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Inositol polyphosphate multikinase: metabolic architect of nuclear inositides

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1. ABSTRACT

The inositides are key cellular second messengers with well established roles in signal transduction pathways initiated by cell surface receptor activation. The recent identification of an evolutionarily conserved nuclear signaling pathway for higher inositol polyphosphates has defined a new signaling paradigm for this diverse class of molecules whose biosynthesis and regulation is mediated by what are likely some of the earliest ancestors of the inositide kinase families. Inositol polyphosphate multikinase (IPMK) represents the most catalytically diverse member of this family with critical roles in nuclear functions including mRNA export, transcriptional regulation, and chromatin remodeling.

2. INTRODUCTION

The inositides define a large family of lipid and water-soluble metabolic and messenger molecules with roles in diverse cellular processes (1-3). Inositide biosynthesis occurs through the modification of the sugar-like, six-carbon ring of *myo*-inositol (Figure 1). Combinatorial phosphorylation of inositides results in more than 30 different inositol phosphates and seven phosphoinositides—lipid inositols, also termed phosphatidylinositol (PtdIns or PI) phosphates, comprised of diacylglycerol (DAG) and inositol phosphates. Signaling roles for inositides were first suggested by studies of receptor-stimulated phosphoinositide metabolism (4), but the first definitive assignment of physiologic

functions for inositides came with the identification of the now well known role of inositol 1,4,5-trisphosphate ($Ins(1,4,5)P_3$) as the calcium releasing factor generated upon cell surface receptor activation (5).

In what has become a classic formulation of receptor-mediated cell signaling, ligand activation of receptor tyrosine kinases or G-protein coupled receptors results in the cellular accumulation of $Ins(1,4,5)P_3$ and DAG via hydrolysis of the membrane bound lipid inositol, $PtdIns(4,5)P_2$, by phosphoinositide-specific phospholipase C (PLC) (6). DAG goes on to activate protein kinase C (PKC) (7) while $Ins(1,4,5)P_3$ binds the $Ins(1,4,5)P_3$-receptor/channel and allosterically mediates the release of Ca^{2+} from intracellular pools within the endoplasmic reticulum (5, 8, 9). Within a short span of years following the characterization of the PLC-initiated "inositol cycle" and second-messenger roles for $Ins(1,4,5)P_3$, inositide metabolism experienced a dramatic expansion initiated by the identification of more phosphorylated D3 derivatives of lipid and water-soluble inositol phosphates (10-13).

Inositol polyphosphates' entrance into the limelight of cell signaling in the mid to late 1980's with the discovery of $Ins(1,4,5)P_3$ and its second messenger role in calcium mobilization was followed by a decade or more of research dominated by the phosphoinositide lipids. To a large degree this was the result of the identification of $PtdIns(3,4,5)P_3$ as a constituent of phosphoinositides extracted from activated neutrophils (12) and the isolation of a phophoinositide-3 kinase (PI3K) activity associated with viral Src and the middle-T antigen of the polyoma virus (13)—discoveries that recast the lipid inositols as signaling molecules in their own right independent of their precursor roles for $Ins(1,4,5)P_3$. This was followed by the characterization of important signaling roles for PI3K in cell migration, growth, proliferation, and survival as well as the identification of lipid inositol receptors and binding domains (14). During this period, while additional water-soluble inositols were identified, the functional significance of "higher" inositol metabolism, the delineation of inositol polyphosphate receptors, and/or the identification of enzymes involved in their metabolism lagged behind. However, the recent cloning and characterization of the kinases responsible for higher inositol polyphosphate synthesis has shed new light on the diverse and complex water-soluble domain of the inositides. In particular, the identification of an evolutionarily conserved, ancient inositol polyphosphate kinase family and its roles in nuclear inositide metabolism has served to expand our understanding of the biological significance of the inositides beyond classical conceptions of their plasma membrane and cytoplasmic signaling roles. In this review, we shall limit our focus to the most catalytically flexible member of this family and its roles in nuclear functions, the inositol polyphosphate multikinase (IPMK).

3. DISCOVERY AND BIOCHEMICAL CHARACTERIZATION

The discovery of D3-hydroxyl phosphorylation of $Ins(1,4,5)P_3$ was followed by the identification of the ubiquitous prevalence of additional more phosphorylated—and in fact the most abundant—eukaryotic inositol polyphosphates, $InsP_5$ and $InsP_6$ (15). Full combinatorial phosphorylation of the inositol ring suggested an upper limit of 63 discrete inositol phosphates with the fully phosphorylated $InsP_6$ as the metabolic endpoint of sequential phosphorylation of $Ins(1,4,5)P_3$. However, the identification of pyrophosphate (diphosphate) inositol species with seven and eight phosphates revealed an expanded potential for the inositol ring (16, 17). The diphosphoinositol pentakisphosphate ($InsP_7$, $PP\text{-}InsP_5$) and bis-diphosphoinositol tetrakisphosphate ($InsP_8$, $PP_2\text{-}InsP_4$) species contain high energy beta-phosphates that exhibit rapid cellular turnover suggesting unique signaling functionality for this class of molecules. Indeed, $InsP_7$ can non-enzymatically transfer its high energy phosphate to proteins in a novel mechanism of protein phosphorylation (18). Even more intriguing, the posttranslational modification appears to be pyrophosphorylation, differentiating it from canonical ATP-dependent protein phosphorylation (R. Bhandari and S.H Snyder, personal communication). The identification and cloning of the $InsP_6$ kinases (IP6Ks) responsible for inositol pyrophosphate synthesis by Snyder and associates revealed them to be conserved from yeast to man (19). Furthermore, sequence database comparisons demonstrated that the IP6Ks, though capable of phosphate-hydroxyl pyrophosphorylation, were in fact part of a larger inositol polyphosphate kinase family defined by a consensus inositol binding motif (P-X-X-X-D-X-K-X-G) (19). Remarkably, the other family members identified included the Ca^{2+}-sensitive $Ins(1,4,5)P_3$ 3-kinases first identified to mediate D3-hydroxyl phosphorylation of $Ins(1,4,5)P_3$ and Arg82 (also known as ArgRIII), a predominantly nuclear, *Saccharomyces cerevisiae* yeast protein previously characterized as an arginine-sensitive transcription factor (20, 21).

Incubation of $InsP_3$ with purified Arg82 revealed it to be an inositol kinase with the ability to generate a series of higher inositol polyphosphates (Figure 1, Table 1). Unlike the $Ins(1,4,5)P_3$ 3-kinases which are limited to the production of $Ins(1,3,4,5)P_4$ (22, 23), Arg82 displays a dual 3-/6-kinase activity that sequentially phosphorylates $Ins(1,4,5)P_3$ to $InsP_4$ to $Ins(1,3,4,5,6)P_5$ (19, 24). The $InsP_4$ formed by Arg82 can be one of two isomers, either $Ins(1,3,4,5)P_4$ or $Ins(1,4,5,6)P_4$, though *in vivo* D6 phosphorylation likely occurs first. Further incubation of $Ins(1,3,4,5,6)P_5$ with Arg82 results in pyrophosphorylation of the molecule and the formation of PP-InsP4 species (25, 26). Noting these multiple activities the Snyder group descriptively redesignated Arg82 as inositol polyphosphate multikinase (IPMK) (19).

IPMK activity was likely first observed in pea extracts (27) and subsequently in budding yeast extracts as an $InsP_3$ D6-kinase activity (28) and as D6- and D3-kinase activities in fission yeast which along with a separable D2-kinase activity constituted a pathway from $Ins(1,4,5)P_3$ to $InsP_6$ (29). *In vivo* confirmation of IPMK's sequential phosphorylation of $Ins(1,4,5)P_3$ to $Ins(1,3,4,5,6)P_5$ came nearly simultaneously to its characterization by Snyder and

Table 1. Characterized activities of IPMK

Species	Name	Enzymatic reactions	Ref
Yeast	IPMK (ARGRIII)	$Ins(1,4,5)P_3 \rightarrow Ins(1,3,4,5)P_4 \rightarrow InsP_5$	19
Yeast	IPMK	$Ins(1,4,5)P_3 \rightarrow Ins(1,3,4,5)P_4$ + $Ins(1,4,5,6)P4 \rightarrow Ins(1,3,4,5,6)P_5$	31
Yeast	IPK2 (ARG82)	$Ins(1,4,5)P_3 \rightarrow Ins(1,4,5,6)P_4 \rightarrow Ins(1,3,4,5,6)P_5$	24
Rat	IPMK	$Ins(4,5)P_2 \rightarrow Ins(1,4,5)P_3 \rightarrow Ins(1,3,4,5)P_4 \rightarrow Ins(1,3,4,5,6)P_5 \rightarrow PP\text{-}InsP_4$	25
Yeast	ARG82	$Ins(1,3,4,5)P_4 \rightarrow Ins(1,3,4,5,6)P_5 \rightarrow PP\text{-}InsP_4$	26
Human	IPMK	$Ins(1,4,5)P_3 \rightarrow Ins(1,3,4,5)P_4$ + $Ins(1,4,5,6)P_4 \rightarrow Ins(1,3,4,5,6)P_5$	37
Arabidopsis	AtIPK2a AtIPK2b	$Ins(1,4,5)P_3 \rightarrow Ins(1,3,4,5)P_4$ + $Ins(1,4,5,6)P_4 \rightarrow Ins(1,3,4,5,6)P5$ $Ins(1,3,4,6)P_4 \rightarrow Ins(1,3,4,5,6)P_5$ $Ins(1,2,3,4,6)P_5 \rightarrow InsP_6$	33
Human	$InsP_4$ 5-kinase	Metabolize $Ins(1,4,5)P_3$, $Ins(1,3,4,5)P_4$, $Ins(1,4,5,6)P_4$ $Ins(1,3,4,6)P_4 \rightarrow Ins(1,3,4,5,6)P_5$	35
Arabidopsis	AtIPK2b	$Ins(1,4,5)P_3 \rightarrow Ins(1,3,4,5)P_4$ + $Ins(1,4,5,6)P_4 \rightarrow Ins(1,3,4,5,6)P_5$	36
Drosophila (Fly)	IPK2	$Ins(1,4,5)P_3 \rightarrow Ins(1,4,5,6)P_4$ + $Ins(1,3,4,5)P4 \rightarrow Ins(1,3,4,5,6)P_5$	34
Rat Yeast	IPMK	$PtdIns(4,5)P_2 \rightarrow PtdIns(3,4,5)P_3$	47
Solanum (Potato)	IPMK	Metabolize $Ins(1,4)P_2$, $Ins(1,4,5)P_3$, $Ins([1/3],4,6)P_3$, $Ins(1,3,4,6)P_4$, $Ins(1,3,4,5)P_4$, $Ins(3,4,5,6)P_4$, $Ins(1,3,4,5,6)P_5$, $InsP_6$	32

The multiple kinase activities ascribed to IPMK across the evolutionary spectrum. Selected references are listed for each description. Ref: references

associates with independent investigations in the budding yeast from York and colleagues (30). A yeast genetic screen aimed at isolating genes contributing to the nuclear export of mRNA identified the requirement of three distinct inositol metabolizing gene products in the cellular production of $InsP_6$ (30). Yeast, like animal cells, initiate inositol polyphosphate metabolism via the hydrolysis of $PtdIns(4,5)P_2$ by the sole yeast isoform of PLC (Plc1). While wild-type yeast display sequential phosphorylation of $Ins(1,4,5)P_3$ to $InsP_6$, mutation of a locus termed *GSL3* resulted in the loss of $Ins(1,4,5)P_3$-initiated higher inositol polyphosphate metabolism. Along with Plc1 and Gsl3, an $Ins(1,3,4,5,6)P_5$ 2-kinase termed Ipk1 was also identified in the screen suggesting a conservative pathway of three gene products was required for $InsP_6$ production in yeast (30). Subsequent studies identified IPMK as *GSL3* (designated Ipk2 by the York group) and confirmed its requirement for mRNA export (24, 31).

Following its identification in yeast, additional IPMK orthologs were characterized in various eukaryotes confirming and expanding on the enzyme's catalytic flexibility (25, 32-37) (Figure 1, Table 1). Like the yeast enzyme, mammalian, plant, and fly orthologs share the capacity for the conversion of $Ins(1,4,5)P_3$ to $Ins(1,3,4,5,6)P_5$. However, while plant and fly IPMKs recapitulate the yeast enzyme's preference for D6-hydroxyl phosphorylation followed by D3-hydroxyl phosphorylation (33, 34), mammalian IPMKs appear to prefer the reverse order (25, 37). The evolutionary conservation of IPMK's $InsP_3$/$InsP_4$ dual-specificity 6-/3-kinase activities suggests the sequential metabolism of $Ins(1,4,5)P_3$ to $InsP_6$ first observed in yeast is likely retained across species. Indeed, the rat, plant, and fly enzymes can rescue the inositol metabolism defects observed in *ipmk* mutant yeast confirming the *in vivo* relevance of these activities (33, 34, 36, 38, 39). The 3-step pathway to $InsP_6$ mediated by Plc1, Ipmk, and Ipk1 contrasts with alternative descriptions of a 5-step pathway proposed to occur in human cells. This latter metabolic pathway can be summarized as follows: $PtdIns(4,5)P_2 \rightarrow Ins(1,4,5)P_3 \rightarrow Ins(1,3,4,5)P_4 \rightarrow Ins(1,3,4)P_3 \rightarrow Ins(1,3,4,6)P_4 \rightarrow Ins(1,3,4,5,6)P_5 \rightarrow InsP_6$ (40-42). The 5-step pathway is suggested to depend on the activities of the $Ins(1,4,5)P_3$ 3-kinases in lieu of IPMK for the initiation of higher inositol metabolism as well as the sequential activities of an $Ins(1,3,4,5)P_4$ 5-phosphatase and $Ins(1,3,4)P_3$ 5-/6-kinase absent in yeast (41). Using kinetic studies Majerus and colleagues suggested human IPMK functions as an $Ins(1,3,4,6)P_4$ 5-kinase rather than as an $InsP_3$/$InsP_4$ dual-specificity 6-/3-kinase (35, 41). Their failure to demonstrate complementation of inositol polyphosphate metabolism using human IPMK in *ipmk* mutant yeast supported this hypothesis. IPMK D5-kinase activities using $Ins(1,3,4,6)P_4$ and $Ins(1,2,3,4,6)P_5$ as substrates has also been identified in *Arabidopsis,* though plant genomes do not contain $Ins(1,4,5)P_3$ 3-kinases (33). However, recent studies in the fly, rat cells, and mice suggest that $Ins(1,4,5)P_3$ 3-kinases likely contribute little to the synthesis of $InsP_5$ and $InsP_6$ (34, 38, 43-45). Indeed mouse fibroblasts devoid of all three mammalian isoforms of $Ins(1,4,5)P_3$ 3-kinase still produce $InsP_5$ and $InsP_6$ suggesting the sufficiency of the 3-step pathway to $InsP_6$ mediated by PLC, IPMK, and IPK1 (46). Perhaps alternative metabolic pathways for higher inositol metabolism in mammals dependent on $Ins(1,4,5)P_3$ 3-kinases and the $Ins(1,3,4)P_3$ 5-/6-kinases and/or IPMK's D5-kinase activity are the result of the evolutionary emergence of separate metabolic pools of $Ins(1,4,5)P_3$ and reflect cytoplasmic versus nuclear signaling as like the yeast ortholog, metazoan IPMKs are primarily localized to the nucleus (34, 36, 38, 47).

4. NUCLEAR FUNCTIONS

4.1 Arginine-dependent transcription in yeast

Though canonical cytoplasmic and plasma membrane functionalities dominate second messenger signaling dogmas for inositide metabolism, signaling roles for nuclear inositides were identified very shortly after the initial characterization of the "inositol cycle" and its role in receptor-mediated Ca^{2+} signaling (48). The functional importance of a separable, nuclear phosphoinositide metabolic pool was suggested by early observations in animal cells of IGF-1-induced decreases in nuclear

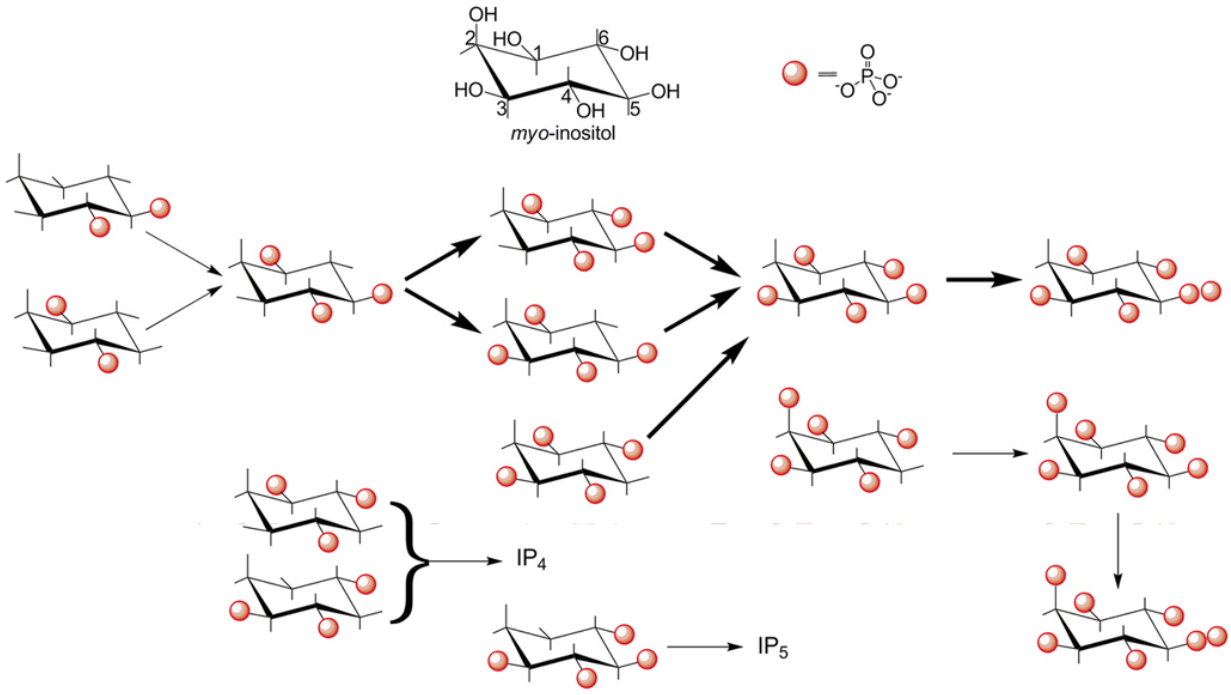

Figure 1. *Myo*-inositol and the multiple water-soluble kinase activities of IPMK. *Myo*-inositol is a 6-carbon cyclitol defined by five equatorial hydroxyls and a single D2 axial hydroxyl. While most characterized IPMK products are the result of D3, D5, or D6 phosphorylation of the inositol ring additional D1-kinase and pyrophosphate synthase activities have also been described.

PtdIns4P and PtdIns(4,5)P_2 accompanied by the production of nuclear DAG and the nuclear translocation of PKC (49, 50). Further studies provided evidence for nuclear functions in cell cycle control and the regulation of nuclear-envelope breakdown prompting the forward-thinking proposal that the nucleus may have been the original site of inositide evolution (51). Nonetheless, the functional significance of nuclear inositide metabolism remained obscure and unappreciated. The identification of IPMK, a nuclear "transcription factor," as the first step in an evolutionarily conserved pathway for inositol metabolism required for mRNA export confirmed the inositides' potential preeminence in nuclear functions.

The characterization of IPMK's inositol kinase activity came after nearly two decades of research had clearly identified the yeast protein (originally named ArgRIII, then subsequently Arg82) as a necessary component of arginine-dependent transcriptional regulation (20, 21, 52) (Figure 2). Yeast are particularly attuned to the nutritional availability of amino acids and nitrogen sources, transcriptionally regulating catabolic and anabolic metabolism in response to environmental changes (21). *IPMK* along with *ARG80*, *ARG81*, and *MCM1* were originally identified in a screen for yeast mutants unable to grow on agar with non-preferred arginine/ornithine as the sole nitrogen source (21). Subsequent studies defined the four gene products as comprising an ArgR/Mcm1 transcriptional complex regulating the suppression of arginine anabolic genes (*ARG1*, *ARG3*, *ARG5,6* and *ARG8*) and the induction of catabolic genes (*CAR1* and *CAR2*) in response to the availability of arginine or optimal nitrogen sources in the environment (53) (Figure 2).

The first investigations examining whether Ipmk's regulatory role in ArgR/Mcm1-dependent functions involves its enzyme activities indicated that arginine-sensitive transcriptional responses did indeed require Ipmk's inositol polyphosphate kinase activities (24). This conclusion was mainly derived from experiments demonstrating the inability of kinase-dead Ipmk to rescue growth defects of *ipmk*-deficient yeast on agar containing arginine/ornithine as the sole nitrogen source. Furthermore, a similar growth phenotype was observed in *plc1*-deficient yeast but not in *ipk1* mutant yeast, suggesting a specific role for either $InsP_4$ or $InsP_5$ in transcriptional regulation (24). In the same study, a kinase-independent role was suggested in the pre-activation assembly of the ArgR/Mcm1 complex on DNA promoter elements (24). However, a subsequent study by Dubois and colleagues challenged the kinase-dependent roles attributed to Ipmk in transcriptional regulation as this group failed to replicate the growth defects of *plc1* mutant yeast (54). More recent studies by Snyder and associates examined arginine-dependent mRNA transcript regulation directly in lieu of growth phenotype analyses and offered an alternative interpretation for Ipmk's functionality in transcriptional regulation based on the identification of Ipmk as an inositide *lipid* kinase (47).

4.2 IPMK is a nuclear PI3K

The surprise addition of a phosphoinositide kinase activity to IPMK's growing list of water-soluble inositol kinase activities was the result of *in vitro* experiments in which brain lipid extracts were incubated with the kinase. The production of a single product identified as PtdIns(3,4,5)P_3 revealed IPMK to be a non-

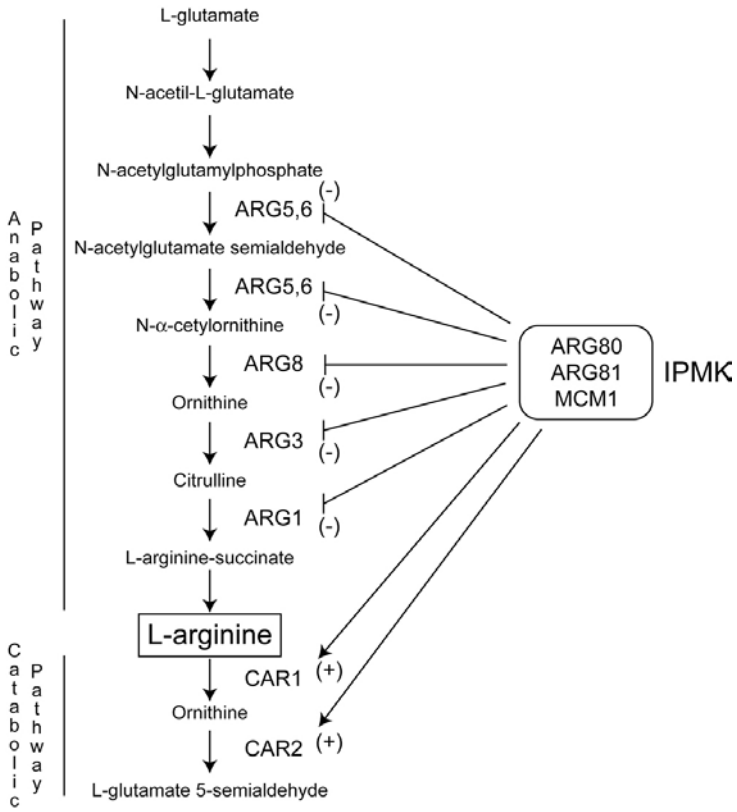

Figure 2. Transcriptional regulatory roles of the ArgR/Mcm1 complex. The ArgR/Mcm1 complex controls (via repression) five anabolic enzymes (*ARG1, ARG3, ARG5,6* and *ARG8*) and (via induction) two catabolic enzymes (*CAR1* and *CAR2*) in an arginine-sensitive manner.

canonical PI3K (Figure 3). Yeast and rat IPMKs display similar kinetic parameters to those previously observed for the canonical PI3Ks but greater substrate specificity as only PtdIns(4,5)P_2 appears to serve as a substrate in contrast to the multiple *in vitro* activities observed for canonical PI3Ks. IPMK PI3K activity is further distinguished from previously characterized PI3Ks in its complete insensitivity to pharmacological inhibition by wortmannin, a potent inhibitor of canonical PI3Ks. Overexpression studies in mammalian cells demonstrated IPMK's ability to dramatically increase nuclear PtdIns(3,4,5)P_3 concentrations suggesting a physiologic relevance for the activity (47).

The discovery of Ipmk's PI3K activity prompted the Snyder group to reevaluate the enzyme's role in arginine-dependent transcription. Monitoring *ARG8* mRNA, normally suppressed in the presence of arginine, they observed derepression and constitutive elevated levels of the transcript in *ipmk* mutant yeast but not in *ipk1*- or *ip6k*-deficient yeast confirming the requirement for Ipmk in the transcriptional regulation of arginine anabolism. A further necessity for kinase activity in *ARG8* regulation was evidenced by the inability of a kinase-dead mutant to restore wild-type suppression of *ARG8* in *ipmk* mutant yeast. However, examination of *ARG8* in *plc1*-deficient yeast, which entirely lack Ins(1,4,5)P_3 and the more phosphorylated higher inositol polyphosphates, revealed wild-type levels of *ARG8* thus implicating Ipmk's lipid kinase activity in ArgR/Mcm1 transcriptional complex regulation (47). The re-appearance of elevated levels of *ARG8* mRNA in a *plc1/ipmk* double knockout confirmed the proposed role for Ipmk's PI3K activity in arginine-dependent transcription. While the mechanism of action of Ipmk's role in ArgR/Mcm1-dependent functions in response to arginine remains to be fully elucidated, more recent studies suggest Ipmk's kinase activities control gene expression in yeast in response to a larger set of environmental and nutritional signals as part of a more general role for nuclear inositol polyphosphate metabolism in transcriptional regulation (55-57) (Figure 4).

4.3 Phosphoinositide 3-kinase related protein kinase regulation

Regulation of arginine catabolism by the ArgR/Mcm1 complex in yeast in response to nitrogen availability is part of a larger catabolic control response mediated by a process termed nitrogen catabolite repression (NCR) in which the absence of preferred nitrogen sources such as ammonia, asparagine, or glutamine results in the transcriptionally initiated catabolism of alternative nitrogen sources (58). DNA microarray analyses of *ipmk* mutant yeast suggest an essential role for Ipmk and its catalytic products in mediating the NCR response beyond arginine metabolism with a large subset of NCR-required genes displaying altered expression (57). Pharmacological

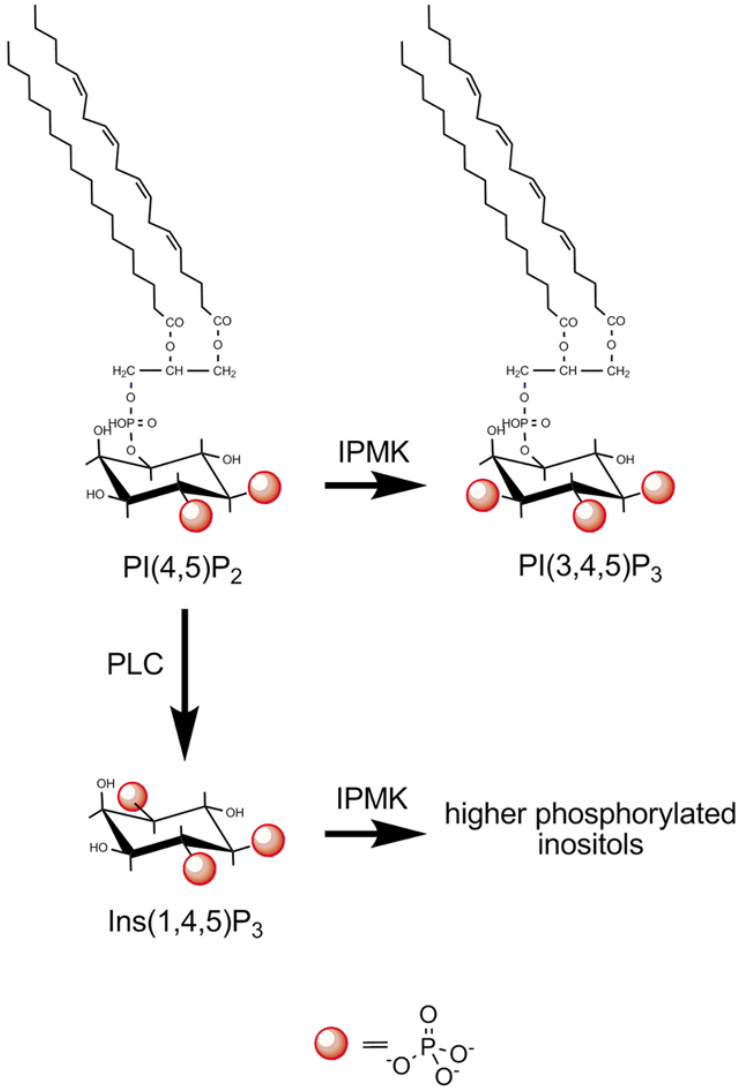

Figure 3. IPMK possesses nuclear PI3K activity. IPMK's lipid kinase activity is somewhat analogous to its water soluble kinase activities as the inositol headgroup of PtdIns(4,5)P_2 is Ins(1,4,5)P_3. Though the related Ins(1,4,5)P_3 3-kinases share the capacity for D3-phosphorylation of Ins(1,4,5)P_3, they are incapable of lipid inositol metabolism.

screens in yeast (59) provide further support for IPMK's role in NCR as *ipmk*-deficient yeast display selective hypersensitivity to rapamycin, an inhibitor of the TOR (Target of Rapamycin) kinases with preeminent regulatory roles in general NCR control (58, 60). TOR kinases belong to a class of phosphoinositide 3-kinase related protein kinases (PIKKs) with roles in cell growth, gene expression, and genome surveillance and repair (61). Intriguingly, in recent years a theme of nuclear inositol polyphosphate regulation of PIKKs has begun to emerge. In yeast, Ipmk was identified as a dosage suppressor of *mec1*, a PIKK yeast homologue of the mammalian ataxia-telangiectasia mutant (ATM) with functional roles in DNA damage response (62). More recently two independent studies identified a role for inositol pyrophosphates in the regulation of telomere length mediated by Tel1, a second PIKK yeast homolog of ATM (63, 64). Lastly, a role for inositol polyphosphates in PIKK-mediated non-homologous end joining (NHEJ) has been proposed by experiments demonstrating $InsP_6$ regulation of the exclusively metazoan DNA-dependent protein kinase (DNA-PK) (65, 66).

4.4 Chromatin remodeling

Recently, work from the O'Shea laboratory has expanded IPMK's role in nutrient sensing to phosphate-limitation response programs (56). In a genetic screen for yeast mutants defective in phosphate-responsive signal transduction pathway elements, O'Shea and colleagues identified *ipmk* mutant yeast as defective in the induction of the phosphate-responsive *PHO5* gene. Additional characterization revealed that expression of Pho5 was repressed in the IPMK mutant strain as a result of the failure of chromatin remodeling complexes to mobilize

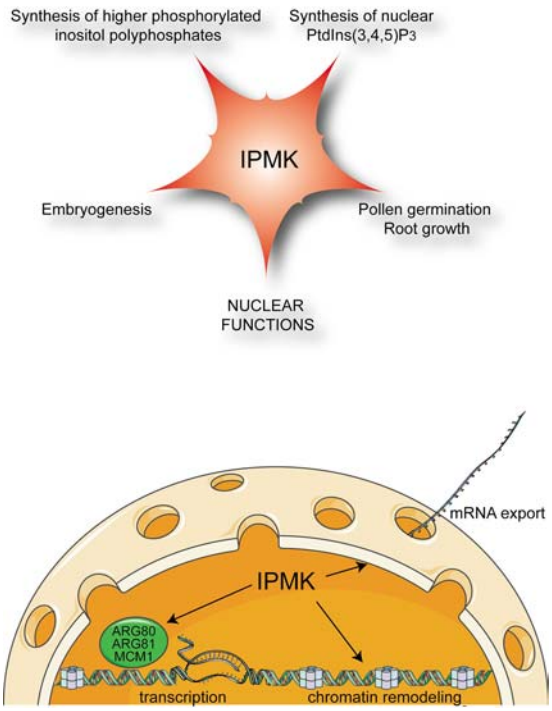

Figure 4. Nuclear processes regulated by IPMK. Yeast genetic screens have implicated IPMK in a number of nuclear processes including mRNA export, arginine-dependent transcriptional regulation, and chromatin remodeling. Additional roles for IPMK are also depicted (artwork provided in part by Servier).

nucleosomes from the promoter, thus preventing access for regulatory factors and machinery necessary for transcription (56). A similar phenotype was observed in *plc1*-deficient strains but not in yeast strains lacking $InsP_6$ suggesting a selective role for $InsP_4$ and $InsP_5$ in the regulation of chromatin remodeling (56). *In vitro* nucleosome mobilization assays by Wu and co-workers proposed direct targeting of the chromatin remodeling complex by inositol polyphosphates (55) though the use of non-physiologic concentrations of inositides in these studies suggests further characterization of the process is necessary (67). Subsequent studies revealed that in addition to $InsP_4$ and $InsP_5$, the inositol pyrophosphates likely also play a role in the phosphate-responsive signal transduction pathway suggesting Ipmk's role in transcription may occur at multiple levels, with both direct and precursor roles for its catalytic products (68-70) (Figure 4).

4.5 Metazoan nuclear roles

While the genetic tractability of the budding yeast has afforded significant insights into the functional roles of Ipmk and its catalytic products (Figure 4), whether or not its roles in transcriptional regulation, chromatin remodeling, and mRNA export are conserved in metazoans and higher species remains to be fully elucidated. Examinations of plants deficient in IPMK suggest roles in pollen germination, root growth, as well axillary shoot branching (71, 72). A recent genome-wide RNA interference (RNAi) screen in cultured *Drosophila melanogaster* haemocyte-like cells identified IPMK as a regulator of the Janus tyrosine kinase/signal transducer and activator of transcription (JAK/STAT) pathway, though the mechanism for this interaction remains undescribed (73). Studies of human cells provide support for a role for inositol polyphosphates in mRNA export as cells expressing the virulence factor SopB, an inositol phosphatase capable of inositol polyphosphate depletion, accumulate mRNA in the nucleus (74). The recent characterization of IPMK knockout mice demonstrates critical roles for IPMK in embryogenesis and central nervous system development (75). Deletion of IPMK in mice results in early lethality with few embryos surviving beyond E8.5. Embryos exhibit a number of abnormal developmental phenotypes including abnormal neural tube folding, failure to turn, a delay and reduction in somite formation, elongation of the anterior/posterior axis, and an absence of allantois and chorion fusion (75). Transcriptional regulatory defects may indeed underlie many of these developmental phenotypes though a role for sequential metabolism mediated by IPK1 and IP6K cannot be excluded. Indeed, deletion of IPK1 also results in early embryonic lethality in mice suggesting a required role for $InsP_6$ and/or the inositol pyrophosphates in mammalian development (76). Though little is known of the physiologic significance of IPMK in mammals beyond the initial characterization of the conventional knockout mouse, the generation of conditional knockout mice bypassing embryonic lethality will likely provide additional inroads to the functional significance of IPMK and downstream metabolites in mammalian biology.

5. PERSPECTIVE

The recent discovery of $PtdIns(3,4,5)P_3$ in fission yeast confirms the early evolutionary emergence of the lipid inositol (77). As yeast do not possess a canonical PI3K homolog capable of $PtdIns(3,4,5)P_3$ synthesis, the identification of yeast Ipmk as a nuclear PI3K suggests IPMK may represent the earliest incarnation of a lipid inositol kinase capable of D3 phosphorylation of $PtdIns(4,5)P_2$. Structure database comparisons of the recently solved crystal structures of $Ins(1,4,5)P_3$ 3-kinase and yeast Ipmk confirm the enzymes' close structural relationship to the lipid inositol kinases despite little primary amino acid sequence similarities (78-80). It also follows that the emergence of $PtdIns(3,4,5)P_3$ functionality in the nucleus may have preceded its cytoplasmic roles associated with the advent of canonical PI3K signaling at the plasma membrane. It is worth noting that nuclear lipids are not restricted to the nuclear membrane and exist in detergent-resistant, yet unidentified components of the nuclear interior (81, 82). Consistent with Ipmk's phosphoinositide-dependent functionality in transcriptional regulation in yeast, studies in mammals have suggested roles for nuclear inositol lipids in the regulation of the BAF chromatin remodeling complex (83). The identification of chromatin-associated proteins as nuclear phosphoinositide receptors lends further support for the role of nuclear inositol lipids in modulating chromatin structure (84).

How IPMK's promiscuous lipid and water-soluble kinase activities are individually regulated remains unknown though *in vivo* phosphorylation of the enzyme has been observed (A.C.R., unpublished observations) suggesting posttranslational modification may regulate these activities. Furthermore, while emphasis is often placed on the $InsP_4$ and $InsP_5$ catalytic products of IPMK, potential roles for its other less characterized products and specifically its pyrophosphate synthase activities should not be ruled out. What "upstream" cell signals impinge on IPMK functionality also remains uncharacterized. In mammalian tissues, components of the canonical PI3K signaling pathway exist in nuclei and nuclear translocation and regulation of canonical PI3K in response to cell surface receptor activation has also been described (82, 85-87). One of the best described downstream targets of canonical PI3Ks is Akt, a serine-threonine kinase with a central role in cancer pathology and regulation of cell survival (88). While much of the functional characterization of Akt has focused on its cytoplasmic roles, additional studies have highlighted roles for its nuclear localization. Once activated in the cytoplasm, Akt translocates to the nucleus where it phosphorylates additional substrates including the FoxO family of transcription factors (89). Recent data from a number of model systems suggest nuclear PI3K/Akt may in fact play integral roles in the regulation of tumorigenesis and apoptosis protection complementing their cytoplasmic functionality, though additional work is necessary to characterize these processes (86). Whether the activities of the canonical PI3K and IPMK function in concert or independently in the regulation of nuclear events is still unclear. Intriguingly, a recent RNAi-based screening of the human kinome identified IPMK as an Akt-cooperating kinase whose knockdown enhanced the efficacy of Akt-directed inhibitors suggesting the targeting of IPMK may hold future therapeutic promise for the treatment of cancer (90).

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7. REFERENCES

1. K. F. Tolias & L. C. Cantley: Pathways for phosphoinositide synthesis. *Chem Phys Lipids*, 98, 69-77 (1999)
2. R.F. Irvine & M.J. Schell: Back in the water: The return of the inositol phosphates. *Nat Rev Mol Cell Biol*, 2, 327-338 (2001)
3. S.B. Shears: The versatility of inositol phosphates as cellular signals. *Biochim Biophys Acta*, 1436, 49-67 (1998)
4. M. R. Hokin & L. E. Hokin: Enzyme secretion and the incorporation of P32 into phospholipides of pancreas slices. *J Biol Chem*, 203, 967-77 (1953)
5. H. Streb, R. F. Irvine, M. J. Berridge & I. Schulz: Release of Ca2+ from a nonmitochondrial intracellular store in pancreatic acinar cells by inositol-1,4,5-trisphosphate. *Nature*, 306, 67-9 (1983)
6. R. F. Irvine: 20 years of Ins(1,4,5)P3, and 40 years before. *Nat Rev Mol Cell Biol*, 4, 586-90 (2003)
7. Y. Nishizuka: Studies and perspectives of protein kinase C. *Science*, 305-312 (1986).
8. C. D. Ferris, R. L. Huganir, S. Supattapone & S. H. Snyder: Purified inositol 1,4,5-trisphosphate receptor mediates calcium flux in reconstituted lipid vesicles. *Nature*, 342, 87-9 (1989)
9. T. Furuichi, S. Yoshikawa, A. Miyawaki, K. Wada, N. Maeda & K. Mikoshiba: Primary structure and functional expression of the inositol 1,4,5-trisphosphate-binding protein P400. *Nature*, 342, 32-8 (1989)
10. R.F. Irvine, A.J. Letcher, D.J. Lander & C.P. Downes: Inositol trisphosphates in carbachol-stimulated rat parotid glands. *Biochem J*, 237-243 (1984).
11. I.R. Batty, S.R. Nahorski & R.F. Irvine: Rapid formation of inositol 1,3,4,5-tetrakisphosphate following muscarinic receptor stimulation of rat cerebral cortical slices. *Biochem J*, 211-215 (1985).
12. A. E. Traynor-Kaplan, A. L. Harris, B. L. Thompson, P. Taylor & L. A. Sklar: An inositol tetrakisphosphate-containing phospholipid in activated neutrophils. *Nature*, 334, 353-6 (1988)
13. M. Whitman, C. P. Downes, M. Keeler, T. Keller & L. Cantley: Type I phosphatidylinositol kinase makes a novel inositol phospholipid, phosphatidylinositol-3-phosphate. *Nature*, 332, 644-6 (1988)
14. L. C. Cantley: The phosphoinositide 3-kinase pathway. *Science*, 296, 1655-7 (2002)
15. J.P. Heslop, R.F. Irvine, A.H. Tashjian Jr. & M.J. Berridge: Inositol tetrakis- and pentakisphosphates in GH4 cells. *J Exp Biol*, 395-401 (1985).
16. L. Stephens, T. Radenberg, U. Thiel, G. Vogel, K.-H. Khoo, A. Dell, T.R. Jackson, P.T. Hawkins & G.W. Mayr: The detection, purification, structural characterization, and metabolism of diphosphoinositol pentakisphosphate(s) and bisdiphosphoinositol tetrakisphosphate(s). *J Biol Chem*, 4009-4015 (1993).
17. F.S. Menniti, R.N. Miller, J.W. Putney Jr. & S.B. Shears: Turnover of inositol polyphosphate pyrophosphates in pancreatoma cells. *J Biol Chem*, 3850-3856 (1993).
18. A. Saiardi, R. Bhandari, A. C. Resnick, A. M. Snowman & S. H. Snyder: Phosphorylation of proteins by inositol pyrophosphates. *Science*, 306, 2101-5 (2004)
19. A. Saiardi, H. Erdjument-Bromage, A.M. Snowman, P. Tempst & S.H. Snyder: Synthesis of diphosphoinositol pentakisphosphate by a newly identified family of higher inositol polyphosphate kinases. *Curr Biol*, 1323-1326 (1999).
20. E. Dubois & F. Messenguy: Pleiotropic function of ArgRIIIp (Arg82p), one of the regulators of arginine metabolism in Saccharomyces cerevisiae. Role in expression of cell-type-specific genes. *Mol Gen Genet*, 315-324 (1994).
21. J. Bercy, E. Dubois & F. Messenguy: Regulation of arginine metabolism in Saccharomyces cerevisiae: expression of the three ARGR regulatory genes and cellular localization of their products. *Gene*, 277-285 (1987).

22. K. Takazawa, J. Vandekerckhove, J.E. Dumont & C. Erneux: Cloning and expression in Escherichia coli of a rat brain cDNA encoding a Ca2+/calmodulin-sensitive inositol 1,4,5-trisphosphate 3-kinase. *Biochem J*, 107-112 (1990).
23. R.F. Irvine, A.J. Letcher, J.P. Heslop & M.J. Berridge: The inositol tris/tetrakisphosphate pathway: Demonstration of Ins(1,4,5)P3 3-kinase activity in animal tissues. *Nature*, 631-634 (1986).
24. A.R. Odom, A. Stahlberg, S.R. Wente & J.D. York: A role for nuclear inositol 1,4,5-trisphosphate kinase in transcriptional control. *Science*, 2026-2029 (2000).
25. A. Saiardi, E. Nagata, H.R. Luo, A. Sawa, X. Luo, A.M. Snowman & S.H. Snyder: Mammalian inositol polyphosphate multikinase synthesizes inositol 1,4,5-trisphosphate and an inositol pyrophosphate. *Proc Natl Acad Sci U S A*, 2306-2311 (2001).
26. T. Zhang, J.J. Caffrey & S.B. Shears: The transcriptional regulator, Arg82, is a hybrid kinase with both monophosphoinositol and diphosphoinositol polyphosphate synthase activity. *FEBS Letters*, 208-212 (2001).
27. J.A. Chattaway, B.K. Drobak, P.A.C. Watkins, A.P. Dawson, A.J. Letcher, L.R. Stephens & R.F. Irvine: An inositol 1,4,5-trisphosphate-6-kinase activity in pea roots. *Planta*, 542-545 (1992).
28. F. Estevez, D. Pulford, M.J.R. Stark, A.N. Carter & C.P. Downes: Inositol trisphosphate metabolism in Saccharomyces cerevisiae: Identification, purification and properties of inositol 1,4,5-trisphosphate 6-kinase. *Biochem J*, 709-716 (1994).
29. P.P. Ongusaha, P.J. Hughes, J. Davey & R.H. Michell: Inositol hexakisphosphate in Schizosaccharomyces pombe: Synthesis from Ins(1,4,5)P3 and osmotic regulation. *Biochem J*, 671-679 (1998).
30. J.D. York, A.R. Odom, R. Murphy, E.B. Ives & S.R. Wente: A phospholipase C-dependent inositol polyphosphate kinase pathway required for efficient messenger RNA export. *Science*, 96-100 (1999).
31. A. Saiardi, J.J. Caffrey, S.H. Snyder & S.B. Shears: Inositol polyphosphate multikinase (ArgRIII) determines nuclear mRNA export in Saccharomyces cerevisiae. *FEBS Letters*, 28-32 (2000).
32. S. E. Caddick, C. J. Harrison, I. Stavridou, S. Johnson & C. A. Brearley: A lysine accumulation phenotype of ScIpk2Delta mutant yeast is rescued by Solanum tuberosum inositol phosphate multikinase. *Biochem J*, 403, 381-9 (2007)
33. J. Stevenson-Paulik, A.R. Odom & J.D. York: Molecular and biochemical characterization of two plant inositol polyphosphate 6-/3-/5-kinases. *J Biol Chem*, 42711-42718 (2002).
34. A.M. Seeds, J.C. Sandquist, E.P. Spana & J.D. York: A molecular basis for inositol polyphosphate synthesis in Drosophila melanogaster. *J Biol Chem*, 47222-47232 (2004).
35. S.-C. Chang, A.L. Miller, Y. Feng, S.R. Wente & P.W. Majerus: The human homolog of the rat inositol phosphate multikinase is an inositol 1,3,4,6-tetrakisphosphate 5-kinase. *J Biol Chem*, 43836-43843 (2002).
36. H.-J. Xia, C. Brearley, S. Elge, B. Kaplan, H. Fromm & B. Mueller-Roeber: Arabidopsis inositol polyphosphate 6-/3-kinase is a nuclear protein that complements a yeast mutant lacking a functional ArgR-Mcm1 transcription complex. *Plant Cell*, 449-463 (2003).
37. M.M. Nalaskowski, C. Deschermeier, W. Fanick & G.W. Mayr: The human homologue of yeast ArgRIII protein is an inositol phosphate multikinase with predominantly nuclear localization. *Biochem J*, 549-556 (2002).
38. M. Fujii & J.D. York: A role for rat inositol polyphosphate kinases rIPK2 and rIPK1 in inositol pentakisphosphate and inositol hexakisphosphate production in Rat-1 cells. *J Biol Chem*, 1156-1164 (2005).
39. A.M. Seeds, R.J. Bastidas & J.D. York: Molecular definition of a novel inositol polyphosphate metabolic pathway initiated by inositol 1,4,5-trisphosphate 3-kinase activity in Saccharomyces cerevisiae. *J Biol Chem*, 27654-27661 (2005).
40. F. S. Menniti, K. G. Oliver, K. Nogimori, J. F. Obie, S. B. Shears & J. W. Putney, Jr.: Origins of myo-inositol tetrakisphosphates in agonist-stimulated rat pancreatoma cells. Stimulation by bombesin of myo-inositol 1,3,4,5,6-pentakisphosphate breakdown to myo-inositol 3,4,5,6-tetrakisphosphate. *J Biol Chem*, 265, 11167-76 (1990)
41. J.W. Verbsky, S.-C. Chang, M.P. Wilson, Y. Mochizuki & P.W. Majerus: The pathway for the production of inositol hexakisphosphate in human cells. *J Biol Chem*, 1911-1920 (2005).
42. T. Balla, L. Hunyady, A. J. Baukal & K. J. Catt: Structures and metabolism of inositol tetrakisphosphates and inositol pentakisphosphate in bovine adrenal glomerulosa cells. *J Biol Chem*, 264, 9386-90 (1989)
43. B. G. Wen, M. T. Pletcher, M. Warashina, S. H. Choe, N. Ziaee, T. Wiltshire, K. Sauer & M. P. Cooke: Inositol (1,4,5) trisphosphate 3 kinase B controls positive selection of T cells and modulates Erk activity. *Proc Natl Acad Sci U S A*, 101, 5604-9 (2004)
44. V. Pouillon, R. Hascakova-Bartova, B. Pajak, E. Adam, F. Bex, V. Dewaste, C. Van Lint, O. Leo, C. Erneux & S. Schurmans: Inositol 1,3,4,5-tetrakisphosphate is essential for T lymphocyte development. *Nat Immunol*, 4, 1136-43 (2003)
45. K. Jun, G. Choi, S. G. Yang, K. Y. Choi, H. Kim, G. C. Chan, D. R. Storm, C. Albert, G. W. Mayr, C. J. Lee & H. S. Shin: Enhanced hippocampal CA1 LTP but normal spatial learning in inositol 1,4,5-trisphosphate 3-kinase(A)-deficient mice. *Learn Mem*, 5, 317-30 (1998)
46. A. Leyman, V. Pouillon, A. Bostan, S. Schurmans, C. Erneux & X. Pesesse: The absence of expression of the three isoenzymes of the inositol 1,4,5-trisphosphate 3-kinase does not prevent the formation of inositol pentakisphosphate and hexakisphosphate in mouse embryonic fibroblasts. *Cell Signal*(2007)
47. A.C. Resnick, A.M. Snowman, B.M. Kang, K.J. Hurt, S.H. Snyder & A. Saiardi: Inositol polyphosphate multikinase is a nuclear PI3-kinase with transcriptional regulatory activity. *Proc Natl Acad Sci U S A*, 102, 12783-12788 (2005)
48. L. Cocco, R.S. Gilmour, A. Ognibene, A.J. Letcher, F.A. Manzoli & R.F. Irvine: Synthesis of polyphosphoinositides in nuclei of Friend cells. Evidence for polyphosphoinositide metabolism inside the nucleus which changes with cell differentiation. *Biochem J*, 765-770 (1987).

49. A. M. Martelli, L. M. Neri, R. S. Gilmour, P. J. Barker, N. S. Huskisson, F. A. Manzoli & L. Cocco: Temporal changes in intracellular distribution of protein kinase C in Swiss 3T3 cells during mitogenic stimulation with insulin-like growth factor I and bombesin: translocation to the nucleus follows rapid changes in nuclear polyphosphoinositides. *Biochem Biophys Res Commun*, 177, 480-7 (1991)
50. N. Divecha, H. Banfic & R. F. Irvine: The polyphosphoinositide cycle exists in the nuclei of Swiss 3T3 cells under the control of a receptor (for IGF-I) in the plasma membrane, and stimulation of the cycle increases nuclear diacylglycerol and apparently induces translocation of protein kinase C to the nucleus. *Embo J*, 10, 3207-14 (1991)
51. N. Divecha, H. Banfic & R.F. Irvine: Inositides and the nucleus and inositides in the nucleus. *Cell*, 405-407 (1993).
52. H. Qiu, E. Dubois, P. Broen & F. Messenguy: Functional analysis of ARGRI and ARGRIII regulatory proteins involved in the regulation of arginine metabolism in Saccharomyces cerevisiae. *Mol Gen Genet*, 192-200 (1990).
53. F. Messenguy, E. Dubois & C. Boonchird: Determination of the DNA-binding sequences of ARGR proteins to arginine anabolic and catabolic promoters. *Mol Cell Biol*, 2852-2863 (1991).
54. E. Dubois, V. Dewaste, C. Erneux & F. Messenguy: Inositol polyphosphate kinase activity of Arg82/ArgRIII is not required for the regulation of the arginine metabolism in yeast. *FEBS Letters*, 300-304 (2000).
55. X. Shen, H. Xiao, R. Ranallo, W.-H. Wu & C. Wu: Modulation of ATP-dependent chromatin-remodeling complexes by inositol polyphosphates. *Science*, 112-114 (2003).
56. D.J. Steger, E.S. Haswell, A.L. Miller, S.R. Wente & E.K. O'Shea: Regulation of chromatin remodeling by inositol polyphosphates. *Science*, 114-116 (2003).
57. M. El Alami, F. Messenguy, B. Scherens & E. Dubois: Arg82p is a bifunctional protein whose inositol polyphosphate kinase activity is essential for nitrogen and PHO gene expression but not for Mcm1p chaperoning in yeast. *Mol Microbiol*, 457-468 (2003).
58. T. G. Cooper: Transmitting the signal of excess nitrogen in Saccharomyces cerevisiae from the Tor proteins to the GATA factors: connecting the dots. *FEMS Microbiol Rev*, 26, 223-38 (2002)
59. T. F. Chan, J. Carvalho, L. Riles & X. F. Zheng: A chemical genomics approach toward understanding the global functions of the target of rapamycin protein (TOR). *Proc Natl Acad Sci U S A*, 97, 13227-32 (2000)
60. T. Beck & M. N. Hall: The TOR signalling pathway controls nuclear localization of nutrient-regulated transcription factors. *Nature*, 402, 689-92 (1999)
61. J. Perry & N. Kleckner: The ATRs, ATMs, and TORs are giant HEAT repeat proteins. *Cell*, 112, 151-5 (2003)
62. B. A. Desany, A. A. Alcasabas, J. B. Bachant & S. J. Elledge: Recovery from DNA replicational stress is the essential function of the S-phase checkpoint pathway. *Genes Dev*, 12, 2956-70 (1998)
63. A. Saiardi, A. C. Resnick, A. M. Snowman, B. Wendland & S. H. Snyder: Inositol pyrophosphates regulate cell death and telomere length through phosphoinositide 3-kinase-related protein kinases. *Proc Natl Acad Sci U S A*, 102, 1911-4 (2005)
64. S.J. York, B.N. Armbruster, P. Greenwell, T.D. Petes & J.D. York: Inositol diphosphate signaling regulates telomere length. *J Biol Chem*, 4264-4269 (2005).
65. L.A. Hanakahi & S.C. West: Specific interaction of IP6 with human Ku70/80, the DNA-binding subunit of DNA-PK. *EMBO J*, 2038-2044 (2002).
66. L.A. Hanakahi, M. Bartlet-Jones, C. Chappell, D. Pappin & S.C. West: Binding of inositol phosphate to DNA-PK and stimulation of double-strand break repair. *Cell*, 721-729 (2000).
67. S. B. Shears: How versatile are inositol phosphate kinases? *Biochem J*, 377, 265-80 (2004)
68. S. Mulugu, W. Bai, P. C. Fridy, R. J. Bastidas, J. C. Otto, D. E. Dollins, T. A. Haystead, A. A. Ribeiro & J. D. York: A conserved family of enzymes that phosphorylate inositol hexakisphosphate. *Science*, 316, 106-9 (2007)
69. Y. S. Lee, S. Mulugu, J. D. York & E. K. O'Shea: Regulation of a cyclin-CDK-CDK inhibitor complex by inositol pyrophosphates. *Science*, 316, 109-12 (2007)
70. C. Auesukaree, H. Tochio, M. Shirakawa, Y. Kaneko & S. Harashima: Plc1p, Arg82p, and Kcs1p, enzymes involved in inositol pyrophosphate synthesis, are essential for phosphate regulation and polyphosphate accumulation in Saccharomyces cerevisiae. *J Biol Chem*, 25127-25133 (2005).
71. J. Xu, C. A. Brearley, W. H. Lin, Y. Wang, R. Ye, B. Mueller-Roeber, Z. H. Xu & H. W. Xue: A role of Arabidopsis inositol polyphosphate kinase, AtIPK2alpha, in pollen germination and root growth. *Plant Physiol*, 137, 94-103 (2005)
72. Z. B. Zhang, G. Yang, F. Arana, Z. Chen, Y. Li & H. J. Xia: Arabidopsis Inositol Polyphosphate 6-/3-kinase (AtIpk2{beta}) Is Involved in Axillary Shoot Branching via Auxin Signaling. *Plant Physiol*(2007)
73. P. Muller, D. Kuttenkeuler, V. Gesellchen, M. P. Zeidler & M. Boutros: Identification of JAK/STAT signalling components by genome-wide RNA interference. *Nature*, 436, 871-5 (2005)
74. Y. Feng, S.R. Wente & P.W. Majerus: Overexpression of the inositol phosphatase SopB in human 293 cells stimulates cellular chloride influx and inhibits nuclear mRNA export. *Proc Natl Acad Sci U S A*, 875-879 (2001).
75. J.P. Frederick, D. Mattiske, J.A. Wofford, L.C. Megosh, L.Y. Drake, S.-T. Chiou, B.L.M. Hogan & J.D. York: An essential role for an inositol polyphosphate multikinase, Ipk2, in mouse embryogenesis and second messenger production. *Proc Natl Acad Sci U S A*, 8454-8459 (2005).
76. J. Verbsky, K. Lavine & P.W. Majerus: Disruption of the mouse inositol 1,3,4,5,6-pentakisphosphate 2-kinase gene, associated lethality, and tissue distribution of 2-kinase expression. *Proc Natl Acad Sci U S A*, 8448-8453 (2005).
77. P. Mitra, Y. Zhang, L. E. Rameh, M. P. Ivshina, D. McCollum, J. J. Nunnari, G. M. Hendricks, M. L. Kerr, S. J. Field, L. C. Cantley & A. H. Ross: A novel phosphatidylinositol(3,4,5)P3 pathway in fission yeast. *J Cell Biol*, 166, 205-11 (2004)

78. W. Holmes & G. Jogl: Crystal structure of inositol phosphate multikinase 2 and implications for substrate specificity. *J Biol Chem*, 281, 38109-16 (2006)
79. G. J. Miller & J. H. Hurley: Crystal structure of the catalytic core of inositol 1,4,5-trisphosphate 3-kinase. *Mol Cell*, 15, 703-11 (2004)
80. B. Gonzalez, M. J. Schell, A. J. Letcher, D. B. Veprintsev, R. F. Irvine & R. L. Williams: Structure of a human inositol 1,4,5-trisphosphate 3-kinase: substrate binding reveals why it is not a phosphoinositide 3-kinase. *Mol Cell*, 15, 689-701 (2004)
81. R. F. Irvine: Nuclear inositide signalling -- expansion, structures and clarification. *Biochim Biophys Acta*, 1761, 505-8 (2006)
82. R. F. Irvine: Nuclear lipid signalling. *Nat Rev Mol Cell Biol*, 4, 349-60 (2003)
83. K. Zhao, W. Wang, O.J. Rando, Y. Xue, K. Swiderek, A. Kuo & G.R. Crabtree: Rapid and phosphoinositol-dependent binding of the SWI/SNF-like BAF complex to chromatin after T lymphocyte receptor signaling. *Cell*, 625-636 (1998).
84. O. Gozani, P. Karuman, D.R. Jones, D. Ivanov, J. Cha, A.A. Lugovskoy, C.L. Baird, H. Zhu, S.J. Field, S.L. Lessnick, J. Villasenor, B. Mehrotra, J. Chen, V.R. Rao, J.S. Brugge, C.G. Ferguson, B. Payrastre, D.G. Myszka, L.C. Cantley, G. Wagner, N. Divecha, G.D. Prestwich & J. Yuan: The PHD finger of the chromatin-associated protein ING2 functions as a nuclear phosphoinositide receptor. *Cell*, 99-111 (2003).
85. K. Ye, K. J. Hurt, F. Y. Wu, M. Fang, H. R. Luo, J. J. Hong, S. Blackshaw, C. D. Ferris & S. H. Snyder: Pike. A nuclear gtpase that enhances PI3kinase activity and is regulated by protein 4.1N. *Cell*, 103, 919-30 (2000)
86. A. M. Martelli, I. Faenza, A. M. Billi, L. Manzoli, C. Evangelisti, F. Fala & L. Cocco: Intranuclear 3'-phosphoinositide metabolism and Akt signaling: new mechanisms for tumorigenesis and protection against apoptosis? *Cell Signal*, 18, 1101-7 (2006)
87. L. M. Neri, D. Milani, L. Bertolaso, M. Stroscio, V. Bertagnolo & S. Capitani: Nuclear translocation of phosphatidylinositol 3-kinase in rat pheochromocytoma PC 12 cells after treatment with nerve growth factor. *Cell Mol Biol (Noisy-le-grand)*, 40, 619-26 (1994)
88. D. P. Brazil, Z. Z. Yang & B. A. Hemmings: Advances in protein kinase B signalling: AKTion on multiple fronts. *Trends Biochem Sci*, 29, 233-42 (2004)
89. K. C. Arden & W. H. Biggs, 3rd: Regulation of the FoxO family of transcription factors by phosphatidylinositol-3 kinase-activated signaling. *Arch Biochem Biophys*, 403, 292-8 (2002)
90. S. Morgan-Lappe, K. W. Woods, Q. Li, M. G. Anderson, M. E. Schurdak, Y. Luo, V. L. Giranda, S. W. Fesik & J. D. Leverson: RNAi-based screening of the human kinome identifies Akt-cooperating kinases: a new approach to designing efficacious multitargeted kinase inhibitors. *Oncogene*, 25, 1340-8 (2006)

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