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Inositol polyphosphate multikinase (IPMK) in transcriptional regulation and nuclear inositide metabolism

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Abstract

Inositol polyphosphate multikinase (IPMK, ipk2, Arg^{82}, ArgRIII) is an inositide kinase with unusually flexible substrate specificity and the capacity to partake in many functional protein–protein interactions (PPIs). By merging these two activities, IPMK is able to execute gene regulatory functions that are very unique and only now beginning to be recognized. In this short review, we present a brief history of IPMK, describe the structural biology of the enzyme and highlight a few recent discoveries that have shed more light on the role IPMK plays in inositide metabolism, nuclear signalling and transcriptional regulation.

Keywords

IPMK; ipk2; ArgR; inositide; multikinase; transcription; gene expression

Introduction

Inositides represent a large family of secondary messengers that are essential in the regulation of various cellular processes. This group of molecules may be classified either as water-soluble inositol polyphosphates (IPs) or inositol lipids [(phosphatidylinositols or phosphoinositides (PIPs)]. These ubiquitous molecules are descendants of *myo*-inositol, the most widely-occurring stereoisomer of inositol. As a six-carbon cyclitol harbouring one axial (C-2) and five equatorial hydroxy groups, *myo*-inositol provides a rich template for single and combinatorial phosphorylation at six positions, generating 63 unique inositol phosphates [1]. This number is magnified as inositol polyphosphates are either further phosphorylated to yield inositol pyrophosphates or conjugated to lipids to yield the phosphoinositides.

Besides their involvement in phospholipid metabolism to maintain cell membrane integrity, inositides are key mediators of diverse cellular signalling pathways. Elucidation of the physiological roles played by both IPs and PIPs in the cell have continued to receive

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increasing attention since the first definitive assignment of inositol 1,4,5-trisphosphate (IP_3) as the calcium-releasing factor generated upon binding to IP_3 receptors [2]. The signalling function of IP_3 arose from the activation of phospholipase C (PLC) to hydrolyse membrane-bound phosphatidylinositol 4,5-bisphosphate (PIP_2) into diacylglycerol (DAG) and IP_3, which in turn activate protein kinase C (PKC) and allosterically stimulates IP_3 receptors to release Ca^{2+} from intracellular stores respectively. These findings ushered in a decade and more of research in inositide metabolism, ranging from identification of more phosphorylated derivatives of lipid inositols and inositol phosphates [3–6], identification of inositide receptors and binding domains, characterization of their signalling roles [7], which may or may not be independent of each other, as well as the nuclear processes that are regulated by inositide–receptor interactions, to name a few. The reader is referred to these excellent reviews on these various themes [1,8–13].

Further investigations of the biochemical roles of inositol phosphates and lipid inositols in the cell have also raised important questions regarding the functional significance of higher inositol phosphate biosynthesis and other metabolic processes. These studies revealed the presence of a family of inositol phosphate kinases that catalyse the phosphorylation of inositol and its phosphorylated derivatives [14–16]. Interestingly, some of these enzymes have activities that are specific to the substrate [17,18] and can therefore be named in a more straightforward manner: inositol (1,4,5) trisphosphate 3-kinase (IP3K) specifically phosphorylates inositol (1,4,5) trisphosphate [(I(1,4,5)P_3] at the 3-hydroxy position of the inositol ring to yield inositol (1,3,4,5) tetrakisphosphate [(I(1,3,4,5)P_4]. Other enzymes however adopt a promiscuous catalytic activity towards several inositol phosphates. Inositol polyphosphate multikinase (IPMK, Figure 1A) is the most catalytically flexible member of this family and it is this enigmatic enzyme that shall be the focus of this short review.

IPMK is a versatile catalyst

IPMK assumed a number of aliases in the course of its history, perhaps, in part due to the variety of function it performs in the cell. It was first known as Arg^{82} (or ArgRIII) and was initially discovered as a transcription factor important in regulation of arginine metabolism in yeast [19–24]. However, the kinase activity of Arg^{82}, detected as phosphorylation of IP_3 at C-6 of the inositol ring, was first reported in pea extracts [25] and subsequently in budding yeast extracts [26]. Phosphorylation of IP_3 to form inositol hexakisphosphate (IP_6) via C-6, C-3 and C-2 kinase activities was also reported for this enzyme in *Schizosaccharomyces pombe* [27]. However, it was only in the late 1990s when independent work by Snyder and colleagues [28] and York and co-workers [29] showed that Arg^{82} displays dual C-3 and C-6 kinase activity that sequentially phosphorylates IP_3 to IP_4 to IP_5 that this enzyme was pushed to the spotlight. Snyder and co-workers [28] aptly renamed the enzyme ‘inositol polyphosphate multikinase’ in honour of its catalytic flexibility. York and co-workers coined the yeast name ‘Ipk2’ for the same enzyme, but have now adopted the IPMK moniker as well [30]. Other investigators have showed that mammalian orthologues of IPMK catalyse pyrophosphorylation of IP_5 to $PPIP_4$ (diphosphorylated inositol phosphate) [31,32].

Besides being an inositol phosphate kinase, investigations showed that IPMK also possesses a nuclear phosphoinositide (phosphatidylinositol) kinase activity, catalysing phosphorylation of the phosphatidylinositol 4,5-bisphosphate (PIP_2) to yield phosphatidyl 3,4,5-trisphosphate (PIP_3) [33]. Interestingly, the related IP_3-kinase shares the capacity to phosphorylate soluble inositol phosphate with IPMK, but phosphorylation of the lipid inositol PIP_2 has only been observed with IPMK and not with IP_3 3-kinase [10,34].

IPMK is a multi-functional regulatory protein

Whereas IPMK proves to be a flexible catalyst essential in inositide metabolism, recent studies show that the versatility of IPMK allows it to play critical roles in other nuclear functions such as transcriptional regulation, mRNA export and chromatin remodelling, among others [35–46]. The following is by no means an exhaustive list, but we present here some examples of the roles of IPMK in metabolic signalling networks.

The characterization of IPMK as an inositol phosphate kinase was preceded with nearly 20-year history as a transcriptional regulator of arginine metabolism [19–24,47]. IPMK is believed to be an important facilitator in the assembling of the transcriptional complex ArgR/Mcm1 that regulates the repression of arginine anabolic genes and the induction of catabolic genes in response to changes in the nitrogen sources in the cellular environment [48]. It remained controversial whether the kinase activity of IPMK is involved in its transcriptional role; however, it now seems clear that IPMK can function in transcriptional regulation simultaneously in both kinase-dependent [35] and kinase-independent [36] roles. The specifics of the kinase-dependence or independence of an isolated system are less important than the overall observations that IPMK can act both dependently and independently of its kinase activity.

The kinase-independent roles of IPMK have been highlighted with several recent discoveries of IPMK acting as an adaptor protein, stabilizing protein complexes that are of the highest biomedical importance. Kim et al. [37,38] identified IPMK as a novel cofactor of mTOR (mammalian target of rapamycin), a serine/threonine protein kinase important in the regulation of processes including cell growth, survival and proliferation, protein synthesis and transcription [39]. It exists as two distinct protein complexes, mTORC1 and mTORC2, each with different affinity toward a separate set of proteins. The mammalian IPMK genes contain N-terminal 60 residues that mediate direct binding of IPMK to mTORC1 and regulate mTORC1 signalling responses to amino acids [37,38].

IPMK has also been demonstrated to function in the physiological regulation of AMPK (AMP-activated protein kinase) [40–42], an enzyme that maintains cellular homoeostasis by triggering catabolic energy and antagonizing ATP-consuming anabolic pathways. Their findings show that IPMK is an AMPK-binding protein whose association with AMPK is controlled by glucose levels. Glucose induces phosphorylation of mouse IPMK at Tyr^{174} (yeast Tyr^{202} and human Tyr^{191}), an amino acid that resides close to the catalytic core of IPMK (Figures 1B and 1C). Phosphorylation at IPMK Tyr^{174} enhances IPMK–AMPK interaction and AMPK signalling responses to glucose; however it remains unclear how

phosphorylation of this tyrosine residue facilitates the IPMK–AMPK interaction, based on the yeast IPMK crystal structure [43].

Steroidogenic factor-1 (SF-1), a nuclear receptor that regulates genes essential for sexual development and production of steroids, has been shown to be an important target of IPMK [35,44]. IPMK interacts with the SF-1 protein and directly phosphorylates PIP_2 bound to the SF-1 protein to yield PIP_3 [35]. The crystal structures of SF-1 bound to PIP_2 (Figure 2A, PDB: 4QK4) and PIP_3 (Figure 2B, PDB: 4QJR) demonstrate high solvent accessibility of the inositide headgroup [44,45]. The interaction of IPMK with SF-1/PIP_2 [35,46] raises interesting structural questions that may shed light on the importance of phosphoinositides in nuclear events such as transcription, as well as the molecular mechanisms by which IPMK can regulate lipid signalling within the nucleus.

IPMK crystallography and catalysis

A glaring hole in our knowledge of IPMK is any structural information of the human orthologue, particularly given recent studies linking IPMK to intestinal carcinoids [49] and Huntington's disease [50], both in studies from human patients. The only IPMK crystal structures that have been solved to date are the *Saccharomyces cerevisiae* (yeast) [43] and *Arabidopsis thaliana* (plant) [30] IPMKs. Despite low sequence homology (only approximately 17%) [30], these two orthologues exhibit high structural homology within their catalytic domains to the IP3-kinase family and both adopt the ATP-grasp fold [30,43]. Structural comparisons between the human IP_3-kinase (PDB: 1W2D) [51], plant IPMK (PDB: 4FRF) [30] and yeast IPMK (PDB: 2IF8) [43] have shown how IPMKs achieve their hallmark substrate flexibility on several inositol phosphate species. This flexibility is contrasted with the high substrate specificity of IP_3-kinases, which fall into the same family of ATP-grasp inositol kinases. In all these structures, it is the inositol phosphate-binding loop (IP loop, Figure 1A) that accounts for substrate selectivity by interacting with phosphorylated inositol species through the amino acid sequence on the ATP-facing surface of the IP loop helix.

The IP loop is the major structural mechanism that allows inositol kinases to achieve substrate specificity. Indeed, in an attempt to instil phosphatidylinositol lipid kinase activity in the human and rat IP_3-kinases [34,51], we observed that removal of the IP loop from IP_3-kinase produces a kinase with little specificity if any, as it will auto-phosphorylate IP_3-kinase protein itself, as well as other proteins and phosphoinositides in the reaction (result not shown). More directed, modulatory experiments attempting to shift IPMK specificity have shown that the substrate specificity of the plant IPMK can be engineered by rational point mutations in the IP loop, dramatically reducing the plant IPMK 3-kinase activity, while not disturbing the 6-kinase activity [30]. Importantly, this uniquely engineered hypomorphic mutant of the plant IPMK can rescue the lethal phenotype of *ipmk* null *Drosophila melanogaster* flies [30], which may suggest that the 6-kinase activity is more important to very basic cellular or developmental IPMK functions, whereas the 3-kinase activity may participate more in fine-tuning cellular signalling. Regardless, the rational mutations afforded by the plant and yeast IPMK crystal structures have been invaluable in assessing how IPMK executes its catalytic functions.

IPMK as a kinase-independent adapter

Where crystallography has provided far less information is in how IPMK acts through catalytically independent mechanisms as an adapter protein in several transcriptional and signalling complexes, in addition to its promiscuous phosphorylated inositol and phosphatidylinositol kinase activities discussed above. Several recent studies have shown that the presence of IPMK stabilizes some very important protein signalling complexes, in a manner independent of the kinase activity of IPMK [37,38,52–55]. The laboratories performing these studies have also had the foresight to begin characterization of which structural domains in IPMK are important for those protein–protein interactions (PPIs). mTOR binds to IPMK via the N-terminal 60 amino acids of IPMK [37,38]. Serum response factor (SRF) requires the IPMK kinase domain itself for interaction (IPMK amino acids 93–182) [52], similar to what is required for the transcription factor and tumour suppressor p53 to interact with IPMK (IPMK amino acids 125–184) [53–55]. It is interesting that the catalytic domain of IPMK would mediate PPIs that do not require the IPMK kinase activity for regulation of the biological output from those complexes. A question that awaits exploration is if those important PPIs inhibit IPMK from executing IPMK kinase-dependent cellular functions in general inositide metabolism and how that may feedback into these signalling systems.

Although IPMK structures gave little insight into kinase-independent functions, the yeast IPMK crystal structure contains several helices (α9 and α5) and a β-sheet (β6) within the C-terminal domain that had been suspected to act as part of a PPI domain [43]. Primary structure analysis revealed that the human and yeast IPMKs contain large inserts immediately following the α9 helix, but that the sequences of those inserts are not conserved between IPMK orthologues in different species, are not ordered in any crystal structures to date and have no identified catalytic activity [30,43]. It remains to be seen if these disordered insert regions in the current IPMK crystal structures can become ordered if IPMK is complexed with proteins that interact with IPMK. Thus, it remains quite an open question as to what function the species-divergent and crystallographically disordered N- and C-terminal regions of IPMK may play in IPMK biology and which co-crystal structures may induce order in these regions?

Future trends in IPMK discovery

The discovery of direct IPMK phosphorylation of PIP_2 bound to the nuclear receptor SF-1 [35,44], as well as the more recent discovery that IPMK effects the RNA-export activity of ALY through generation of nuclear PIP_3 [56], suggests that IPMK may have an expansive, novel function specific to cellular nuclei, the phosphorylation of protein–inositide complexes. This function would unify IPMK catalytic flexibility with its ability to bind proteins, the inositide-binding proteins perhaps allowing the chemical inositide substrate to be optimally positioned for IPMK phosphorylation. Indeed, the enzyme kinetic parameters describing IPMK activity on PIP_2 bound in micelles compared with PIP_2 bound by the nuclear receptor SF-1 demonstrates that IPMK phosphorylates PIP_2 in micelles with higher apparent velocity than PIP_2 in SF-1, but with a ‘worse’ apparent K_M (Figure 3) [35]. Those data are consistent with a model whereby IPMK PPIs with SF-1 enhance the K_M, but slow

down the enzyme when compared with PIP_2 in micelles (perhaps due to slower substrate off rate).

The details of the molecular interface between IPMK, PIP_2 and SF-1 that enhance the overall activity of IPMK while still allowing substrate release will reveal how systems like this work in the nucleus and perhaps why they were selected for evolutionary reasons [46]. Further, the identification of novel protein–inositide complex substrates of IPMK will broaden the scope of IPMK influence in the nucleus, perhaps explaining why nuclear inositides are ubiquitous in all eukaryotic species examined to date, from yeast to plants to humans. The multiple roles IPMK plays in such central processes highlights the importance of this protein to eukaryotic cell physiology (Figure 4). Given that structure-based mutations introduced into the plant IPMK were able to decouple two of the most important enzyme activities of IPMK [30], a better understanding of IPMK structure and function will open the door for specific pharmacological targeting of these important IPMK activities.

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Abbreviations

AMPK	AMP-activated protein kinase
IP loop	inositol phosphate-binding loop
IPMK	inositol polyphosphate multikinase
mTOR	mammalian target of rapamycin
PIP_2	phosphatidylinositol 4,5-bisphosphate
PIP_3	phosphatidylinositol 3,4,5-trisphosphate
PPI	protein–protein interaction
$PPIP_4$	diphosphorylated inositol phosphate
SF-1	steroidogenic factor-1

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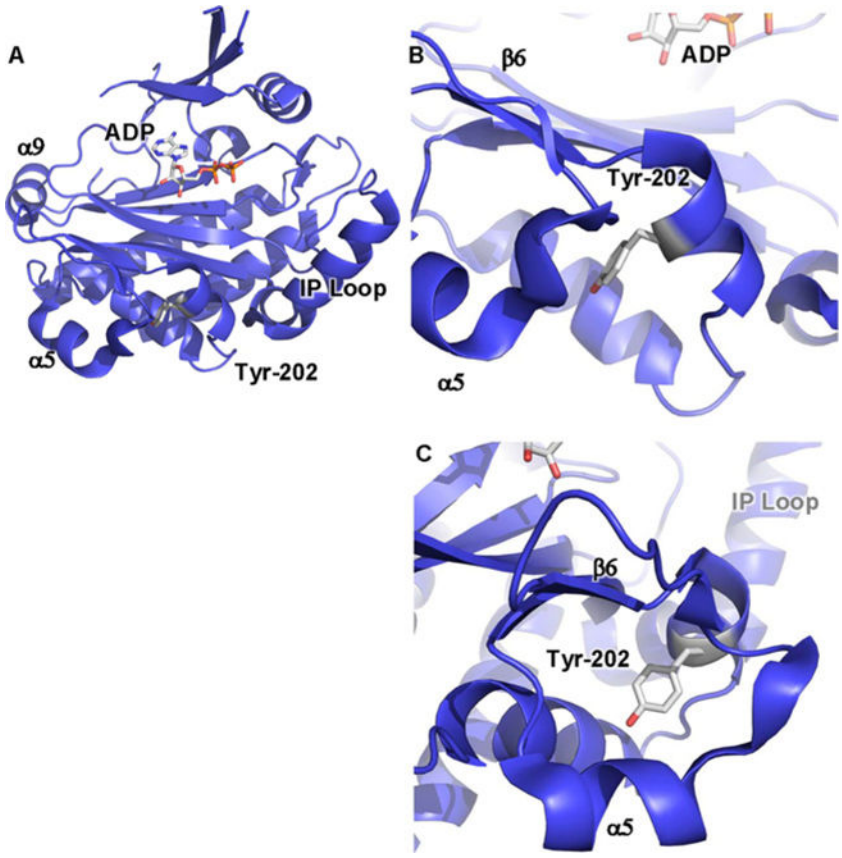


Figure 1. Crystal structure of yeast IPMK reveals positions of amino acids involved in IPMK substrate specificity and PPIs

(**A**) Ribbon cartoon of the 2.0 Å crystal structure of *S. cerevisiae* IPMK [43] (PDB: 2IF8), one of only two IPMK structures solved to date, the other being the *A. thaliana* plant IPMK [30] (result not shown, PDB: 4FRF). The helix in the lower right labelled as 'IP loop' is the inositol binding helix, instilling much of the substrate specificity to the enzyme. ADP indicated as sticks. α9, α5 and β6 elements have been suggested to be a PPI surface. (**B**) Tyr^{174} of the mouse IPMK is phosphorylated in response to glucose and mediates IPMK interaction with AMPK, through an unknown mechanism. Tyr^{174} is conserved in human IPMK (Tyr^{191}) and the depicted yeast IPMK (Tyr^{202}), as indicated. (**C**) As in (B), rotated 90°.

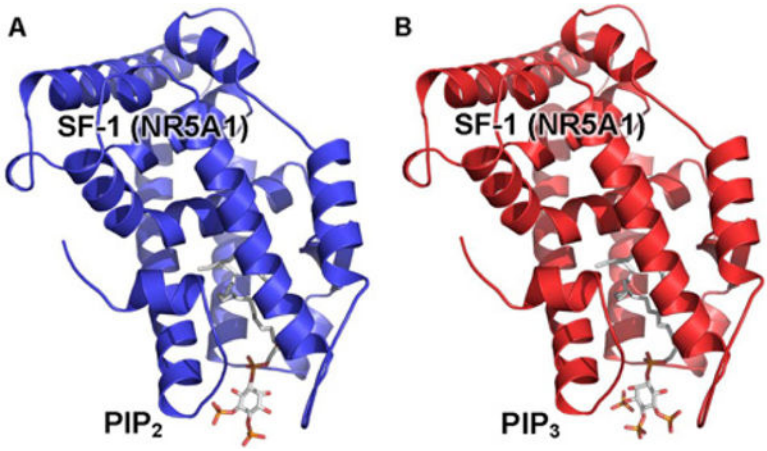
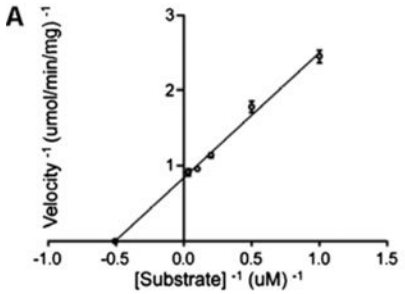


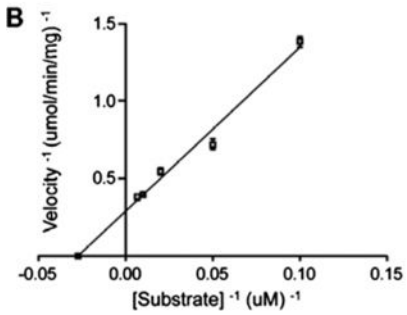
Figure 2. Co-crystal structures of SF-1/PIP_2 and SF-1/PIP_3 demonstrate high solvent accessibility of the inositide headgroups

(**A**) Ribbon cartoon of the 2.8 Å co-crystal structure of the ligand-binding domain (LBD) of the nuclear receptor SF-1 bound to PIP_2 (PDB: 4QK4), PIP_2 indicated as sticks, revealing the solvent accessibility of the PIP_2 headgroup. (**B**) Ribbon carton of the 2.4 Å crystal structure of the SF-1 LBD bound to PIP_3 (PDB: 4QJR), PIP_3 indicated as sticks. In both structures, the transcriptional co-activator peptide has been removed for clarity.



H.s. IPMK on SF-1/PIP2

curve fit (r^2)	0.995 (+/-0.03)
KM (μM)	1.97 (+/-0.55)
VMAX ($\mu mol/min/mg$)	1.19 (+/-0.09)
kcat (s^{-1})	1.02 (+/-0.28)
kcat/KM ($s^{-1} M^{-1}$)	519,490



H.s. IPMK on PIP2 micelles

curve fit (r^2)	0.988 (+/-0.05)
KM (μM)	36.4 (+/-6.1)
VMAX ($\mu mol/min/mg$)	3.45 (+/-0.21)
kcat (s^{-1})	2.97 (+/-0.18)
kcat/KM ($s^{-1} M^{-1}$)	81,511

Figure 3. Enzyme kinetic parameters of human IPMK activity on PIP_2 in SF-1 or micelles

(**A**) Kinetic parameters of human IPMK activity on PIP_2 bound by the nuclear receptor SF-1 [35]. (**B**) Kinetic parameters of human IPMK activity on PIP_2 in micelles [35]. Although SF-1 slows the enzyme down, SF-1 improves the apparent K_M, consistent with a PPI between IPMK and SF-1 facilitating IPMK phosphorylation of PIP_2 bound by SF-1.

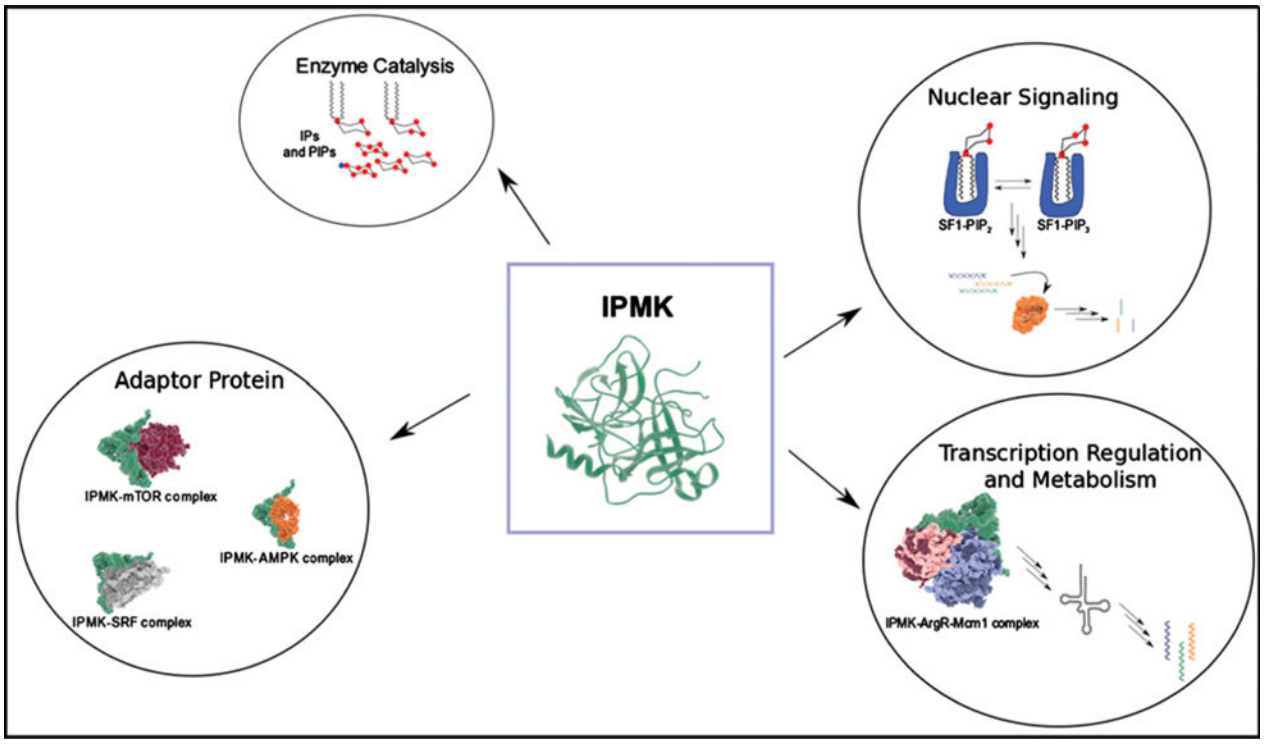


Figure 4. IPMK has many cell physiological roles

IPMK has well-established roles in inositol phosphate metabolism, in nuclear signalling through protein phosphoinositide complexes such as SF-1/PIP_2, as a transcriptional co-regulator and in the cytoplasm as an adapter protein mediating several metabolic responses. The central role of IPMK in such a broad array of cellular processes highlights its importance to all eukaryotic cells.