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Nuclear Phosphoinositide Kinases and Inositol Phospholipids

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Abstract The presence of inositol phospholipids in the nuclei of mammalian cells has by now been well established, as has the presence of the enzymes responsible for their metabolism. However, our understanding of the role of these nuclear phosphoinositides in regulating cellular events has lagged far behind that for its cytosolic counterpart. It is clear, though, that the nuclear phosphoinositide pool is independent of the cytosolic pool and is, therefore, likely to be regulating a unique set of cellular events. As with its cytosolic phosphoinositides, many nuclear phosphoinositides and their metabolic enzymes are located at distinct sub-cellular structures. This arrangement spatially limits the production and activity of inositol phospholipids and is believed to be a major mechanism for regulating their function. Here, we will introduce the components of nuclear inositol phospholipid signal transduction and discuss how their spatial arrangement may dictate which nuclear functions they are modulating. J. Cell. Biochem. 97: 252–260, 2006.

Key words: phosphoinositide; diacylglycerol; nucleus; signal transduction; nuclear matrix; phosphoinositide kinase

Phosphoinositides are lipid second messengers, which regulate a diverse array of cellular functions. Originally, phosphoinositide signaling has been thought to occur at the plasma membrane where a stimulus promotes the generation of inositol phospholipid second messengers that in turn modulate signaling pathways within the cells. However, it has become quite clear that phosphoinositides are also present in the nucleus of mammalian cells, as are the enzymes responsible for their generation. Initially, it was thought that nuclear phosphoinositide generation was occurring at the nuclear membrane [Smith and Wells, 1983]. However, it was found that mouse erythroleukemia (MEL) cell nuclei, stripped of their nuclear membrane by detergent, still maintained the ability to synthesize phosphoinositides. Additionally, it was determined that when these cells were induced to differentiate, the levels of non-membranous nuclear phosphatidylinositol 4,5-bisphosphate ($PI4,5P_2$) increased, while the total cellular amount of $PI4,5P_2$ remained unchanged [Cocco et al., 1987]. This suggests that signaling by inositol phospholipids occurs within the nucleus and that nuclear phosphoinositide metabolism and the events it regulates are independent from its cytosolic counterpart. Since these early studies, the nuclear localization of phosphoinositide kinases responsible for the generation of nuclear $PI4,5P_2$ has been confirmed, as has the nuclear localization of kinases, which produce phosphatidylinositol phosphates (PIPs) and various 3-phosphorylated phosphoinositides, as well as phosphoinositide specific phospholipase C's (PI-PLCs), which can produce diacylglycerol (DAG) and inositol 3,4,5-trisphosphate (IP_3), all of which provide the potential for inositol phospholipid signaling within the nucleus (Fig. 1, Table I).

It is clear that nuclear phosphoinositides and phosphoinositide kinases exist independently of the nuclear membrane. When purified nuclei are stripped of their membranes by detergent, they retain significant amounts of DAG, PI4P, and $PI4,5P_2$, as well as PI 4-kinase and PI4P 5-kinase activities [Vann et al., 1997]. When nuclear phosphoinositides are visualized by

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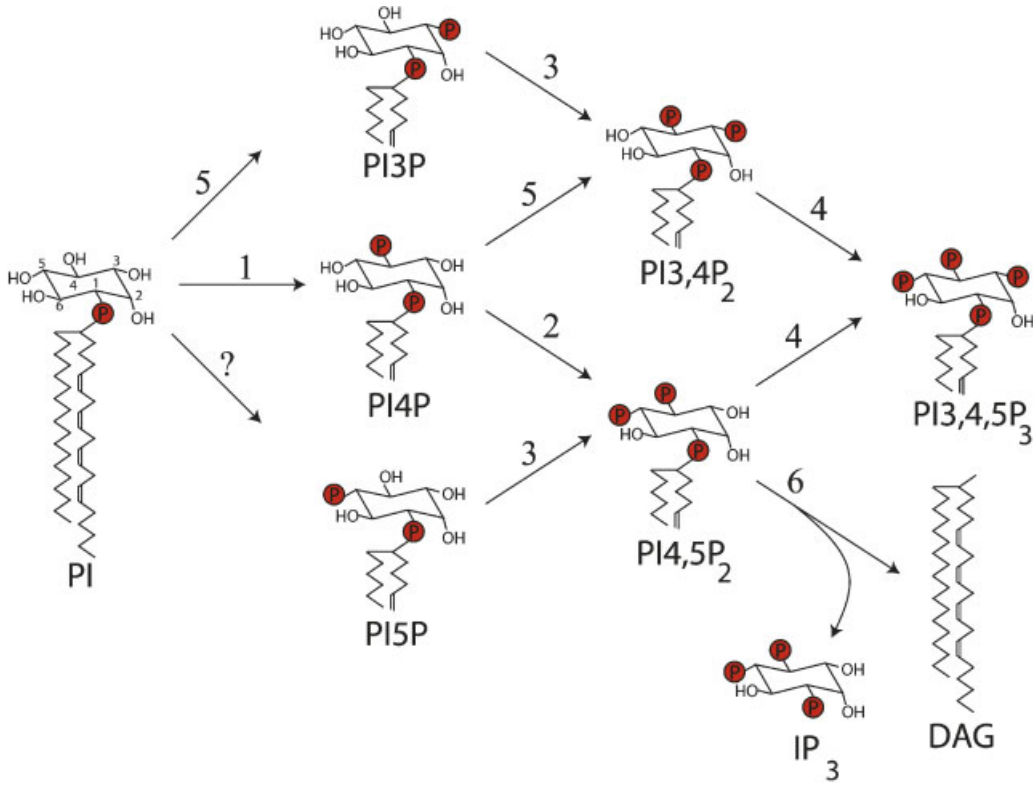

Fig. 1. Substrates, products, and activities of identified nuclear phosphoinositide lipid kinases. The acyl chains have been abbreviated to save space. The kinases catalyzing each reaction are numbered as in Table I. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

electron microscopy, they are found in fibrillar structures that are distinct from the nuclear membrane [Gillooly et al., 2000; Watt et al., 2002]. What remains unclear is the actual environment of nuclear phosphoinositides. The current belief is that nuclear phosphoinositides may be a part of a unique sub-nuclear structure and may exist in an environment that is different from classic membrane bilayers but the nature of this structure remains unknown.

The nucleus of mammalian cells is organized into multiple compartments, which are responsible for performing various nuclear functions [Spector, 2001]. The organization of many if not all of these domains is thought to be determined by a scaffold called the nuclear matrix, a filamentous structure that is analogous to the actin cytoskeleton. Actin cytoskeletons contain and are regulated by phosphoinositides and there is an increasing evidence that actin is actually an important part of the nuclear matrix [Rando et al., 2000]. It is then conceivable that nuclear phosphoinositides may be important components of the nuclear matrix. It also appears that components of the nuclear phosphoinositides pathway have different subnuclear distributions as indicated by the partitioning of PI 4-kinase versus DAG kinase, PI4P 5-kinase, and PI-PLC activities [Payrastre et al., 1992].

TABLE I. Nuclear Phosphoinositide Kinases and PLCs

#	Enzyme	Product(s)
1	PI 4-Kinase92 (PI4Kβ)	PI4P
2	PI4P 5-Kinase type Iα	$PI4,5P_2$
3	PI5P 4-Kinase type II α/β	$PI4,5P_2$
4	PI3-Kinase class IA/IB	PIP_3
5	PI3-Kinase class II α/β	PI3P, $PI3,4P_2$
6	$PLC\beta_{1-4}$, δ_1, δ_4, γ_1, ζ	IP_3 and DAG

As a whole, phosphoinositides regulate a wide array of cellular events through recruitment and/or activation of their effectors. Furthermore, the same lipid species can affect a diverse set of processes. For example, $PI4,5P_2$ can modulate cell adhesion, vesicular trafficking, and cytoskeletal organization. Because of the broad signaling potential of inositol phospholipids, a mechanism to ensure regulation of specific pathways is required. This is achieved through the spatial regulation of phosphoinositide generation. Phosphoinositides are gener-

ated primarily by families of phosphoinositide kinases, which contain multiple isoforms, each with a unique sub-cellular distribution. Regulating the location of specific kinases allows for the generation of inositol lipid second messengers at specific sites within the cell, which in turn allows for precise targeting of individual phosphoinositide sensitive pathways. As is the case with their cytosolic counterparts, the location of nuclear phosphoinositide signaling components is likely vital to their nuclear function. In this review, we will discuss the presence of inositol phospholipids and the kinases responsible for their generation in the nuclei of mammalian cells. Additionally, we will highlight how the known sub-nuclear distribution of some of these components may give clues to their nuclear functions.

NUCLEAR PI KINASES AND PIPs

The simplest inositol phospholipids are the singly phosphorylated phosphatidylinositol phosphates (PIPs): PI3P, PI4P, and PI5P. PIPs are intermediates in the synthesis of PIP_2 and PIP_3, however, they are also able to directly influence cellular events [Clarke, 2003].

PI3P can be synthesized from PI by PI 3-kinases. PI3P has been detected in the nucleus by electron microscopy with a PI3P specific probe. Using this approach, PI3P was found in a dense fibrillar component of the nucleus and not dispersed in the nucleoplasm or in the nuclear membrane, suggesting a specific organization and possible association with the nuclear matrix [Gillooly et al., 2000].

PI4P, the most abundant PIP both in whole cells and the nucleus, is synthesized from PI through phosphorylation by the PI 4-kinase family. PI 4-kinase activity has been detected in the nuclei of cells [Payrastre et al., 1992] and the kinase responsible has been identified as PI4K92 (PI4Kβ) [de Graaf et al., 2002]. PI4P levels change with progression through the cell cycle, peaking as the cell enters G_1 and then decreasing through S phase [Clarke et al., 2001]. Despite an understanding of PI4P synthesis in the nucleus, there is still no evidence that it directly regulates any nuclear functions. The primary role for PI4P in the nucleus may be as a $PI4,5P_2$ precursor. $PI4,5P_2$ turnover also changes through the cell cycle with a peak at the G_1/S transition. By this time, however, nuclear PI4P levels are already decreasing [Clarke et al., 2001]. This indicates that the relationship between nuclear PI4P and $PI4,5P_2$ is more complex than a direct precursor–product relationship. This may be related to the observation that PI4P and $PI4,5P_2$ generating enzymes are found in separate nuclear compartments [Payrastre et al., 1992].

PI5P is also present in the nucleus of mammalian cells, although its metabolism and regulation remains a matter of debate [Roberts et al., 2005]. Nuclear PI5P mass has also been shown to change during progression through the cell cycle peaking in G_1 [Clarke et al., 2001], but the significance of this remains unclear. The strongest evidence for the role of PI5P in nuclear signaling events has come from the identification of ING2 as a nuclear PI5P-binding protein [Gozani et al., 2003]. ING2 is a member of the ING family, which associates with and modulates the activity of histone acetylases and deacetylases as well as inducing apoptosis through p53 acetylation [Feng et al., 2002]. ING2 is able to bind to PI5P both in vitro and in vivo. This interaction is mediated by the plant homeodomain (PHD) finger motif of ING2, which is structurally similar to the PI3P-binding FYVE finger domain [Pascual et al., 2000]. ING2 was found to be associated with the chromatin containing nuclear matrix and it was suggested that PI5P is required for this association. Additionally, it was shown that ING2 regulation of p53 acetylation and apoptosis required both PI5P and an intact PI5P-binding domain in ING2 [Gozani et al., 2003]. The ING2 association with chromatin implies that PI5P is also found at these sites and that PI5P signaling may link chromatin to p53 responses. It was also shown that other nuclear PHD domain containing proteins can bind PIPs with varying specificities and affinities [Gozani et al., 2003]. PHD domains are commonly found in chromatin regulatory proteins, indicating that the PIP/PHD interaction may be a more general link between phosphoinositides and chromatin.

NUCLEAR PIP KINASES AND $PI4,5P_2$

Phosphatidylinositol 4,5-bisphosphate ($PI4,5P_2$) is produced from PIP by PIP kinases. Both the type I and II families of PIP kinases can catalyze the conversion of PIP to $PI4,5P_2$ but differ in their substrate preference. Type I PIP kinases use PI4P as a substrate to create $PI4,5P_2$. The type II kinases prefer PI5P as a substrate but

produce chemically identical $PI4,5P_2$. Because PI4P is found in cells at much higher levels than PI5P, it is thought that the type I kinases are responsible for the majority of cellular $PI4,5P_2$ production. However, it is likely that the type II kinases are capable of making significant amounts of $PI4,5P_2$ at specific sub-cellular sites.

There are three isoforms that make up the type I PIP kinase family. PIPKIα, β, and γ are each unique gene products and each has its own distinct sub-cellular distribution. Of the three isoforms, only PIPKIα is found in the nucleus. We have shown that nuclear PIPKIα resides in a detergent insoluble nuclear fraction, suggesting an association with the nuclear matrix. Type II PIP kinases also exist as α, β, and γ isoforms. Our lab has shown that PIPKIIβ is found in the nucleus, also in association with the nuclear matrix [Boronenkov et al., 1998]. This nuclear localization is dependent on a 17-amino-acid alpha-helix that is unique to the β isoform of the type II PIP kinases [Ciruela et al., 2000]. The sub-cellular localization of PIPKIIα is still a matter of debate. We have shown PIPKIIα to be in the nucleus using both antibodies to the endogenous protein as well as overexpression studies [Boronenkov et al., 1998]. However, others have shown PIPKIIα to be excluded from the nucleus when overexpressed [Ciruela et al., 2000]. The presence of both type I and II PI4P 5-kinases in the nucleus suggests that they may be producing distinct and independent pools of $PI4,5P_2$ which could be used to regulate different nuclear functions. Indeed, it appears that there may be at least two pools of $PI4,5P_2$, as well as PI4P and DAG in the nucleus [Vann et al., 1997]. Alternatively, it could be that the function of the nuclear type II kinases is to attenuate PI5P signaling by conversion of PI5P to $PI4,5P_2$.

$PI4,5P_2$ can be found in detergent-insoluble nuclear fractions, suggesting an association with the nuclear matrix, and co-localizes with small nuclear ribonucleoprotein particles (snRNPs) in nuclear speckles, structures that are involved in pre-mRNA processing [Boronenkov et al., 1998]. Detection of $PI4,5P_2$ by electron microscopy using the PH domain of $PLC\delta_1$ as a probe revealed that in the nucleus, $PI4,5P_2$ can be found at distinct electron dense structures [Watt et al., 2002], which may be the same as the nuclear speckles observed by immunofluorescence. The presence of PIPKIα and $PI4,5P_2$ at nuclear speckles suggests a role for nuclear phosphoinositides in pre-mRNA splicing. This is supported biochemically by the observation that nuclear $PI4,5P_2$ is associated with snRNPs as well as RNA polymerase II, and that immunodepletion of $PI4,5P_2$ from nuclear extracts also depletes these proteins. Furthermore, immunodepletion of $PI4,5P_2$ from nuclear extracts ablates their ability to splice pre-mRNA in an in vitro assay [Osborne et al., 2001].

There is also evidence that $PI4,5P_2$ may be involved in regulating the state of chromatin in the nucleus. Exogenous $PI4,5P_2$ can reduce histone H1 transcriptional inhibition in an in vitro *Drosophila* embryo transcription system. $PI4,5P_2$ can bind to both histone H1 and H3 and this is thought to be a part of the mechanism for the observed relief of transcriptional inhibition [Yu et al., 1998]. Another piece of evidence linking $PI4,5P_2$ to the regulation of chromatin structure is the observation that $PI4,5P_2$ can regulate the interaction of the chromatin remodeling complex BAF with the nuclear matrix. BAF is related to the yeast SWI/SNF complex and can bind specifically to $PI4,5P_2$ containing micelles. During T-cell activation, the BAF complex transitions from a soluble to an insoluble nuclear fraction. Addition of $PI4,5P_2$ to unstimulated T-cell nuclei can cause the same shift in association, suggesting that $PI4,5P_2$ may help to regulate the association of BAF with chromatin and/or the nuclear matrix [Zhao et al., 1998]. Actin and actin-related proteins are an important part of the BAF complex and are found in mammalian nuclei. The actin binding subunit of the BAF complex is the protein BRG-1. BRG-1 contains two actin binding sites, one of which is also the $PI4,5P_2$ binding site for the BAF complex. It has been proposed that $PI4,5P_2$ modulates the partitioning of BAF between the soluble and insoluble nuclear fractions by regulating its ability to interact with actin filaments through BRG-1. It has been reported that when BRG-1 is bound to $PI4,5P_2$ it becomes capable of binding to and stabilizing nuclear actin filaments, thereby promoting the association of BAF with the nuclear matrix [Rando et al., 2002]. Additionally, the retinoblastoma protein Rb, which recruits the BAF complex to transcription sites, can interact with and activate PIPKIα [Divecha et al., 2002]. This may be a mechanism for promoting $PI4,5P_2$ production at sites where it can specifically affect BAF activity. In addition

to providing another link between phosphoinositides and chromatin, these studies show that PI4,5P_2 is capable of affecting nuclear actin and may, therefore, be an important regulator of the nuclear matrix.

NUCLEAR PLCs AND DAG

While PI4,5P_2 directly modulates many cellular functions, it is also the substrate for phosphoinositide specific phospholipase C's (PI-PLCs), which can hydrolyze the inositol head group of PI4,5P_2 to produce IP_3 and DAG. IP_3 and other soluble inositides are likely playing their own roles in nuclear signaling but this is beyond the scope of this review. One of the functional consequences of DAG production is the recruitment and activation of protein kinase C (PKC) isoforms. There are 13 isoforms of PI-PLC's divided into 5 families β, γ, δ, ε, and ζ, several of which can be found in the nucleus. All four members of the PLCβ family contain a C-terminal NLS and can be found in the nucleus [Manzoli et al., 2005]. PLCβ1 exists as two splice variants PLCβ1a and PLCβ1b, with the b splice variant being primarily nuclear and a being primarily cytosolic [Bahk et al., 1998]. Additionally, PLC γ1, γ2, δ1, δ4, and PLCζ have been reported in the nucleus [Manzoli et al., 2005]. One key role of PI-PLC produced nuclear DAG is the recruitment and activation of the various protein kinase C (PKC) isoforms that have been shown to shuttle in and out of the nucleus. There are three events that coincide with nuclear production of DAG. They are cell proliferation, cell cycle progression, and differentiation.

Stimulation of proliferation in Swiss 3T3 fibroblasts by the mitogen insulin-like growth factor-1 (IGF-1) has been shown to cause a rapid increase in the mass of nuclear DAG along with a corresponding decrease in nuclear PI4,5P_2. Bombesin, another mitogen that signals through PI-PLCs is only able to affect DAG mass at the plasma membrane [Divecha et al., 1991], which further illustrates the separation of nuclear and cytosolic phosphoinositide signaling events. This increase in nuclear DAG mass is primarily due to the activity of PLCβ1, as blocking PLCβ1 expression with anti-sense RNA greatly reduces the DAG mass increase in response to IGF-1. Similarly, overexpression of PLCβ1 can cause an increase in nuclear DAG levels, even in the absence of IGF-1 stimulation [Manzoli et al., 1997]. Blocking IGF-1-induced nuclear DAG production with a PI-PLC specific inhibitor sufficient to block PKCα translocation to the nucleus, indicating that one role of IGF-1-mediated nuclear DAG production is the recruitment of PKCα to the nucleus [Neri et al., 1998]. Little is known about the regulation of nuclear phosphoinositide metabolism but there are some data suggesting a mechanism for regulation of nuclear PLCβ1 in proliferation. In the cytoplasm, PLCβ1 is regulated by heterotrimeric G proteins. However, there is no evidence that this is the case in the nucleus. Alternatively, it appears that nuclear PLCβ1 is regulated by MAP kinase signaling pathways. ERK can phosphorylate nuclear PLCβ1 at serine 982 in response to IGF-1. Mutation of serine 982 to glycine prevents phosphorylation by ERK, and expression of this mutant in cells can block both IGF-1 stimulated nuclear DAG increase and cellular growth. Phosphorylation of PLCβ1 on serine 982 does not affect its activity; however, the direct consequences of this phosphorylation remain unknown [Xu et al., 2001a]. PKCα has also been reported to be involved in the regulation of nuclear PLCβ1. PCKα phosphorylates nuclear PLCβ1 in response to IGF-1 stimulation and this phosphorylation is sufficient to terminate the IGF-1-induced increase in nuclear PLCβ1 activity [Xu et al., 2001b]. This may represent a mechanism to insure the transient nature of nuclear PLCβ1 activation as PLCβ1-produced DAG will recruit and activate PKCα, which will in turn inactivate PLCβ1.

Nuclear DAG levels have also been shown to change with progression through the cell cycle. There are two points in the cell cycle where nuclear DAG mass peaks. The first occurs around late G_1 or early S phase and the second occurs at the G_2/M boundary [Banfic et al., 1993; Sun et al., 1997]. Treatment with a PI-PLC specific inhibitor can reduce the G_1/S DAG mass peak and progression through S phase [Lukinovic-Skudar et al., 2005]. PLCδ1 and PLCδ4 have been reported to accumulate in the nucleus at the G_1/S transition [Stallings et al., 2005]. PLCδ4 is found in the nucleus of regenerating rat liver and tumor cells, and its expression increases at the G_1/S transition as well as in response to serum stimulation [Liu et al., 1996]. In addition, nuclear PLCβ1 activity increases at the G_1/S transition and this activity also appears to be regulated by ERK signaling as inhibition of ERK activation can block the

increase in PLCβ1 activity at the G_1/S boundary [Lukinovic-Skudar et al., 2005]. It is interesting to note that the G_1/S transition is also a time of increased PIP_2 turnover [Clarke et al., 2001], which may be related to the increased PLC activity at this transition. The G_2/M DAG mass increase can also be inhibited by PI-PLC inhibitors and this is sufficient to block the cells at the G_2/M boundary [Sun et al., 1997]. The importance of the DAG increase at G_2/M may be related to the role of PKCβII at this transition. PKCβII is also required for progress through the G_2/M boundary where it can phosphorylate nuclear lamins to regulate nuclear envelope breakdown [Thompson and Fields, 1996]. PKCβII is targeted to the nucleus at G_2/M as well as at other times of increased DAG mass, including IGF-1 stimulation [Neri et al., 1999b]. This suggests a pathway where the G_2/M DAG increase serves to recruit PKCβII to the nucleus and promotes nuclear envelope breakdown. PLCβ1 is the only PLC isoform to be reported in the nucleus at this time where it also appears to be regulated by ERK activity [Lukinovic-Skudar et al., 2005].

Finally, nuclear PI-PLCs have also been implicated in cell differentiation. However, the actual role of nuclear PI-PLCs, whether promoting or inhibiting differentiation, seems to vary by cell type. Erythroid differentiation of MEL cells corresponds with a decrease in nuclear PLCβ1 activity and nuclear DAG mass [Martelli et al., 1994; Divecha et al., 1995]. Conversely, when C2Cl2 myoblasts are induced to differentiate, there is a dramatic increase in both PLCβ1 expression and activity. Additionally, expression of a PLCβ1 mutant that is confined to the cytoplasm by destroying its nuclear localization signal is able to repress C2Cl2 cell differentiation [Faenza et al., 2003]. Another interesting role for nuclear PLCγ1 is its ability to act as a guanine nucleotide exchange factor (GEF) for PIKE (phosphatidylinositol 3-kinase enhancer), a GTPase that can activate nuclear PI 3-kinase, which will be discussed in more detail below.

NUCLEAR PI 3 KINASES AND 3-PHOSPHORYLATED LIPIDS

Phosphoinositide 3 kinases (PI3 kinases) are divided into three classes (I, II, and III) based on their substrate specificity, composition, and mechanism of regulation. Class I PI3Ks are further divided into A and B sub-classes. Class IA PI3 kinases, which primarily produce phosphatidylinositol 3,4,5-trisphosphate (PIP_3) from $PI4,5P_2$, consist of a p110 catalytic subunit and one of a number of regulatory subunits derived from the alternative splicing of the p85α, p85β, or p55γ genes. The regulatory subunits of class I PI3-kinases are important for regulating both enzyme activity and localization. p85/p110 PI3-kinases have been detected in the nucleus by immunochemical detection of the p85 regulatory subunit and the presence of p110 catalytic activity [Lu et al., 1998]. One role class IA kinases may play in nuclear signaling is in cellular differentiation. When HL-60 pre-myeloblasts are induced to differentiate, they show an increase in nuclear matrix associated p85 and PI3-kinase activity [Neri et al., 1999a], placing PI3-kinases in proximity to other members of the nuclear phosphoinositide signaling pathway. An interesting mechanism for the regulation of nuclear class IA PI3-kinases has been reported involving PLCγ1 and the nuclear GTPase PIKE. GTP bound, active PIKE is able to activate class IA PI3-kinases while a dominant negative form of PIKE is able to prevent nerve growth factor (NGF)-induced increase in nuclear PI3 kinase activity [Ye et al., 2000]. PLCγ1 can act as a GEF for PIKE and as such can also modulate the NGF-induced increase in nuclear PI3 kinase activity. Specifically, the SH3 domain of PLCγ1 is required for its GEF activity [Ye et al., 2002]. This provides one explanation for the observation that the mitogenic properties of PLCγ1 does not require its catalytic activity [Smith et al., 1998]. The p55γ regulatory of class I PI3-kinases subunit has also been found in the nucleus where it interacts with Rb, a regulator of cell cycle progression. The interaction between Rb and p55γ prevents cell cycle progression by inhibiting Rb-regulated transcription [Xia et al., 2003]. It is unclear if regulation of this mechanism requires PI3 kinase activity or inositol phospholipids but it is interesting to remember that pRB also interacts and regulates PIPKIα as discussed above. Class IB PI3Ks consists of a p110g catalytic subunit and a p101 regulatory subunit. The p110γ catalytic subunit has been shown to translocate to the nucleus in response to serum stimulation in HepG2 hepatoma cells. This translocation requires the Gβγ subunit of heterotrimeric G proteins. Pertussis toxin can

TABLE II. Species, Location, and Proposed Functions of Nuclear Phosphoinositides

Phosphoinositide	Nuclear location	Proposed nuclear function
PI3P	Matrix, speckles(?)	Cell cycle, PDX domain regulation(?)
PI4P	Matrix	PDX domain regulation(?)
PI5P	Matrix, chromatin	ING2/PDX domain regulation
$PI3,4P_2$	Matrix, nuclear speckles(?)	Splicing(?)
$PI4,5P_2$	Matrix, nuclear speckles, chromatin	Splicing, chromatin
PIP_3	Matrix	Cell cycle, differentiation, proliferation
DAG	Matrix	Cell cycle, differentiation, proliferation

Entries followed by (?) have been proposed but remain unconfirmed.

inhibit the serum-stimulated translocation while Gβγ overexpression can induce the translocation in the absence of serum. Regulation of this translocation also requires the interaction between p110γ and the p101 regulatory subunit as truncation of the p101 interacting region on p110γ that abolishes the p110γ/p101 interaction resulting in a constitutively nuclear p110γ [Metjian et al., 1999].

The three Class II PI3-kinases are the monomeric enzymes C2α, β, and γ. Class II enzymes exhibit a strong preference for PI4P as a substrate and produce PI 3,4 P_2, but can also produce PI3P from PI. Both C2α and β have been detected in the nucleus of mammalian cells. C2α has been shown to not only be in the nucleus but to be found at nuclear speckles [Didichenko and Thelen, 2001], again implicating a role for phosphoinositides in pre-mRNA splicing. C2β is also found in membrane-stripped nuclei but not at nuclear speckles, suggesting distinct functions for the two nuclear class II PI3 kinases. In fact, C2β is activated at the G_2/M transition, similar to PLCβ1, resulting in an increase in nuclear PI3P at this time [Visnjic et al., 2003]. Class III PI3-kinases are also heterodimeric enzymes that phosphorylate PI to create PI3P. To date, there is no report of class III enzymes in the nucleus of mammalian cells.

Unfortunately, there is still very little known about nuclear phosphoinositide signaling. However, there are several promising prospects. The changes in inositol phospholipids as well as DAG with progression through the cell cycle suggest that there are multiple roles for phosphoinositide signaling in this process. The colocalization of $PI4,5P_2$ and phosphoinositide-generating enzymes at nuclear speckles suggests a role in the processing of pre-mRNA. Similarly, the association of inositol phospholipids with chromatin suggests a role for modulating its structure and therefore, possibly transcription. Finally, the strong association of phosphoinositides with the nuclear matrix may indicate that they play a fundamental role in regulating the organization of the nucleus. It is important to recognize that there are other proteins besides lipid kinases and PI-PLCs that participate in phosphoinositide signaling. The phosphatases SHIP and PTEN, which can dephosphorylate inositol phospholipids both as a way to attenuate their signaling and to create unique second messengers, are at specific sites within the nucleus [Deleris et al., 2003]. Additionally, inositol kinases are also present in the nucleus. These kinases can phosphorylate IP_3 created by PI-PLCs to create the higher inositides including IP_6, IP_7, and IP_8 [Irvine and Schell, 2001]. The association of nuclear phosphoinositides with specific nuclear components indicates that like its cytosolic counterpart, nuclear phosphoinositide signaling may require spatial regulation of phosphoinositide production (Table II). This idea is supported by the presence of apparently redundant kinase and phospholipase activities in the nucleus, which may in fact be generating separate pools of phosphoinositide second messengers. Overall, our current understanding of nuclear inositol signaling is quite limited. Identification of the phosphoinositide-sensitive components of nuclear signaling pathways will allow for a much more detailed view of the functions of nuclear phosphoinositides.

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