and 3-PI phosphatases that regulate the level of nuclear Ptdlns(3,4,5)$P_3$. We will also discuss on its potential nuclear targets and its impact on cell biology.

## NUCLEAR Ptdlns(3,4,5)$P_3$ SYNTHESIS

Many isoforms of PI3K have been cloned in mammalian cells and three main classes (referred to as I, II, and III) have now been identified on the basis of their structural features, in vitro substrate specificity and mechanism of regulation [Fruman et al., 1998; Wymann and Pirola, 1998; Vanhaesebroeck et al., 2001]. The class I enzymes phosphorylate in vitro Ptdlns, Ptdlns(4)P, and Ptdlns(4,5)$P_2$, but display a strong preference for Ptdlns(4,5)$P_2$ in vivo, and is responsible of cellular Ptdlns(3,4,5)$P_3$ synthesis.

Class I PI3Ks form a heterodimeric complex and are subdivided according to the adaptor protein associated with the catalytic subunit. Class IA PI3Ks consist of a 110 kDa catalytic subunit (p110 α, β, δ) [Hiles et al., 1992] and a 85-, or 55-kDa adaptor subunit (p85α, β and p55γ). All these adaptors contain two tandem Src homology 2 (SH2) domains that link class IA PI3Ks to tyrosine kinase signaling. In contrast, class IB PI3K or PI3Kγ defines a G protein-coupled receptor-regulated PI3K. It is made of a p110γ catalytic subunit and a p101 regulatory subunit, unrelated to p85. Activation of PI3K γ seems to predominantly involve interactions with βγ subunits of heterotrimeric G proteins and also possibly Gα subunits. Its activation is considerably enhanced by the p101 adaptor [Stephens et al., 1997].

PI3Ks also possess a serine protein kinase activity [Fruman et al., 1998; Wymann and Pirola, 1998; Vanhaesebroeck et al., 2001]. In vitro and in vivo studies showed that class I PI3Ks down-regulate their lipase kinase activity by phosphorylation of their catalytic and/or regulatory subunits. Thus, the $Ser^{608}$ phosphorylation of the p85α regulatory subunit by p110α causes an 80% decrease in PI3K activity [Dhand et al., 1994]. This regulation occurs in vivo in response to stimulation of cell lines and animal tissue with insulin and platelet-derived growth factor [Foukas et al., 2004]. In vitro activity of other class IA PI3K isoforms, p110β and p110δ, is regulated by catalytic subunit autophosphorylation on $Ser^{1070}$ [Czupalla et al., 2003] and $Ser^{1039}$ [Vanhaesebroeck et al., 1999], respectively. Moreover, it was demonstrated that the serine kinase activity of PI3K could be involved in various signaling pathway. Class IA PI3K phosphorylates IRS-1 (insulin receptor substrate-1) in insulin-stimulated rat adipocytes [Lam et al., 1994] and in interferon-treated myeloid cells [Uddin et al., 1997]. In addition, protein kinase activity of the class IB PI3Kγ may be involved in the activation of serine/threonine kinase MAPK [Bondeva et al., 1998].

### Nuclear Class IA PI3K

A number of reports have highlighted the presence of active class IA PI3K in the nucleus of different cell types. Thus, class IA PI3K has been reported to be active in the nucleus of rat liver [Lu et al., 1998b], in human hepatocarcinoma Hep-G2 cells [Kim, 1998] and osteoblast-like MC3T3-E1 cells [Martelli et al., 2000]. However, it is of note that most of these studies focused on the p85α/p110 heterodimer. Also, we cannot exclude the presence of other class IA PI3K isoforms in the nuclear compartment.

The regulation of nuclear class IA PI3K is not well-understood, but very interesting data clarified its activation in NGF-treated PC12 cells [Ye et al., 2000, 2002]. Ye et al. [2000] identified a brain specific GTPases, PIKE-S (phosphoinositide 3-kinase enhancer), as a novel physiological regulator of nuclear PI3K. PIKE-S localizes exclusively to the nucleus and is characterized by three proline-rich domains in the N-terminus, followed by a GTPase domain and a partial pleckstrin homology (PH) domain in C-terminus. Retroviral infection of PC12 cells showed that NGF-induced PI3K activity was blocked by a dominant-negative form of PIKE, and that PI3K activation by PIKE was GTP-dependent and required the presence of both p85 and p110 subunits [Ye et al., 2000]. Recently, PLC-γ1 was identified as a physiological guanine nucleotide exchange factor for PIKE-S [Ye et al., 2002]. Indeed, the Src-homology 3 (SH3) domain of PLC-γ1 directly bind the third proline-rich domain (353–362 residues) of PIKE-S. This interaction stimulates GDP dissociation and markedly enhances GTP binding to PIKE-S, independently of its lipase catalytic activity. Moreover, the down-regulation of nuclear PI3K activity seems involve the interaction between PIKE-S and the protein 4.1N, a neuronal-specific isoform of erythrocyte membrane cytoskeleton protein 4.1R [Ye et al., 2000]. In NGF-treated PC12 cells, protein 4.1N translocates to the nucleus over a period of hours, lagging behind PI3K nuclear translocation and PIKE activation that occurs within 30 min of stimulation.

Moreover, overexpression of protein 4.1N abolishes activation of PI3K by PIKE-S [Ye et al., 2000]. In addition to PI3K and PLC-γ1, PIKE-S also associates with protein 4.1N. The interaction between PIKE-S and protein 4.1N is dependent on the N-terminal 23 residues, to which the regulatory p85 subunit also binds. Thus, in PC12 cells, NGF triggers nuclear translocation of PLC-γ1 and its binding to PIKE-S. PLC-γ1 acts as a guanine nucleotide exchange factor for PIKE-S through its SH3 domain and the active GTP-bound PIKE-S subsequently provokes nuclear PI3K activation. Then protein 4.1N translocates to the nucleus and the binding of protein 4.1N to PIKE-S prevents its interaction with p85 and may down-regulate PI3K activity. However, it remains to determine whether this regulatory loop of nuclear PI3K activity is relevant in other systems. Protein 4.1N is constitutively located within the nucleus in many cell types [Correas, 1991; De Career et al., 1995] but PIKE-S is brain specific. To date, two other forms of PIKE, PIKE-L, and PIKE-A have been identified [Rong et al., 2003; Ahn et al., 2004a]. However, PIKE-L, a longer isoform, occurs in both the cytoplasm and the nucleus, but like PIKE-S seems brain specific, while PIKE-A distributes in various tissues but lacks N-terminal domain which binds PI3K. However, Klein et al. [2004] identified a 98 kDa protein recognized by anti-brain PIKE antibody suggesting that some isoforms of PIKE could be present in other cell nuclei.

Several years ago, it was reported in HepG2 cells that insulin-induced nuclear translocation of the p85/p110 PI3K was mediated by association between PI3K and insulin receptor substrate-1 (IRS-1) [Kim, 1998]. Recently, Boylan and Gruppuso [2002] demonstrated that IRS-1 and p85 were constitutively present in rat liver nuclei and further observed that both IRS-1 and p85 were more expressed in adult rat nuclei (24% and 15% of total IRS-1 and p85, respectively) compared to fetal liver (about 3% of total IRS-1 or p85). Moreover, insulin administration in adult rat liver was unable to stimulate nuclear translocation of IRS-1 and p85, but induced tyrosine phosphorylation of nuclear IRS-1, which was correlated both to an increase in the amount of p85 associated with IRS-1 and PI3K activity [Boylan and Gruppuso, 2002]. As previously described for cytoplasmic PI3Ks, the phosphorylated tyrosine residues of nuclear IRS-1 may thus recognize the SH2 domain of p85 and activate nuclear class IA PI3K. These observations could be very important since a wide range of phosphotyrosine proteins like EGF (epidermial growth factor) receptor translocate from cell surface to nuclear compartment [Wells and Marti, 2002] (see Section Nuclear Translocation of Transmembrane Receptors and Nuclear Pi3k Activity).

Furthermore, a unique actin-regulating protein, the nuclear CapG, might also participate in nuclear PI3K regulation in view of its unique ability to bind Ptdlns(4,5)$P_2$ in a $Ca^{2+}$-dependent manner [Lu et al., 1998b]. In contrast to cytoplasm, CapG was highly phosphorylated in the nucleus [Onoda and Yin, 1993] and this phosphorylation increased its affinity for Ptdlns(4,5)$P_2$. Therefore, the phosphorylated CapG might inhibit nuclear PI3K through Ptdlns(4,5)$P_2$ sequestration in a similar fashion to gelsolin-mediated PLC-γ1 inhibition [Banno et al., 1992].

### Nuclear Class IB PI3K

In addition to class IA PI3Ks, the nucleus also contains class IB PI3Ks. Interestingly, Metjian et al. [1999] performed immunolocalization of tagged-p110γ catalytic subunit in HepG2 cells. They found that p110γ was present in cytoplasm under resting conditions, but translocated to the cell nucleus after serum stimulation. This effect was pertussis-toxin sensitive and was mimicked by overexpression of Gβγ heterodimers, implying that the nucleocytoplasmic transport of p110γ was regulated by the release of Gβγ heterodimers downstream a $G_1/G_0$ protein-coupled receptor. The p101 regulatory subunit seems also to regulate the intranuclear location of p110γ since a p110γ variant which was deleted of the first 82 amino-terminal residues completely failed to associate with p101 and was constitutively localized in the nucleus. Therefore, p101 subunit could maintain p110γ in the cytoplasm of Hep-G2 cells [Metjian et al., 1999]. More recently, we identified an endogenous G protein-regulated PI3K activity in membrane-free nuclei isolated from pig aorta smooth muscle cells, a relevant model in vascular proliferative disorders [Bacqueville et al., 2001]. This enzyme was immunologically related to p110γ and was able to produce Ptdlns(3,4,5)$P_3$ from intranuclear Ptdlns(4,5)$P_2$. Furthermore, the nuclear class IB PI3K activity was sensitive to pertussis toxin and immunoblot

experiments showed the presence of $G_0$ proteins inside nuclei, indicating that nuclear heterotrimeric $G_1/G_0$ protein are likely involved in its activation. Unfortunately, all available antibodies were ineffective to study subnuclear localization of PI3K$\gamma$ [Metjian et al., 1999; Bacqueville et al., 2001]. In contrast to Metjian et al., we observed a constitutive expression of class IB PI3K in smooth muscle cell nuclei but serum did not change the amount of nuclear p110$\gamma$, suggesting the existence of cell specific mechanisms to regulate nuclear class IB PI3K. In this respect, these observations may account for the great diversity of G protein-coupled receptors to transmit signals to the nucleus.

## NUCLEAR Ptdlns(3,4,5)$P_3$ PHOSPHATASES

Although much attention focused on nuclear Ptdlns(3,4,5)$P_3$ synthesis, their catabolism remains obscure. Ptdlns(3,4,5)$P_3$, as other 3-Pls, is not substrate for PLC, so a counter-regulation by specific phosphatases has emerged as a crucial process to control Ptdlns(3,4,5)$P_3$-dependent signaling. The only way known to degrade Ptdlns(3,4,5)$P_3$ is the dephosphorylation of its inositol head by specific phosphatases. Indeed, the PTEN (phosphatase and tensin homolog deleted on chromosome 10) tumor suppressor dephosphorylates specifically the D-3 position of Ptdlns(3,4,5)$P_3$ thus antagonizing PI3K signaling pathways [Downes et al., 2001; Vanhaesebroeck et al., 2001], whereas SHIP (Src homology-2 (SH2) domain-containing inositol phosphatase) proteins are phosphoinositide 5-phosphatases that dephosphorylate Ptdlns(3,4,5)$P_3$ into Ptdlns(3,4)$P_2$. Thus, Ptdlns (3,4,5)$P_3$ phosphatases may modulate downstream targets of 3-PI by either increasing Ptdlns(3,4)$P_2$ production or inactivating Ptdlns (3,4,5)$P_3$ [Rohrschneider et al., 2000].

### Nuclear PTEN

PTEN is the only 3-PI phosphatase known expressed in the nucleus. Indeed, nuclear PTEN was identified in neurons [Lachyankar et al., 2000], thyroid tissue [Gimm et al., 2000], endocrine pancreatic cells [Perren et al., 2000], skin [Whiteman et al., 2002], esophageal squamous cell carcinoma [Tachibana et al., 2002], and vascular smooth muscle cells [Deleris et al., 2003].

**PTEN protein.** PTEN is a dual-specificity protein phosphatase and a lipid phosphatase. Although PTEN is able to remove in vitro all phosphorylated amino acids (serine, threonine, and tyrosine), its preferential substrates seem to be Ptdlns(3,4,5)$P_3$ and Ptdlns(3,4)$P_2$ in vivo [Maehama and Dixon, 1998; Myers et al., 1998; Stambolic et al., 1998]. The activity of PTEN toward Ptdlns(3,4,5)$P_3$ is 5-fold higher than for Ptdlns(3,4)$P_2$ and 1,000-fold higher than for inositol 1,3,4,5 *tetraki*sphosphate [Maehama and Dixon, 1998]. PTEN consists of an N-terminal phosphatase domain which includes the classical motif of the dual specificity protein phosphatase (HCXXGXXRS/T), a lipid-binding C2 domain and a C-terminal tail that contains two putative PEST sequences (350–375 and 379–386) characterized by multiple consensus phosphorylation sites and a PDZ (*P*SD-95, *o*isc-large, Zonula Occludens-1) domain-binding sequence [Maehama et al., 2001; Waite and Eng, 2002]. The phosphatase domain and the C2 domain form together a minimal functional catalytic unit, in which the C2 domain may play a dual role of promoting membrane recruitment and assisting a productive orientation of the catalytic site [Georgescu et al., 2000]. In contrast, the C-terminal tail is not required for the phosphatase activity, but is critical for controlling membrane binding and activation of PTEN. Several groups have reported that C-terminal domain could be involved in membrane localization of PTEN through the interaction of its PDZ-binding sequence with PDZ domain-containing protein such as MAGI (membrane-associated guanylate kinase with inverted orientation) [Wu et al., 2000a,b]. Moreover, the phosphorylation/dephosphorylation of clustered serine/threonine within the C-terminal tail regulates the PTEN activity by directly modulating both its membrane targeting and its stability [Vazquez et al., 2000, 2001; Das et al., 2003]. It was observed that the mutation of second cluster, $Ser^{380}$, $Thr^{382}$, or $Thr^{383}$, reduces the half-time and increases the catalytic activity of PTEN [Vazquez et al., 2000]. The phosphorylated form of the C-terminal tail is thought to wrap unto the C2 and catalytic domains of PTEN, probably by interacting directly with the cationic residues in the phosphatase and/or C2 domains. This conformation change appears to mask PDZ-binding site and thereby prevents PTEN interaction with PDZ domain-containing proteins and the membrane-targeting of PTEN [Vazquez et al., 2001; Das et al., 2003]. In contrast, the

dephosphorylation of the C-terminal tail could trigger PTEN targeting to the plasma membrane via electrostatic interactions between the polybasic N-terminal domain and high anionic phospholipids of the membrane and protein–protein interactions through its PDZ-binding site [Vazquez et al., 2001; Das et al., 2003]. Thus, PTEN activity could be regulated by balance between kinase(s) and phosphatase(s). Several groups have proposed that CK2 (caseine kinase 2) is the kinase involved in PTEN phosphorylation [Torres and Pulido, 2001; Miller et al., 2002]. Nevertheless, very recently, Al-Khouri et al. [2005] showed that multiple kinases, including CK2 and GSK3$\gamma$ (glycogen synthase kinase 3 $\gamma$) participate in PTEN phosphorylation in human embryonic kidney 293T-cells. CK2 and GSK3$\gamma$ phosphorylate non-overlapping sites and seem to synergize rapid changes in the phosphorylation state of PTEN. However, both kinases seem unable to phosphorylate $Ser^{380}$, which is one of the crucial phosphorylation sites necessary for stabilizing PTEN, suggesting that a third kinase is responsible for PTEN phosphorylation at $Ser^{380}$ in these cells [Al-Khouri et al., 2005]. Okahara et al. [2004] have recently identified a protein PICT-1 (protein interacting with carboxyl terminus-1) that bind to the C-terminus of PTEN. Using an RNA interference method, they demonstrated that down-regulation of PICT-1 enhances the degradation of PTEN with a concomitant decrease of phosphorylation of $Ser^{380}$ in MCF-7 cells. Thus, it is possible that PICT-1 may regulate PTEN stability by activating a kinase responsible for $Ser^{380}$ phosphorylation. These results indicate a complex regulation of PTEN by several kinases, which act in concert or in response to different conditions, or in different cell type.

In cell, PTEN exists in a predominantly phosphorylated, inactive state. Thus, the identification of phosphatase(s) and/or pathways responsible for dephosphorylating C-terminal tail is crucial. However, treatments of Jurkat T-cells by PI3K inhibitors result in reduced phosphorylation and accelerated degradation of PTEN [Birle et al., 2002] indicating that the substrates of PTEN could regulate its activity in a negative feedback mechanism through the activation of a PI3K-dependent kinase or the inhibition of a PI3K dependent phosphatase. A recent study showed that protein but not lipid phosphatase activity of PTEN is required to inhibit the migration of U373 glioma cells [Raftopoulou et al., 2004]. This inhibition depends on the C2 domain of PTEN and is controlled by the dephosphorylation of a single residue of C-terminal tail, $Thr^{383}$. These results suggested that $Thr^{383}$ dephosphorylation could be mediated by PTEN itself through autodephosphorylation or that the protein phosphatase activity of PTEN could activate another protein phosphatase or inactivate a kinase, leading to dephosphorylation of $Thr^{383}$.

**Nuclear import of PTEN.** A burning question is to know how PTEN shuttle to the nucleus since PTEN lacks a traditional nuclear localization sequence (NLS). Chung et al. [2005] identified two bipartite NLS sequences for nuclear import of PTEN and showed that NLS, amino acids 265–268 KKDK, together with NLS, amino acids 159–164 RTRDKK, or NLS, amino acids 232–236 RREDK, is required for nuclear localization of PTEN. The authors also indicated that importin proteins, which are classical proteins involved in nuclear import, are not implicated in the nuclear entry of PTEN, and proposed that PTEN transport to the nucleus is driven by the major vault protein (MVP). In fact, MVP is the major structural component of vault particle, the largest ribonucleoprotein particle, and is localized to the nuclear membrane and/or the nuclear pore complex [Chugani et al., 1993]. Moreover, MVP binds to the C2 domain of PTEN in HeLa cells [Yu et al., 2002] and vault particle has been proposed as carrier molecule for nuclear-cytoplasmic transport [Mossink et al., 2003]. Chung et al. [2005] also indicated that MCF-7 cells transfected with phosphatase dead mutants (PTEN C124S and G129E), or transfected with dephosphorylation-mimicking mutants of the C-terminal tail, display no nuclear localization defect, suggesting that PTEN import into the nucleus is independent of the protein and lipid phosphatase activities of PTEN and of the phosphorylation of clustered serine/threonine within the C-terminal tail.

At the same time Liu et al. [2005] proposed that PTEN enter the nucleus by passive diffusion rather than active transport. Using tsBN2 cells, a temperature-sensitive RCC1 mutant cell line in which the nuclear import of proteins containing SV40 T antigen NLS is suppressed at non-permissive temperatures, they observed that GFP–PTEN was present in both the nucleus and cytoplasm, whereas protein in

fusion with large tags (GFP–GFP–PTEN or PK (pyruvate kinase)–PTEN) did not enter the nucleus.

**Function of nuclear PTEN.** The function of nuclear PTEN is still being elucidated. Various somatic mutations and deletions of PTEN were found in cancer [Simpson and Parsons, 2001; Waite and Eng, 2002]. An interesting observation is that PTEN subcellular partitioning might play a significant role in the tumoral progression. Indeed, PTEN was mainly localized in the nucleus of normal cells whereas its level expression decreased in neoplastic cell nuclei and became predominantly cytoplasmic [Gimm et al., 2000; Perren et al., 2000; Tachibana et al., 2002; Whiteman et al., 2002]. Gimm et al. [2000] analyzed 139 thyroid tumors and revealed that PTEN expression in the nucleus is closely related to the degree of malignancy. Although the global expression of PTEN was reduced in advanced and aggressive malignant tumors, this effect was more pronounced in the nucleus compared to the cytoplasm. PTEN was also undetectable in both nucleus and cytoplasm in 20% of undifferentiated thyroid carcinomas [Gimm et al., 2000]. Perren et al. [2000] obtained same results in endocrine pancreatic tumors. They showed that an altered subcellular localization of PTEN is frequent in malignant cells (cytoplasmic vs. nuclear in 83% tumors) while PTEN mutations are rare events (only 3%). Finally, similar observations were recently reported in primary cutaneous melanoma where only one-third of 92 individuals were found to have PTEN silencing [Whiteman et al., 2002]. Altogether, these studies strongly suggest that dysregulated nucleocytoplasmic distribution of PTEN could be an early event toward neoplasia. Furthermore, multivariant analysis of prognostic factors for survival in patients with esophageal squamous cell carcinoma correlated nuclear PTEN expression with clinicopathologic parameters, and revealed that the absence of PTEN in the nucleus is a significant indicator of poor survival [Tachibana et al., 2002]. Thus, nuclear PTEN expression could be a useful predictor of prognosis in some cancers. Moreover, we have recently demonstrated that nuclear endogenous PTEN, immunopurified from membrane depleted vascular smooth muscle cell nuclei, dephosphorylates [$^{32}$P]-Ptdlns(3,4,5)$P_3$ specifically labeled on the D-3 position of the inositol ring to produce $^{32}$P [Deleris et al., 2003]. This result indicates that PTEN is active in the nuclear compartment and could regulate intranuclear Ptdlns(3,4,5)$P_3$ level and play a role in transducing antiproliferative signals within the nucleus.

An exciting study reported very recently that the C-terminal tail of PTEN can regulate cellular transformation though protein–protein interaction and that PTEN could play a nuclear role as a docking protein [Okumura et al., 2005]. Using yeast two-hybrid system, the authors showed a physical interaction between the PTEN C-terminal tail and the nucleolar protein MSP58 (58-kDa microspherule protein). MSP58 contains a forkhead-associated (FHA) domain known to be a modular phosphopeptide recognition domain with a striking specificity for phosphothreonine containing epitope [Durocher and Jackson, 2002]. In fact, Thr$^{366}$ of PTEN C-terminal tail is critical for the interaction with the MSP58 FHA domain. In eukaryotes, FHA domain is found almost exclusively in nuclear proteins linked to the control of transcription, DNA repair and cell-cycle progression [Durocher and Jackson, 2002]. MSP58 is known to directly interact with p120, a proliferation-related nucleolar protein expressed at high level [Ren et al., 1998] and Daxx, a transcriptional repressor and signal transducer for Fas [Lin and Shih, 2002], and has been implicated in cell transformation [Bader et al., 2001]. Moreover, Okumura et al. [2005] showed that an inactive mutant of PTEN (PTEN G129R) is as efficient as wt-PTEN in suppressing MSP58-mediated cell transformation, whereas PTEN T366A mutant, which does not interact with MSP58, is unable to effect this suppression.

Thus, PTEN could interfere with processes of tumorigenesis not only through its catalytic activity, but also through its interaction with nuclear protein involved in cellular transformation such as MSP58.

## Nuclear SHIP2

SHIP-2 (SH2-containing domain inositol polyphosphate 5-phosphatase-2) is a member of the type II inositol polyphosphate 5-phosphatase family that includes INPP5P (inositol polyphosphate 5-phosphatase), OCRL (oculo cerebro-renal Lowe syndrome), synaptojanine and the SHIP1/2 proteins. These proteins are sharing a conserved 300 amino acid phosphatase domain, and dephosphorylate both inositol polyphosphates and PI at the 5′ position of the

inositol ring. However, the 5′-phosphatase activity of SHIP proteins is specific for substrates, which are also phosphorylated at the 3′ position. Moreover, in vitro studies showed that SHIP1 dephosphorylates both lns(1,3,4,5)$P_4$ and Ptdlns(3,4,5)$P_3$ [Lioubin et al., 1996; Giuriato et al., 1997] whereas SHIP2 utilizes Ptdlns (3,4,5)$P_3$ substrate only [Wsniewski et al., 1999]. Compared to SHIP1, that is restricted to hematopoietic cells, SHIP2 is expressed ubiquitously [Pesesse et al., 1997].

By their ability to dephosphorylate Ptdlns (3,4,5)$P_3$, the SHIP proteins were thought to down-regulate PI3K signaling. To date, only SHIP2 has been reported to be expressed and active in the nucleus of vascular smooth muscle cells [Deleris et al., 2003]. Although SHIP2 and PTEN are present in nuclei of vascular smooth muscle cells, immunocytochemistry experiments showed a different subnuclear localization of both 3-PI phosphatases: SHIP-2, but not PTEN, concentrates in nuclear speckles. Moreover, SHIP-2 might directly interact with speckles since the phosphatase reorganized identically with speckles when transcriptional activity was inhibited [Deleris et al., 2003]. These interchromatin granule clusters contain factors involved in the transcription and processing of pre-mRNA including RNA polymerase II and splicing factors. Moreover, some elements of the PI cycle, such as Ptdlns(4,5)$P_2$ and Ptdlns(4)P 5-kinases (lα and IIα), and class II PI3K C2α, are associated with nuclear speckles [Spector, 1993; Didichenko and Thelen, 2001; Osborne et al., 2001]. These data are very intriguing because class II PI3Ks phosphorylate Ptdlns and Ptdlns(4)P but are unable to phosphorylate Ptdlns(4,5)$P_2$ while active SHIP2 dephosphorylates Ptdlns(3,4,5)$P_3$. However, class I PI3Ks which display a preference in vivo for Ptdlns(4,5)$P_2$ were present in the nucleus (see Section Nuclear Ptdlns(3,4,5)$P_3$ Synthesis), and might transiently localize to speckles and produce Ptdlns(3,4,5)$P_3$. The remarkable subnuclear localization of SHIP2 suggests that 3-PI could be critical regulators of transcription. This hypothesis is supported by the observations that Ptdlns(3,4,5)$P_3$ and Ptdlns(4,5)$P_2$ can reverse the inhibition of transcription caused by histone H1 [Walker et al., 2001].

Actually, the mechanism of SHIP2 transport into the nuclear compartment and its targeting to the speckles are unknown. SHIP2 does not possess a classical NLS but contains several identifiable motifs important for protein–protein interactions. In its N-terminal part, SHIP2 contains an SH2 domain interacting with numerous intracellular proteins via phosphotyrosine containing motifs. The C-terminal domain comprises a NPXY motif which, when phosphorylated, may interact with PTB (phosphotyrosine binding domain) containing proteins, and a proline-rich region binding to SH3 containing proteins [Erneux et al., 1998]. These domains could allow the interaction of SHIP2 with a carrier protein for its nucleo-cytoplasmic transport. Another hypothesis would be that SHIP2 contains and bipartite NLS, that remains to be identified, for its nuclear targeting, as described for PTEN.

## NUCLEAR TRANSLOCATION OF TRANSMEMBRANE RECEPTORS AND NUCLEAR PI3K ACTIVITY

Evidence is emerging that demonstrates that nuclear translocation of cell surface receptors could be involved in nuclear PI3K-dependent signaling. Studies have localized tyrosine kinase receptors including epidermal growth factor (EGF) [Carpentier et al., 1986], fibroblast growth factor (FGF) [Maher, 1996], insulin [Vigneri et al., 1978], nerve growth factor (NGF) [Yankner and Shooter, 1979] receptors, and G protein-coupled receptors including angiotensin type 1 ($AT_1$) [Lu et al., 1998a], endothelin [Boivin et al., 2003], lysophosphatidic acid type 1 ($LPA_1$) [Gobeil et al., 2003], metabotropic glutamate mGlu5 [O'Malley et al., 2003], platelet-activating factor (PAF) [Marrache et al., 2002], prostaglandin $E_2$ [Bhattacharya et al., 1998, 1999] receptors to the membrane nucleus and/or inside the nucleus. However, the lack of a defined mechanism for nuclear import of full-length transmenbrane receptors has raised some concerns about the relevance of nuclear receptors. Putative NLS sequences have been reported for several receptors such as $AT_1$ [Lu et al., 1998a] and $LPA_1$ [Moughal et al., 2004] receptors but most transmembrane receptors lack typical NLS. However, a recent report established that the nuclear import of FGF receptor-1, which does not contain an NLS, occurs via an importin-β-dependent mechanism [Reilly and Maher, 2001]. Thus, NLS-bearing receptors may be directed to the nucleus through the importin/RanGTP mechanism in association with

another NLS-containing protein(s) that could be their ligand.

Recent evidence has only just begun to shed light on the functional role of these nuclear receptors. The agonist-induced translocation of cell surface receptor seems to mediate transcriptional signals of immediate early response genes and nuclear calcium transients. Using dot hybridization of RNA, Bhattacharya et al. [1998] revealed that exposure of nuclei isolated from Swiss 3T3 cells to $PGE_2$ EP1 receptor agonist (17-phenyltrinor $PGE_2$) increased transcription of *c-fos* to a greater extent than that observed after stimulation of whole cells and that this effect was augmented in cells overexpressing EP1 and was abolished by the EP1 antagonist AH6809. In the other hand, Swiss 3T3 cells expressing an NLS-tagged FGF receptor 1, constitutively localized to the nucleus, showed elevated expression of *c-jun* compared with cells transfected with the wild type receptor 1 while the basal level of *c-fos* and *c-myc* were unaffected [Reilly and Maher, 2001]. Direct nuclear stimulation of $PGE_2$ $EP_3$ receptors [Bhattacharya et al., 1999; Gobeil et al., 2002], PAF receptor [Marrache et al., 2002], or $LPA_1$ receptor [Gobeil et al., 2003] with selective agonists, can also induce the expression of proinflammatory genes iNOS (inductible nitric-oxide synthase) [Bhattacharya et al., 1999; Marrache et al., 2002; Gobeil et al., 2003], eNOS (endothelial nitric-oxide synthase) [Gobeil et al., 2002], and COX-2 (cyclooxygenase-2) [Marrache et al., 2002; Gobeil et al., 2003] in porcine cerebrovascular endothelial cells. Moreover, an interesting study showed that EGF receptor contains a transactivation domain and can bind directly to the promoter region of cyclin D1 in vivo suggesting that EGF receptor might function as a transcription factor or co-activator and might activate the genes required for its mitogenic effects [Lin et al., 2001].

Several lines suggest that nuclear $Ca^{2+}$ plays a critical role in many cellular functions such as protein import across the nuclear envelope [Stehno-Bittel et al., 1995; Perez-Terzic et al., 1996] and the transcription of some genes [Hardingham et al., 1997]. Recently, O'Malley et al. [2003] demonstrated that direct stimulation of nuclei isolated from mGlu5 receptor transfected HEK cells by glutamate triggered $Ca^{2+}$ oscillations that were blocked by the addition of the mGlu5-specific antagonist MPEP (2-methyl-6-(phenylethynyl)-pyridine) or EGTA.

At present there is little known concerning the nuclear transduction mechanism induced by the agonist-dependent translocation of cell surface receptors to the nucleus. It should be pointed out that key components of various signaling pathways associated with these receptors are present in nuclei, including enzymes involved in classical PI- and 3-PI-dependent signaling pathways (see Sections Nuclear Ptdlns(3,4,5)$P_3$ Synthesis and Nuclear Ptdlns(3,4,5)$P_3$ Phosphatases), and also phospholipases $PLA_2$ [Peters-Golden and McNish, 1993; Fayard et al., 1998] and PLD [Balboa and Insel, 1995; Baldassare et al., 1997], heterotrimeric G-proteins [Wllard and Crouch, 2000], which suggest a variety of downstream signaling pathway available for nuclear tyrosine kinase receptors and G protein-coupled receptors. First studies showing PI3K activation downstream nuclear receptor were recently published. Gobeil et al. [2002] demonstrated that direct stimulation of nuclei isolated from cerebral microvessel endothelial cells by selective $PGE_2$ $EP_3$ receptor agonist (M&B28767) induces the phosphorylation of Akt and Erk1/2 and resultant activation of nuclear PI3K/Akt- and MEK-dependent pathways. Moreover, the addition of PI3K and MEK inhibitors, wortmannin and PD98059, respectively, suppresses the transcription of the constitutive endothelial nitric-oxide synthase (eNOS) induced by $EP_3$ receptor agonists. In a same way, iNOS expression induced by direct stimulation of isolated rat liver nuclei by $LPA_1$ is prevented by pertussis toxin, $Ca^{2+}$ chelator and channel blockers, EGTA and SK&F96365, respectively, and wortmannin, showing that $LPA_1$ receptor regulates iNOS gene expression through $G_1/G_0$ proteins, calcium channels and PI3K activation [Gobeil et al., 2003]. More recently, Klein et al. [2004] characterized a 120 kDa protein, a cleaved 150 kDa PLC$\gamma$1 moiety in the liver nuclei in vivo, activated by EGF with a time-course similar to that of nuclear membrane EGF receptor phosphorylation. This PLC$\gamma$1 fragment interacts with activated nuclear membrane EGFR, binds PIKE and stimulated nuclear PI3K activity in response to EGF.

Thus, the regulated translocation of transmembrane receptors to nuclear membrane and/or inside the nucleus represents an additional mode of signal transduction, complementing the well-characterized cytoplasmic pathways.

## TARGETS AND FUNCTIONS OF NUCLEAR 3-PHOSPHOINOSITIDES

Information concerning the targets and the functions of nuclear 3-PI are yet very limited. The class I PI3K activation into nuclei leads to Ptdlns(3,4,5)$P_3$ synthesis. Then, target proteins are thought to be recruited (probably via PH domain) and to induce specific responses in the nucleus. Among the wide range of Ptdlns (3,4,5)$P_3$ downstream effectors the serine/threonine kinase Akt and the atypical PKCζ are of special interest. Indeed, these two kinases were identified in nuclei of numerous cells such as NGF-treated PC12 cells, cardiac cells [Mizukami et al., 1997], cardiomyocytes [Mizukami et al., 2000], vascular smooth muscle cells [Bacqueville et al., 2001], and hepatocytes [Calcerrada et al., 2002].

However, a first study suggesting that protein kinase activity of nuclear PI3K may be involved in specific nuclear function has been recently published [Drakas et al., 2004]. These authors proposed that nuclear PI3K phosphorylates and activates the nucleolar protein upstream binding factor (UBF) 1, a regulator of RNA polymerase I activity. They observed that stimulation of myeloid cells or fibroblasts by insulin-growth factor-1 (IGF-1) induces the migration of IRS-1 to nucleoli and the phosphorylation of UBF-1. Moreover, they identified a complex including IRS-1, the p110 catalytic subunit of PI3K and UBF-1 in the nucleus and demonstrated that highly purified p110 subunit directly phosphorylates in vitro UBF-1.

### Nuclear PKCζ

Nuclear translocation of PKCζ upon mitogenic stimulation was associated to PI3K activation in the nucleus. Thus, in PC-12 cells [Neri et al., 1999b] and rat hepatocytes [Calcerrada et al., 2002] treated with NGF and C2 ceramides, respectively, the translocation of PKCζ followed a progressive and sustained nuclear translocation of active PI3K, suggesting that nuclear Ptdlns(3,4,5)$P_3$ level may control nuclear PKCζ targeting. It must be reminded that a similar mechanism was previously proposed to explain the nuclear translocation of classical PKCs (α, β) in response to diacylglycerol production in the nucleus [Neri et al., 1998]. Nuclear substrates of PKCζ include the heterogeneous ribonucleoprotein A1 (RNPA1) [Municio et al., 1995] and the nucleolin, a major nucleolar protein [Zhou et al., 1997]. In vitro studies indicated that RNPA1 phosphorylation by PKCζ inhibits dramatically it is binding to single-stranded RNA [Municio et al., 1995]. Moreover, nucleolin was implicated in chromatin structure, rRNA transcription and maturation, pre-ribosome assembly and finally nucleocytoplasmic transport [Ginisty et al., 1999]. Therefore, nuclear PKCζ could be involved in the regulation of translation and ribosome biogenesis.

### Nuclear Akt and Apoptosis

Akt requires a double phosphorylation to be fully active: Ptdlns(3,4,5)$P_3$ drives the phosphorylation on $Thr^{308}$ through the phosphoinositide-dependent kinase 1 (PDK1) whereas $Ser^{473}$ phosphorylation by a yet to be identified kinase depends on Ptdlns(3,4)$P_2$ [Scheid et al., 2002]. Akt was thought to translocate to the nucleus after these phosphorylation steps. However, recent studies demonstrated that mitogens, including insulin [Lim et al., 2003], IGF-1 and PDGF [Scheid et al., 2005] stimulated the nuclear shuttling of PDK1 in various cell lines. Moreover, PDK1 nuclear localization is increased in PTEN deficient cells and in cells treated with PI3K inhibitors, LY-294002 or wortmannin, indicating that PI3K pathway is involved in PDK1 nuclear shuttling [Lim et al., 2003]. It was also shown that $Ser^{396}$ phosphorylation of PDK1 is necessary for its nuclear translocation [Scheid et al., 2005]. PDK1 possess a functional nuclear export signal (NES) (residues 382–391), which, when mutated, induces nuclear accumulation of PDK1. Interestingly, the NES-deleted mutant of PDK1, which accumulates in the nucleus, is as active as wt-PDK1 [Lim et al., 2003]. Moreover, cotransfection experiments in MCF-7 cells revealed that nuclear PDK1 colocalizes with Akt, and inhibits the transcriptional activity and the nuclear localization of the transcription factor FOXO (Forkhead box, class O) 3 [Scheid et al., 2005]. Forkhead transcription factors of FOXO subfamily are important downstream targets of nuclear Akt. Direct phosphorylation of FKHR/FOXO1, FOXO2, FKHRL1/FOXO3, and AFX/FOXO4 transcription factors by Akt results in their nuclear export and inhibition of transcription. FOXO proteins play an essential role in many of the effect of Akt on cell proliferation and survival. FOXO proteins control the transcription of pro-apoptotic genes,

such as Fas ligand or Bim (Bcl-2 interacting mediator) and anti-apoptotic genes, such as the transcriptional repressor Bcl-6 (for review, see [Birkenkamp and Coffer, 2003; Song et al., 2005]). Phosphorylation of cAMP-response element-binding protein (CREB), another nuclear transcription factor, by Akt results in increasing affinity of CREB to its co-activator CRE and transcriptional activity of CREB. CREB mediates Akt-induced expression of some anti-apoptotic genes, such as bcl-2 and mcl-1 [Song et al., 2005].

Recently, very interesting studies showed that NGF elicits nuclear Ptdlns(3,4,5)$P_3$-dependent anti-apoptotic signaling in PC12-cells. In these cells, NGF induced the translocation of PI3K and its activation by the nuclear GTPase PIKE (see Section Nuclear Class IA PI3K). PI3K generates Ptdlns(3,4,5)$P_3$, which in turn leads to the activation of nuclear Akt [Ahn et al., 2004b]. However, nuclear Akt is necessary, but not sufficient, to mediate the anti-apoptotic action of NGF. In fact, the B23 protein, a major nucleolar phosphoprotein, interacts with Ptdlns(3,4,5)$P_3$ and the nuclear B23-Ptdlns (3,4,5)$P_3$ complex directly associates with CAD (caspase-activated DNase), inhibiting its DNA fragmentation activity [Ahn et al., 2005]. PIKE nuclear signaling in mediating the anti-apoptotic actions of NGF has been very recently reviewed in this journal [Ye, 2005].

### Nuclear Ptdlns(3,4,5)$Pg_3$ and Cell Differentiation

The involvement of nuclear Ptdlns(3,4,5)$P_3$ in cell differentiation was mainly studied in the human promyelocytic leukemia HL-60 cells. These cells are an interesting model because they differentiate into granulocytic cells, monocyte, or macrophage according to the differentiating agent used. The differentiation of HL-60 cells into granulocytic cells and monocytes by all-*trans*-retinoic acid (ATRA) and vitamin D3, respectively, was correlated to PI3K up-regulation and increase of intranuclear Ptdlns (3,4,5)$P_3$ level, whereas cytoplasmic PI3K activity did not vary significantly [Neri et al., 1999a]. Moreover, HL-60 cells treated with wortmannin or p85$\alpha$ antisense were unable to differentiate after ATRA treatment [Bertagnolo et al., 1999]. In the same way, the intranuclear expression and activity of p85/p110 PI3K increased during differentiation of erythroleukemia K562 cells treated with erythropoietin, while the PI3K inhibitor LY-294002 blocked the K562 cell differentiation [Neri et al., 2002]. Moreover, the association of PI3K with Vav seems important for its nuclear activity in neutrophil-like maturation of myeloid cells [Bertagnolo et al., 1999, 2004]. It was found that in ATRA-treated HL-60 cells tyrosine-phosphorylated Vav associates with p85 regulatory subunit of PI3K in both cytoplasmic and nuclear compartments, and that association is essential for the ATRA-induced PI3K activity and for association of PI3K with actin, particularly in the nucleus [Bertagnolo et al., 2004]. A possible role of Vav-related activation of PI3K in differentiating HL-60 cells may consist in the rearrangement of cytoskeleton, and in particular of nucleoskeleton, characterizing the maturation of myeloid precursors to granulocytes.

### Nuclear Ptdlns(3,4,5)$P_3$ and Cell Cycle

Although the importance of PI3Ks in cell cycle control [Roche et al., 1994; Bacqueville et al., 1998] and the translocation of class I PI3Ks into nuclei of serum and growth factors-stimulated cells is now well-established (see Section Nuclear Ptdlns(3,4,5)$P_3$ Synthesis), the role of nuclear Ptdlns(3,4,5)$P_3$ in the progression of cell cycle remains unclear.

However, experiments concerning the subcellular compartmentalization of the 3-PI 3-phosphatase, PTEN, throughout the course of a cell cycle of MCF-7 breast cancer cells [Ginn-Pease and Eng, 2003]. Using biochemical approach and direct visualization of exogenous expressed GFP–PTEN, Ginn-Pease et al. showed that nuclear and cytoplasmic PTEN levels increased when cells begin to leave $G_2$-M phase and enter in $G_1$ phase. In the other hand, in membrane-depleted nuclei isolated from vascular smooth muscle cells endogenous PTEN expression increased during late $G_1$ phase with a maximum in S phase and decreased in $G_2$-M phase, while cellular PTEN level remained stable throughout cell cycle [Deleris et al., 2003]. This inconsistency can be due to differences in the experimental system. Nevertheless, cytosolic and nuclear PTEN could have a specific role in cell cycle control, dependent on cell type. It is also interesting to note that PI3K inhibitors, as PTEN or SHIP-2 overexpressions, down-regulate the cell cycle kinase inhibitor $p27^{kip}$ and blocked cell cycle progression in G1 phase [York and Majerus, 1994; Volinia et al., 1995; Simonsen et al., 1998]. Concerning PTEN,

it was believed that only the lipid phosphatase activity of PTEN mediated growth suppression. However, in MCF-7 cells, PTEN inhibits cell cycle progression in $G_1$ phase through the cooperation of its protein phosphatase, which down-regulates the positive cell cycle regulator, cyclin D1, and its lipid phosphatase activity, which up-regulates the CDK (cyclin dependent kinase) inhibitory protein, $p27^{kip}$ [Weng et al., 2001]. Further experiments will be necessary to determine, which of the lipid and protein phosphatase activities of PTEN and/or lipid and protein kinase activities of PI3K, or even the non-enzymatic effects of both proteins, are relevant to regulate cell proliferation within cell nuclei. Moreover, as described above, nuclear PTEN can regulate cellular transformation through its interaction with the nucleolar protein MSP58 [Okumura et al., 2005].

## CONCLUSIONS

Evidence reviewed here show that Ptdlns $(3,4,5)P_3$ could be a major player in nuclear signaling. However, numerous questions concerning nuclear Ptdlns$(3,4,5)P_3$ remain unanswered. The temporal and spatial aspects in which nuclear Ptdlns$(3,4,5)P_3$ is produced and metabolized are crucial. The development of specific anti-Ptdlns$(3,4,5)P_3$ antibodies and Ptdlns$(3,4,5)P_3$ affinity probes, will be useful tools to improve our understanding of nuclear Ptdlns$(3,4,5)P_3$ regulation [Chen et al., 2002; Prestwich, 2004]. Another burning question is to determine the specific role of lipid and protein kinase activities of PI3K, and lipid and protein phosphatase activities of PTEN in cell functions. Moreover, the emerging isoform-selective inhibitors of the different class I PI3K [Prestwich, 2004] could be used to explore the role of different nuclear class I PI3Ks.

Over the past decade, the translocation of transmembrane receptors to the nucleus has gained credibility, and recent evidence indicates that translocated receptors induce nuclear PI3K activity. An important challenge is now to understand the functional specificity of the activation of nuclear Ptdlns$(3,4,5)P_3$-dependent signaling pathways by translocated receptors.

There is now a substantial body of in vitro evidence, which point to a unique role for nuclear Ptdlns$(3,4,5)P_3$ and its metabolizing enzymes in cell biology. More effort will be spent on trying to discern the function of these processes in vivo and to identify interesting targets for the development of new therapeutic strategies.

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