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Nuclear Phosphoinositides: A Signaling Enigma Wrapped in a Compartmental Conundrum

Christy A. Barlow*, **Rakesh S. Laishram***, and **Richard A. Anderson**[1]
University of Wisconsin-Madison, Department of Pharmacology, 1300 University Ave. University of Wisconsin Medical School, Madison, Wisconsin, 53706

Abstract

While the presence of phosphoinositides in the nuclei of eukaryotes and the identity of the enzymes responsible for their metabolism have been known for some time, their functions in the nucleus are only now emerging. This is illustrated by the recent identification of effectors for nuclear phosphoinositides. Like the cytosolic phosphoinositide signaling pathway, nuclear phosphatidylinositol 4,5 bisphosphate ($PI4,5P_2$) is at the center of the pathway and acts both as a messenger and as a precursor for many additional messengers. Here, recent advances in the understanding of nuclear phosphoinositide signaling and its functions are reviewed with an emphasis on $PI4,5P_2$ and its role in gene expression. The compartmentalization of nuclear phosphoinositide phosphates (PIP_n) remains a mystery, but emerging evidence suggests that phosphoinositides occupy several functionally distinct compartments.

Keywords

Phosphatidylinositol-4; 5-bisphosphate; Nuclear phosphoinositide cycle

Introduction

Phosphoinositides are lipid messengers that regulate many cellular processes in eukaryotic cells. Phosphatidylinositol (PI) is a negatively charged phospholipid that can be phosphorylated on the 3, 4, and 5 hydroxyls of the *myo*-inositol ring in all possible combinations. The resulting phosphatidylinositol phosphate (PIP), phosphatidylinositol bisphosphate (PIP_2), or phosphatidylinositol trisphosphate (PIP_3), collectively called phosphoinositides (PIP_n), are direct messengers and precursors to messengers (Figure 1A). The PI signaling cycle was discovered in the 1950s by the Hokins (Box 1)[1]. In the canonical cytoplasmic phosphoinositide cycle, an extracellular stimulus triggers the generation of phosphoinositide signals via an array of kinases, phosphatases and phospholipases. The phosphoinositide kinases are integrated into signaling pathways that generate phosphoinositide signals in specific subcellular compartments that regulate effector proteins at these sites[2]. This distribution is regulated by specific protein-protein interactions unique to each kinase. This site specific targeting allows

1Corresponding Author Richard A. Anderson Department of Pharmacology 1300 University of Wisconsin-Madison Madison, WI 53706 Phone: 608-262-3573 Fax: 608-262-1257 raanders@wisc.edu.
*These authors contributed equally to this work and are listed alphabetically.

for the generation of lipid messengers at specific cellular compartments and results in spatial specific phosphoinositide signaling pathways[2].

Most of our knowledge of phosphoinositide signaling is derived from cytosolic signaling pathways, yet over the past several decades much evidence has established the existence of a nuclear phosphoinositide cycle. Although the roles played by phosphoinositides in the nucleus are only now emerging, it is clear that some aspects of nuclear phosphoinositide signaling are regulated differentially from that in the cytosol and on the plasma membrane[3]. This is emphasized by reports showing phosphoinositides in the inner nuclear envelope and also in nuclear compartments that are separate from known membranes[4-7]. The presence of phosphoinositides in the nucleus itself raises several questions: what are the functional implications attributed to the different phosphoinositides in the nucleus?; how is their function and metabolism regulated?; and how is their subnuclear localization achieved? Several functions have been proposed for nuclear phosphoinositides in regulating nuclear signaling processes. However, there is little mechanistic data defining how phosphoinositides regulate nuclear events. To date, nuclear phosphoinositides and derived inositol phosphates have been implicated in a wide range of functions including differentiation, proliferation, apoptosis, stress responses, and gene expression[4-6, 8, 9]. This review will focus on functions attributed to nuclear phosphoinositides with an emphasis on PIP_n effectors and mechanisms.

Nuclear phosphoinositide kinases

Nuclear and cytoplasmic phosphoinositide signaling share common enzymes. The phosphoinositide cycle involves the ordered phosphorylation of PI by PI kinase and PIP kinases forming all possible PIP_2 isomers and PIP_3 (Figure 1A). Specific isoforms of phosphoinositide kinases, phosphatases, and phospholipase C (PLC) are located within the nucleus (Figure 1B) [10-14]. Some of these enzymes are imported into the nucleus upon stimulation of cells, and in this context the roles of PI-PLCs, diacylglycerol (DAG), and PI3K have been reviewed extensively [5, 10, 13, 15].

Nuclear phosphoinositide signaling revolves around the generation of $PI4,5P_2$ by phosphatidylinositol phosphate kinases (PIPK). There are three classes of PIPKs, types I, II and III, and of these type I and II both generate $PI4,5P_2$ although by utilizing different substrates, PI4P and PI5P, respectively[16, 17]. At least four members of this family of kinases, type Iα phosphatidylinositol 4-phosphate 5-kinase (PIPKIα), type Iγ isoform 4 phosphatidylinositol 4-phosphate 5-kinase (PIPKIγ_i4) and type II and IIβ phosphatidylinositol 5-phosphate 4-kinase (PIPKIIα and PIPKIIβ) are localized to the nucleus[7, 18, 19]. These kinases are not exclusively localized to the nucleus and appear to have cytoplasmic functions as well[7, 18, 20]. The presence of type I and type II PIPKs in the nucleus suggest that different pools of $PI4,5P_2$ are generated and these may regulate distinct nuclear functions as discussed below.

The targeting of phosphoinositide metabolizing enzymes to the nucleus is achieved by a number of mechanisms. While some of the enzymes contain defined nuclear localization sequences (NLS), others do not. For example, PIPKIIβ lacks a NLS, but is targeted to the nucleus through the “kinase insert region”, a nonhomologous sequence that separates the kinase domain of type I and II PIPKs[7, 16, 19]. PIPKIIβ is associated with the nuclear protein SPOP (speckle-type POZ domain protein) via the insert sequence and this interaction may modulate nuclear localization 21. Nuclear localization of the $PI3,4,5P_3$ 3-phosphatase PTEN depends upon a putative NLS and major vault protein-mediated import. However, PTEN has also been shown to diffuse through the nuclear pore. Importation of PTEN is regulated by monoubiquitinylation and by other pathways including oxidative stress[22, 23]. As a group the

import of PI3K and PI-PLC isoforms is diverse occurring through an NLS or associated proteins and is modulated by growth factors and other stimuli[5, 10].

In nuclei, both type I and II PIPKs are targeted to structures called interchromatin granule clusters or nuclear speckles[7]. Speckles are separated from known membrane in the interchromatin regions of the nucleoplasm. Yet, significantly, $PI4,5P_2$ appears to be present at these sites, shown in Figure 1C[7, 24]. Nuclear speckles are also enriched in pre-messenger RNA processing factors suggesting a role in pre-mRNA processing[7, 12, 24-26]. Other enzymes involved in the nuclear PI cycle are localized at nuclear speckles or are diffuse throughout the nucleus[8, 27]. There is also evidence that phosphoinositides are generated on the inner nuclear envelope[28-30] and are highly concentrated on nuclear envelope remnants[31]. The combined data indicate that there are distinct PI cycles within the nucleus both associated with and separate from membranes and this will be discussed in more detail below.

Role of Nuclear Type II PIP Kinase Isoforms in Stress Signaling

In response to oxidative and UV damage in mammalian cells PI5P, $PI3,5P_2$ and $PI3,4,5P_3$ are generated depending on the cell type and specific stimulus, indicating the activation of specific pathways[32-34]. PIPKIIβ and its substrate PI5P have been linked to nuclear stress response pathways[34, 35]. PIPKIIβ signaling within the nucleus putatively connects PI5P and type I $PI4,5P_2$ 4-phosphatase (type I 4-pptase) to p38-mediated stress response signaling[34]. As depicted in Figure 2, in response to oxidative or UV stress, PIPKIIβ is phosphorylated by p38 mitogen-activated protein kinase (MAPK). This phosphorylation inhibits PIPKIIβ, resulting in the accumulation of nuclear PI5P[34]. Upon cellular stress, type I 4-pptase translocates to the nucleus and creates PI5P by dephosphorylation of $PI4,5P_2$, indicating that PIPKIIβ and type I 4-pptase work together to regulate PI5P levels[36, 37]. Increased PI5P causes translocation of ING2, a nuclear PI5P binding protein to a chromatin-enriched fraction[38]. ING2 associates with and modulates the activity of histone acetylases and deacetylases, and induces apoptosis through p53 acetylation[38, 39]. Additionally, it was shown that ING2 regulation of p53 acetylation and apoptosis required both PI5P generation and an intact PI5P binding domain in ING2. The accumulation of nuclear PI5P facilitates the ING2-p53 apoptotic pathway by promoting ING2-dependent p53 acetylation[38] (Figure 2).

Recently, a novel mechanism has been revealed by which PIPKIIβ and PI5P accumulation regulates a nuclear ubiquitin ligase complex[21]. We identified an interaction between PIPKIIβ and SPOP, an adaptor protein that recruits substrates to Cul3-based ubiquitin ligases[21]. Ubiquitin ligases covalently attach ubiquitin to lysine residues on proteins to target for degradation or to modify activity[40]. Bunce and colleagues described a mechanism where type I 4-pptase generates PI5P leading to the stimulation of a p38-MAPK pathway that activates the Cul3-SPOP ubiquitin ligase complex. The Cul3-SPOP ubiquitin ligase complex ubiquitinylates PIPKIIβ and other proteins. PIPKIIβ down-regulates this pathway by converting PI5P to $PI4,5P_2$. The expression of a PIPKIIβ kinase dead mutant stimulated the ubiquitinylation of itself and other Cul3-SPOP targets demonstrating a dominant negative effect and supporting a model where PI5P generation leads to activation of Cul3-SPOP activity[21]. As both PIPKIIβ and type I 4-pptase modulate SPOP activity this may also be a mechanistic connection between PIPKIIβ and insulin signaling. The major phenotype of the PIPKIIβ knockout mouse is enhanced insulin sensitivity[34]. PIPKIIβ and type I 4-pptase modulate the activity of SPOP toward ubiquitinylation of Pdx1[21]. In turn, Pdx1 is a transcription factor that plays key roles in pancreatic beta-cell function and is modulated by oxidative stress[41, 42].

One physiological function for PIPKIIβ appears to be the regulation of PI5P by conversion to $PI4,5P_2$. Consistent with this model, PIPKIIβ and type I 4-pptase work synergistically to generate PI5P which in turn modulates apoptosis[36] and ubiquitinylation by the Cul3-SPOP

complex[21]. Such a mechanism is an intriguing contrast to the type I PIPK signaling pathways in which $PI4,5P_2$, the product of the kinase, is the key regulatory species modulating effector proteins. Although PIPKIIβ may function by removal of PI5P, PIPKIIβ is also positioned to differentially regulate effectors through the generation of $PI4,5P_2$, as shown in Figure 2. Since PIPKIIβ and SPOP are speckle targeted, both PI5P and $PI4,5P_2$ could be in the same compartment positioned to regulate effectors differentially, as shown in Figure 2.

Gene expression and Nuclear Phosphoinositides

In eukaryotes, the generation of messenger RNAs (mRNAs) involves a series of events from transcription to export from the nucleus. Precursor RNAs (pre-mRNAs) are co-transcriptionally capped at the 5′-end, spliced, and processed at the 3′-end before they are exported to the cytoplasm as mature mRNA[43]. Each step in mRNA synthesis is subjected to regulatory events and requires macromolecular complexes consisting of many proteins and enzymes. Nuclear phosphoinositide signaling has been linked to mRNA processing, including splicing, 3′-end processing and export[24, 38, 44-46] (Figure 3). There is emerging evidence that some actively transcribed genes localize to the nuclear periphery (inner nuclear membrane) and nuclear pore complexes, positioning them for modulation by phosphoinositides[47].

RNA processing and phosphoinositides

Nuclear speckles contain a number of components of the cellular pre-mRNA processing machinery including splicing factors, small nuclear ribonucleoproteins (nRNPs), and RNA polymerase II[25]. $PI4,5P_2$ is present at speckles[7] and the depletion of $PI4,5P_2$ from a splicing efficient extract blocked splicing[24]. Reconstitution with exogenous $PI4,5P_2$ failed to restore mRNA splicing to $PI4,5P_2$-depleted fractions[24], suggesting that factors associated with $PI4,5P_2$ that are required for mRNA processing may also have been removed. As a result, the role of $PI4,5P_2$ in pre-mRNA splicing remain ambiguous.

Splicing is coupled to the 3′-end processing of eukaryotic pre-mRNAs48. The 3′-end processing of pre-mRNA is key for gene expression and consists of two steps: cleavage followed by addition of a poly (A) tail43. Cleavage and polyadenylation are ordered processes involving the assembly of a large multimeric 3′-end formation complex, including poly (A) polymerase (PAP)49. The poly(A) tail of eukaryotic mRNA is required for export from the nucleus, stability, and translation43.

A recent link has been established between 3′-end processing and nuclear phosphoinositide signaling[12]. PIPKIα and its product $PI4,5P_2$ localize at nuclear speckles[7]. Based on the hypothesis that $PI4,5P_2$ signaling specificity is dependent on the interaction of PIPKs with PIP effectors[2, 16], a yeast two-hybrid screen was performed to identify PIPKIα interacting proteins that may be PI4,5P effectors[50]. One interacting protein was a poly (A) polymerase that was named Star-PAP (Speckle Targeted PIPKIα Regulated-Poly(A) Polymerase) and this enzyme functions with PIPKIα to regulate pre-mRNA processing[12].

Star-PAP is distinct from other members of the PAP family in domain architecture[12]. Unlike other PAPs, Star-PAP contains a polymerase domain that is split by a proline rich sequence and also has two nucleotide recognition motifs - a zinc finger and an RNA binding domain. Star-PAP and PIPKIα directly interact *in vitro* and *in vivo* and $PI4,5P_2$ stimulated both recombinant and cell purified Star-PAP activity greater than 10-fold[12]. $PI4,5P_2$ stimulated both the initiation (short poly(A) tails) and elongation (longer poly(A) tails) steps in polyadenylation. Thus, $PI4,5P_2$ stimulated Star-PAP's ability to increase the length of the poly (A) tail by enhancing processivity of Star-PAP[12].

Microarray analysis demonstrated that Star-PAP was required for the expression of a subset of mRNAs, many of which encode proteins involved in oxidative stress responses. Star-PAP and PIPKIα function together to control expression of these mRNAs[12]. However, Star-PAP is required for 3′-end cleavage, but PIPKIα is not. Star-PAP assembles into a stable 3′-end processing complex that also contains unique signaling components, such as PIPKIα and the $PI4,5P_2$ sensitive protein kinase CKIα, but lack other PAPs[12, 26]. Functionally, PIPKIα and CKIα are required for the expression of specific Star-PAP target mRNAs[12, 26]. CKIα directly phosphorylates Star-PAP[26], indicating that this kinase controls Star-PAP's ability to process target pre-mRNAs. These data indicate a phosphoinositide pathway that controls expression of specific mRNAs via 3′-end processing.

There is emerging evidence that the localization of genes to the nuclear periphery modulates the transcriptional activity of these genes[47, 51, 52]. Although the nuclear periphery is often associated with gene repression, in yeast some genes activated by cellular stress pathways are localized near the nuclear pore complex [47]. In mammalian cells, it remains unclear if activation of these stress response pathways results in gene expression at the nuclear pore. However, such a system could be extrapolated to a mammalian system if Star-PAP-dependent genes where localized near the nuclear pore resulting in $PI4,5P_2$-stimulated polyadenylation at the envelope. This would in turn position these inducible genes for rapid export of their mRNAs.

Star-PAP activity is specifically regulated by $PI4,5P_2$ binding. Only a few nuclear proteins have been identified that bind nuclear phosphoinositides, including ING2, histone H1, BAF complex, the nuclear export factor Aly, and the nuclear receptors SF-1 and LRH-1[38, 45, 46, 53]. These proteins are regulated by $PI4,5P_2$ indicating that they are $PI4,5P_2$ effectors. For example, $PI4,5P_2$ binds histone H1 and H3 and contributes to chromatin unfolding and transcription[45]. $PI4,5P_2$ binding to histone H1 reversed histone H1-mediated repression of RNA polymerase II transcription *in vitro*[45]. Further, the nuclear receptors SF-1 and LRH-1 require phosphoinositide binding for maximal activity[54]. These are emerging examples of phosphoinositide nuclear effectors, however to date, Star-PAP is the only enzyme identified that is specifically activated by $PI4,5P_2$.

RNA export and phosphoinositides

In eukaryotes, mRNAs are exported from the nucleus to the cytoplasm for translation. Like other nuclear trafficking events, mRNA export is a multi-step process that involves the generation of carrier-cargo complexes in the nucleus, transportation through the nuclear pore complex (NPC), and recycling of the carrier55, 56. In yeast, Mex67, (TAP in the mammalian system) acts as the primary mRNA nuclear export factor55. Mex67 heterodimerizes with Mtr2 and facilitates the export of mature nRNPs through the NPC55.

In addition to acting as a direct lipid messenger, $PI4,5P_2$ serves as a precursor for the generation of higher inositol phosphates. $PI4,5P_2$ is hydrolyzed PI-PLC to generate DAG and IP_3. IP_3 makes the direct precursor for higher inositol polyphosphates (IPs) such as inositol 1,3,4,5-tetrakisphosphophosphate (IP_4), inositol 1,3,4,5,6-pentakisphosphate (IP_5) and inositol 1,2,3,4,5,6- hexakisphosphate (IP_6). In yeast, an *in vivo* role for IP_6 and Gle1, a component of the cytoplasmic filament of the NPC, in mRNA export has been identified[57]. Mutation in PLC displayed defects in mRNA export and IP_6 synthesis demonstrating the requirement for $PI4,5P_2$ cleavage[57]. Similarly, inositol polyphosphate kinase 2 (Ipk2) or inositol polyphosphate kinase 1 (Ipk1) which convert IP_3 to IP_6 also have mRNA export defects[57]. IP_6 was proposed as a positive regulator of Gle1-mediated mRNA export. Recent studies suggested that Gle1 and IP_6 act together to stimulate the ATPase activity of Dbp5, a DEAD-box helicase that binds to Gle1 and remodels nRNP proteins58, 59. Mutations of ARGIII (Ipk2) that lowered the conversion of IP_3 to IP_6 also impaired mRNA export[60]. Thus, higher IP_n messengers are generated from products of $PI4,5P_2$ hydrolysis indicating its central role.

In yeast, the nuclear export factor Yra1 interacts with Mex67 and is required for mRNA export[61, 62]. The Yra1 mammalian isoform, Aly, is regulated by nuclear PI3K signaling and interacts with $PI4,5P_2$ and $PI3,4,5P_3$, which is required for its localization to nuclear speckles[53]. Disruption of the $PI3,4,5P_3$ association with Aly diminished speckle association and mRNA export, making Aly a putative $PI3,4,5P_3$ target in regulating mRNA export[53]. This positions nuclear phosphoinositides for regulation of mRNA export directly through a target export factor or indirectly via hydrolysis of $PI4,5P_2$ and generation of IP_ns.

Nuclear Actin and Phosphoinositides

Actin has been identified as a central component of the nuclear matrix and is present as both G- and F-actin[63-65]. Nuclear actin has been implicated in transcription, chromatin remodeling, mRNA processing, regulation of transcription factors and intranuclear motility[66, 67]. Actin is a key cytoplasmic component of the eukaryotic cytoskeleton and is critical for cell motility, membrane dynamics, cytokinesis, organelle transport, and many other processes. It is not clear how actin gets into the nucleus as is does not have a classical NLS, and specific import receptors have not been reported. It is postulated that actin binds to nuclear actin binding proteins such as cofilin, CapG, and MAL, which contain NLS motifs, and that these proteins piggy-back actin into the nucleus[66].

Phosphoinositides are key regulators of actin dynamics in the cytoplasm[68]. $PI4,5P_2$ modulates the activity of many regulatory proteins that control actin polymerization and association with other proteins. For example, PIP_2 activates N-WASP-Arp2/3 complex-induced actin filament nucleation and inhibits the actin-binding activity of cofilin[66, 67]. $PI4,5P_2$ also mediates uncapping of actin where $PI4,5P_2$ induces capping proteins, such as those in the gelsolin family and CapZ, to disassociate from the actin filaments[66, 67]. Considering that $PI4,5P_2$ is a major regulator of actin in the cytoplasm, one could speculate the nuclear actin binding proteins would be prime $PI4,5P_2$ targets in the nucleus[66, 67].

The regulation of nuclear actin has been linked with PI signaling. The nuclear equivalent of the cytoskeleton, i.e. the nuclear matrix, and nuclear cytoskeletal proteins bind to $PI4,5P_2$[66, 67]. Profilin I, a regulatory component of actin organization in the nucleus, is required for efficient mRNA synthesis and is regulated by $PI4,5P_2$[66, 67]. Profilin I localizes to nuclear speckles along with $PI4,5P_2$ and has also been implicated in pre-mRNA splicing[69]. The Arp2/3 complex is regulated by $PI4,5P_2$ in the cytoplasm and localizes to the nucleus, where it appears to directly interact with RNA polymerase II and participate in transcription[70]. In addition, myosin I regulates transcription by RNA polymerase I and II[71]. Most recently, the p53-cofactor JMY was discovered to be a multifunctional actin nucleation factor[72]. A theme among these processes is that many nuclear actin-binding proteins are regulated by $PI4,5P_2$ or indirectly by PI signaling.

Chromatin remodeling complexes, such as the BAF (Brahma related gene association factor) and INO80 complexes contain β-actin as an integral component[73]. $PI4,5P_2$ modulates chromatin remodeling possibly through regulation of the $PI4,5P_2$ actin binding site on the chromatin remodeling protein BRG1[74], suggesting that one function of $PI4,5P_2$ could be to stabilize these complexes within the nuclear matrix and contribute to gene expression[75]. In resting T-lymphocytes, the chromatin remodeling complex BAF is primarily soluble. Upon stimulation, the BAF complex translocates to an insoluble fraction by association of the complex with chromatin[46]. Association with chromatin is mediated by $PI4,5P_2$ levels in T lymphocytes[46]. Association of the BAF complex with the nuclear matrix requires BRG1 (a SWI/SNF2-like ATPase core subunit), β-actin and BAF53, an actin-related protein[76, 77]. BRG1, has two actin binding domains one of which contains a lysine-rich region that is required for function and can bind $PI4,5P_2$[74, 77]. It was proposed that the actin monomer is bound to

both domains and $PI4,5P_2$ association disrupts actin binding to one domain, allowing a previously occluded site of actin to interact with components of the nuclear matrix[74]. This mechanism is analogous to $PI4,5P_2$-mediated uncapping of actin during actin polymerization[78]. $PI4,5P_2$ binding to BRG1 may facilitate recruitment to chromatin and stabilize the chromatin remodeling complex by an increased interaction with matrix. Alternatively, $PI4,5P_2$ could stimulate the interaction of BRG1 with other BAF components, or by engaging actin as a bridge for the remodeling complex to the chromatin through its increased interaction with nuclear matrix. This could lead to the stable association of the remodeling complex with an active promoter on a condensed chromatin.

The initial identification of chromatin associated lipid molecules was by Rose and colleagues in 1965[79]. Since then various studies have suggested the regulation of chromatin remodeling by IP_ns that are derived from IP_3[80]. Ipk2 phosphorylates IP_3 to generate IP_4 and IP_5[57, 81] and strikingly Ipk2 acts as a transcriptional regulator known as Arg82[81]. In an *arg82* deficient yeast strain, chromatin remodeling at PHO5, a phosphate responsive promoter, is impaired. In a complementary study, various ATP dependent chromatin remodeling complexes including NURF, ISWI, SWI-SNF, and INO80 were shown to be sensitive to various IP_ns[82, 83]. In addition, the SWI-SNF and INO80 chromatin remodeling factors were not recruited to the phosphate responsive promoters suggesting the role of IP_ns in chromatin organization[84]. Actin is required for efficient DNA binding, ATPase activity, and nucleosome mobilization in the INO80 complex, as INO80 complexes lacking actin were deficient in these activities[77]. It appears that a major function of actin is to allosterically regulate remodeling of the chromatin remodeling assemblies that are sensitive to IP_ns[82]. The presence of actin in the chromatin remodeling complexes and the regulation of chromatin remodeling by IP_ns supports a triangular relation between IP_ns, chromatin and nuclear actin[82].

The Compartment Conundrum

Nuclear phosphoinositides and their synthetic enzymes regulate nuclear processes, but the mechanisms and the organization of phosphoinositides in the nucleus is poorly understood (Table 1). $PI4,5P_2$ has long been considered a membrane anchored precursor of soluble inositol phosphates (IP_3 and higher IP_ns)[85]. However, the retention of $PI4,5P_2$ in detergent stripped nuclei[86] and the evidence that phosphoinositides and their synthetic enzymes are localized at speckles and other sub-nuclear sites lacking defined membranes insinuates a unique compartment[7, 12, 24]. If nuclear phosphoinositides are not in a membrane, there must be a mechanism to shield the hydrophobic acyl chains from solvent. The amphipathic structure of $PI4,5P_2$ is suited for membrane anchorage, but renders it energetically and thermodynamically unfavorable to freely move within the nucleus. The differential localization of $PI4,5P_2$ within nuclei suggests that there are different pools and these could be used to regulate diverse nuclear functions. The data suggest that there are at least two pools of phosphoinositides: a nuclear envelope pool and another within the nucleus that is separate from known membrane (Figure 4).

A fraction of nuclear PIP_2 is present in the inner envelope and it is reasonable to assume that a component of $PI4,5P_2$ hydrolysis by nuclear PLCs would also occur within the nuclear envelope generating DAG and IP_3[29]. Echevarria and colleagues demonstrated that the inner face of the nuclear envelope and invaginations of the envelope contain IP_3 receptors that release Ca^{2+} from the endoplasmic reticulum and nuclear envelope[87]. This location is consistent with the release of Ca^{2+} into the nucleus via the IP_3 receptor channel[4, 5, 30, 87]. At the inner nuclear envelope the generation of DAG could activate PKC and PI3K may generate 3-phosphorylated phosphoinositides.

PI4,5P_2 and phosphoinositide generating enzymes that are present at nuclear speckles are separate from known membrane structures[2, 7-9, 37]. The motifs of PI4,5P_2 binding proteins contain charged residues that the head group of inositol lipids interact with[69]. This would leave the hydrophobic tails free; however it seems unlikely that the acyl chains would be solvent exposed. There are other lipids in the nucleus such as phosphatidylcholine[88] and it is possible that the phosphoinositides and other lipids form a mixed micelle structure, thus eluding the unfavorable constraints of free acyl chains. Such structures would have to be resistant to detergents and would not be detectable by current electron microscopy approaches.

Another possibility is that the phosphoinositides are associated with carrier proteins in the nucleus that contain phosphoinositide acyl chain binding pockets. Such proteins would integrate the hydrophobic acyl chain in the binding cleft exposing only the charged inositol head group. These proteins could move freely in the nucleus to deliver PI specifically to the effector protein complex. It has been previously suggested that phosphoinositide transfer proteins (PITPs) are involved in nuclear import of PI in mammalian cells[89-91] and hypothetical carrier proteins may function similar to the PITPs (Figure 4). Solution of the crystal structure for the yeast PITP Sec14p revealed a large hydrophobic pocket into which PI is inserted[92]. The incorporation of phosphoinositides into individual binding proteins may modulate folding or structure and the bound phosphoinositide could be modified by kinases, phosphatases or phospholipases leading to changes in binding protein activity or localization. The existence of such a system will provide solutions to both the energetic constraints and the functioning of PI4,5P_2 at non-membranous sites in the nucleus. A similar presentation has been considered for the soluble inositides, in which IP_6 bound to Gle1 was proposed to act as co-effector for Dbp5 in a Dbp5:Gle1 complex during RNA export[56, 59]. Additionally, a PIP_n carrier protein could itself be a PIP_n effector. Such a model would be similar to the nuclear receptors SF-1 and LRH-1 that integrate phosphoinositides into a hydrophobic pocket where the lipid is required for activity[54]. This model is attractive, as the bound PIP_n could be phosphorylated or hydrolyzed which could control functional changes in the effector protein (Figure 4). Such a paradigm could be important for proteins that perform multiple functions in gene expression.

Future perspectives and concluding remarks

It is evident from current literature that nuclear phosphoinositides and in particular PI4,5P_2 regulate aspects of gene expression and other functions. Despite increasing information from chromatin remodeling, mRNA splicing, and recently mRNA 3′-end processing, there remain many questions. Additional PIP_n effectors, like Star-PAP, will be key to unraveling the intricate mechanisms involved in the regulation of the nuclear PI cycle. Identification of the PI-sensitive components of nuclear signaling pathways will reveal insights of phosphoinositide function in the nucleus. The identification of putative PIP_n carrier/effector proteins will be central for understanding how phosphoinositides regulate nuclear events.

Phosphoinositides are components of the nuclear interior but the environment of nuclear phosphoinositides remains ambiguous. The localization of nuclear phosphoinositides has been mapped by a variety of techniques. From these approaches, phosphoinositides and their metabolizing enzymes have been found in the nuclear envelope. However, many studies have localized nuclear phosphoinositides and the enzymes that synthesize or metabolize phosphoinositides at nuclear speckles, cajal bodies, nucleoli, nuclear matrix, and chromatin[8, 9]. The current evidence supports a model where nuclear phosphoinositide signaling is compartmentalized into either the nuclear envelope or a unique subnuclear protein-lipid compartment(s). A clear objective for the field is to characterize how the distinct pools of phosphoinositides and PI metabolizing enzymes are maintained in separate compartments in the nucleus and define the function of the lipid messengers in these compartments.

Box 1

Historical Perspective

The phosphatidylinositol cycle was discovered in the 1950s by Lowell and Mabel Hokin[1]. Soon after, they discovered that PI could be phosphorylated sequentially on its myoinositol ring to generate $PI4,5P_2$[93]. While $PI4,5P_2$ was originally thought of only as a metabolic precursor of soluble inositides, $PI4,5P_2$ is now recognized as a potent second messenger that has been implicated in a diverse array of cellular processes[2, 8, 50, 78, 94, 95]. Over the last decades, evidence has accumulated indicating that there is a distinct nuclear PI cycle[4, 6, 96].

The presence of lipids within the nucleus and in the nuclear membrane was described in the late 1960s79. During the 1970s and early 1980s, Manzoli and co-workers started to define the various lipid components within the nucleus and link them to nuclear processes97¯99. It was first proposed by Smith and Wells (1983) that nuclear phosphoinositide generation occurred at the nuclear membrane[28]. They described the activities of DAG kinase, PI kinase and PIP kinase in isolated rat nuclear envelope. Cocco and colleagues showed the direct evidence of a nuclear phosphoinositide cycle in mouse erythroleukemia (MEL) cell nuclei stripped of their nuclear membrane by detergent. They reported that the detergent stripped nuclei maintained the ability to synthesize phosphoinositides and in fact, retained significant amounts of DAG, PI4P and $PI4,5P_2$, as well as PI 4-kinase, and PI4P 5-kinase activities[86]. Additionally, it was determined that when MEL cells were induced to differentiate, the levels of non-membranous nuclear $PI4,5P_2$ increased, while total cellular $PI4,5P_2$ levels remained unchanged[86] The existence of an autonomous nuclear PI cycle was also evident from further studies in 3T3 human fibroblast cells using differential cellular stimuli, bombesin and insulin-like growth factor-1 (IGF-1)[3, 100]. Stimulation by IGF-1 has been shown to cause a rapid increase in the mass of nuclear DAG along with a corresponding decrease in nuclear $PI4,5P_2$, whereas bombesin was only able to affect DAG mass at the plasma membrane[3].

Phosphatidylinositol-phosphate (PIP) kinase activity was identified by the Hokins in the early 1960's101, however, characterization of the PIP kinases was not pursued until nearly three decades later when the PIP kinases were successfully purified from erythrocytes102¯104. PIP kinases are classified as type I, II, or III PIPK (PIPKI, PIPKII, PIPKIII) based on their biochemical properties, substrate specificity and sequence7. To date, PIPKIα, PIPKIγ_i4,

PIPKIIβ, and possibly PIPKIIα are found in the nucleus, in association with the nuclear matrix[7, 18, 19].

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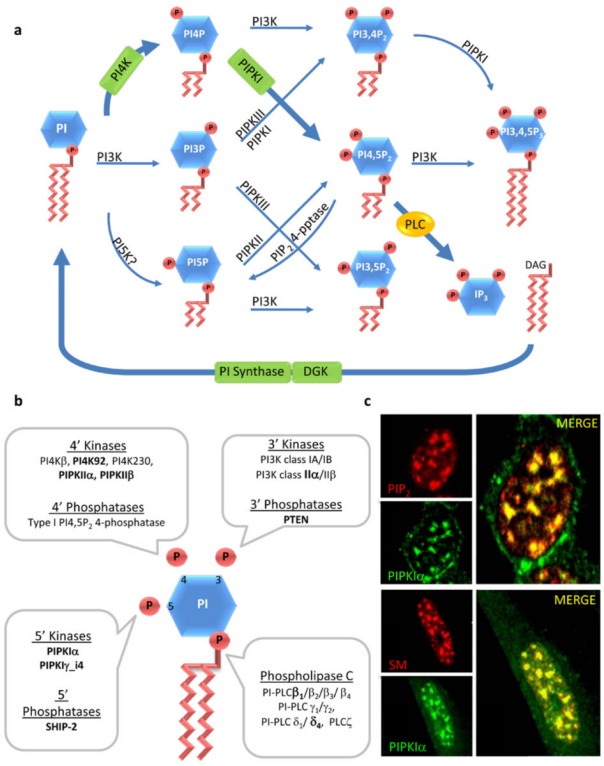

Figure 1.

Phosphoinositide kinases, phosphatases, and phospholipases. **A.** Canonical PI cycle. Inositol phospholipids are named according to the number and position of phosphate groups on the inositol headgroup. The singly phosphorylated PIPs (PI3P, PI4P and PI5P) are created by the phosphorylation of PI at the 3, 4 and possibly 5 positions. PI5P can also be generated by the type I $PI4,5P_2$ 4-phosphatase (type I 4-pptase). Theses PIPs act as intermediates for the synthesis of inositol bis- and tris-phosphates (PIP_2 and PIP_3). PIP_2 is generated by phosphorylation of PI3P, PI4P or PI5P by the indicated PIP kinases and $PI4,5P_2$ is hydrolyzed by PLC to form IP_3 and DAG. PIP_3 is generated by phosphorylation of $PI4,5P_2$ by PI3K. The classical PI cycle is highlighted with bold arrows and enzymes. **B**. A graphic representation of PI and the kinases, phosphatases, and phospholipases that to date have been shown to be localized in the nucleus according to the position the on inositol head group where they act. Nuclear speckle targeted enzymes are indicated in bold. **C.** PIPKIα colocalizes with components of the mRNA-processing machinery in nuclear speckles and PIP_2. Top panel: Cells were double labeled with an anti-PIPKIα polyclonal antibody and anti-PIP_2 monoclonal antibody. Bottom panel: Cells were double-labeled with an anti-PIPKIα polyclonal antibody and human Sm antiserum (a nuclear speckle marker).

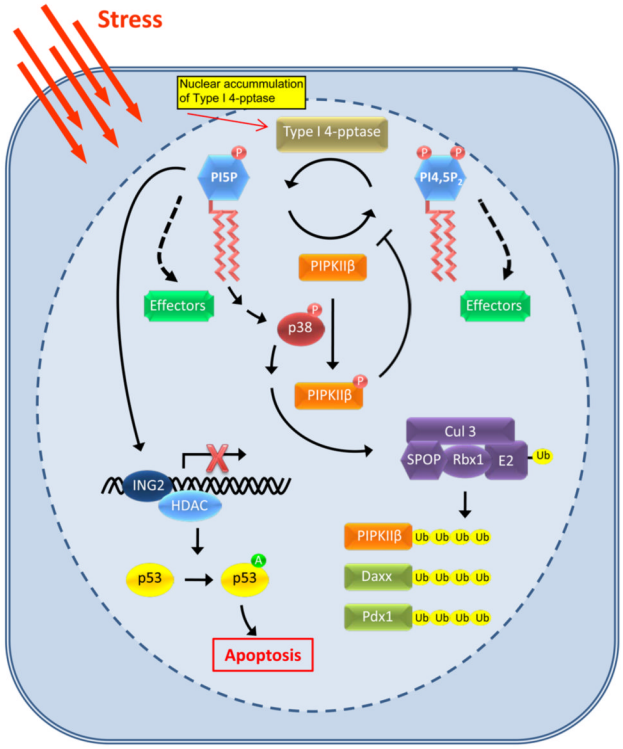

Figure 2.

A nuclear phosphoinositide-mediated stress response pathway. Under resting conditions, PIPKIIβ controls PI5P levels by its synthesis of $PI4,5P_2$. Upon cellular stress, PIPKIIβ activity is attenuated via type 1 $PI4,5P_2$ 4-phosphatase and p38 MAPK activity resulting in the accumulation of PI5P. Specifically, in response to cellular stress, such as oxidative stress or UV irradiation, type 1 $PI4,5P_2$ 4-phosphatase translocates to the nucleus where it hydrolyzes $PI4,5P_2$ into PI5P. Concurrently, PIPKIIβ is phosphorylated by activated p38 MAPK, inhibiting its lipid kinase activity and resulting in increased nuclear levels of PI5P. The accumulation of PI5P recruits ING2 to chromatin and promotes ING2-dependent p53 acetylation. Acetylation of p53 enhances its activity and stability and therefore increases apoptotic death. PI5P also may modulate an upstream activator of p38 MAPK, resulting in the activation of the Cul3-SPOP ubiquitin ligase complex toward multiple substrates, including PIPKIIβ. Represented are the defined functions of PI5P and $PI4,5P_2$; however, both PI5P and $PI4,5P_2$ may bind as of yet unidentified effectors (green boxes), which could play diverse roles in nuclear signaling. Ub = ubiquitin; A = acetylation

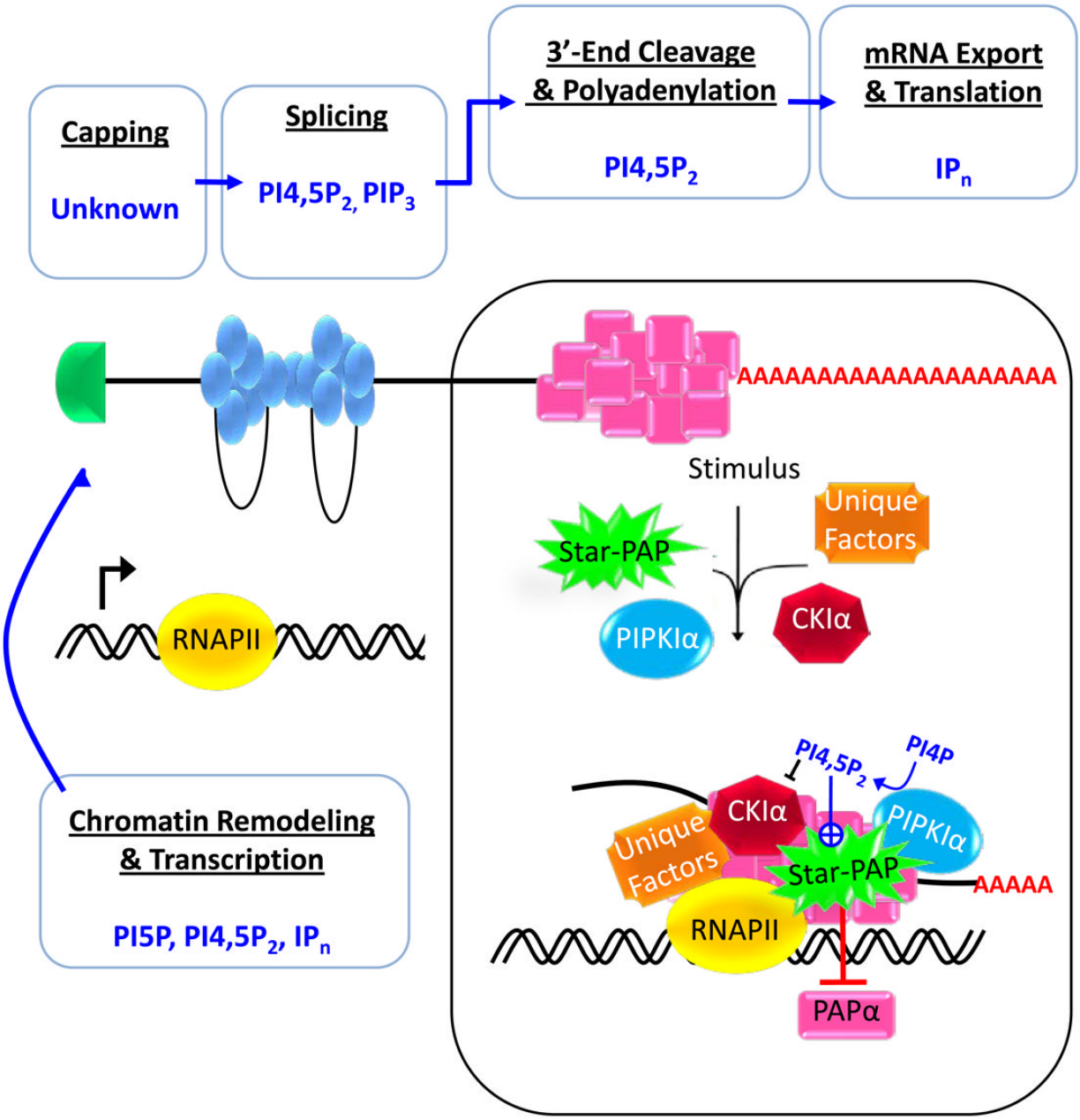

Figure 3.

Phosphoinositides in eukaryotic mRNA transcription and processing. A diagram depicting the events of mRNA generation in eukaryotes, including chromatin remodeling and transcription, mRNA processing (5′-end capping, splicing, and cleavage and polyadenylation), and mRNA export and translation, and the PIP_n or IP_n molecules that have been implicated in each step. Phosphoinositides have been implicated in most aspects of mRNA synthesis except for 5' capping. Phosphoinositide species and the process regulated are indicated.

This schematic also highlights the processing of mRNA at the 3′-end modulated by Star-PAP, the only nuclear effector identified to date that is directly activated by $PI4,5P_2$. Star-PAP is a nuclear poly(A) polymerase that is required for the expression of select mRNAs. Star-PAP

assembles into a complex with RNA polymerase II (RNAPII) and known 3′-processing factors, but the complex is notably devoid of canonical PAPα. The Star-PAP complex contains unique components, such as PIPKIα and the $PI4,5P_2$ sensitive protein kinase CKIα. Star-PAP is necessary for the 3′-processing of its target mRNAs and functionally both PIPKIα and CKIα are required for the maturation of a subset of Star-PAP target mRNAs.

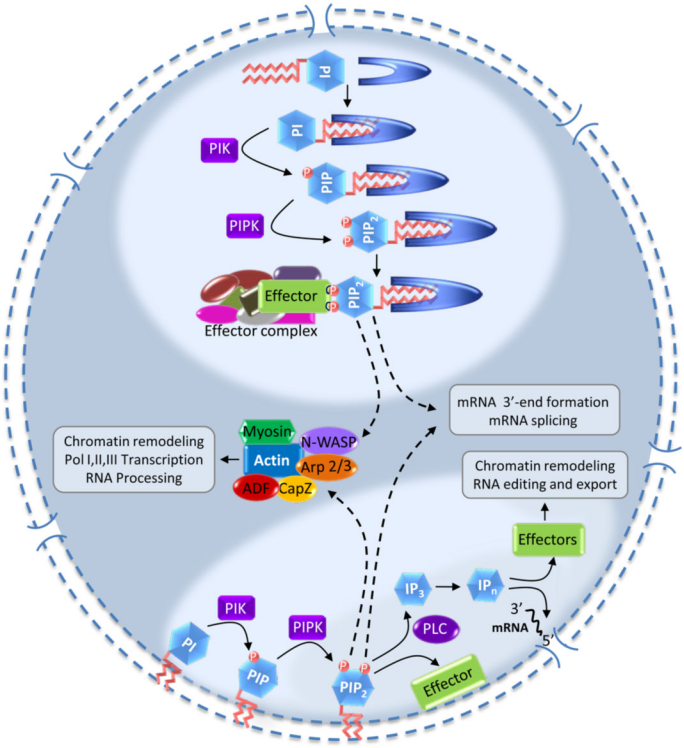

Figure 4.

A model illustrating the compartmentalization of phosphoinositide signaling in the nucleus. Current data suggests two compartments for the nuclear phosphoinositide cycle: One associated with the nuclear envelope and another in a subnuclear compartment separate from known membrane structures. In both compartments, PI is sequentially phosphorylated by PI kinases (PIK) and PIP kinases (PIPK) to generate PIP_2, which could then be metabolized by PLC to generate IP_3 and then higher inositol phosphates (IP_n) or phosphorylated by PI 3-kinase creating PIP_3. In subnuclear compartments, phosphoinositides are hypothesized to be associated with carrier or effector proteins. Such proteins could be specific for certain functions and/or could present phosphoinositides to other effectors. In addition, regulation of nuclear actin polymerization and actin binding proteins, such as N-WASP, CapZ and ADF (actin/cofilin depolymerising factor), either from envelope-bound or endonuclear phosphoinositides (shown by dashed arrows) has been shown to affect many aspects of gene expression.

Table 1

Nuclear targeted phosphoinositides: Location, Effectors, and Proposed Function.

Inositol molecules	PI Enzymes	Nuclear Location	Effectors	Functions	References
PI3P	PI3-kinase	Nucleolus Nuclear Matrix	Unknown	Cell cycle regulation	10, 105
PI4P	PI4-kinase	Nuclear Matrix	Unknown	Cell cycle, Precursor of PI4,5P2	11
PI5P	PI5-kinase	Nuclear Matrix Chromatin	ING2 Others?	Chromatin organization, Apoptosis, DNA damage	38
PI3,4P_2	SHIP-2, PIP Kinase IIβ	Membrane Nuclear Speckle	Unknown	Pre-mRNA splicing	14, 106
PI4,5P_2	PIP Kinase Iα, PIP Kinase IIβ	Membrane Nuclear Speckle Nuclear Matrix Chromatin	Star-PAP, ING2, Aly, BRG1	3′-end processing, Splicing, Chromatin organization, precursor for IP3	12, 24
PI3,4,5P_3	PIP Kinase I, PIP3 Kinase	Nuclear Matrix	PIP3BP Others?	Cell cycle, Differentiation, proliferation	107, 108
DAG	PI-PLC	Nuclear Matrix	Unknown	Cell cycle, Differentiation, Proliferation	13
Ins1,4,5P_3	PI-PLC,	Nuclear Matrix	Unknown	Calcium signaling	87
IP_n (IP_3, IP_4, IP_5, IP_6)	IP_n kinases	Nuclear Matrix Chromatin	Unknown	mRNA export, Chromatin structure	84, 109