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# Inositol pyrophosphates as multifaceted metabolites in the regulation of mammalian signaling networks

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## ABSTRACT

Inositol pyrophosphates (PP-IPs) such as 5-diphosphoinositol pentakisphosphate (5-IP<sub>7</sub>) are inositol metabolites with high-energy phosphoanhydride bonds. The formation of PP-IPs is catalyzed by two groups of enzymes, the IP<sub>6</sub> kinases and the PPIP5 kinases, which both phosphorylate inositol hexakisphosphate (IP<sub>6</sub>). In mammals, PP-IPs are implicated in diverse biological phenomena including cellular growth, vesicular trafficking, apoptosis, and metabolic homeostasis. Mechanistically, all the diverse actions of PP-IPs proceed in one of two ways: the PP-IPs modulate the activity of their target proteins either through allosteric binding or protein pyrophosphorylation. In this review, we highlight recent advances in our understanding of the pleiotropic functions of the mammalian PP-IPs and the metabolic enzymes that produce them. We also discuss some future challenges in the exploration of areas where PP-IPs play important but unknown roles in physiology and disease.

## ARTICLE HISTORY

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Metabolism; inositol pyrophosphate; IP<sub>6</sub>K; PPIP5K; cell signaling

## Introduction

Since the early 1990s, more than 30 different inositol pyrophosphates (PP-IPs) have been identified as phosphorylated derivatives of myo-inositol, which is itself an isomer of cyclohexanehexol. Many of these inositol pyrophosphates play regulatory roles in controlling a wide range of biological processes such as telomere length control, vesicular trafficking, chemotaxis, and insulin signaling (Tsui and York 2010; Chakraborty et al. 2011; Wilson et al. 2013; Shah et al. 2017). The most well-studied PP-IPs in mammals are diphosphoinositol pentakisphosphate (PP-IP<sub>5</sub> or IP<sub>7</sub>) and bis-diphosphoinositol tetrakisphosphate, ([PP]<sub>2</sub>-IP<sub>4</sub> or IP<sub>8</sub>). These small, water-soluble molecules are produced by two classes of inositol kinases, the IP<sub>6</sub> kinases (IP<sub>6</sub>Ks) and the PPIP5 kinases (PPIP5Ks), which add high-energy phosphate groups to inositol hexakisphosphate (IP<sub>6</sub>). The steady-state cellular concentration of IP<sub>7</sub> in mammals varies from 0.5 to 5 μM (Stephens et al. 1993; Barker et al. 2004; Lin et al. 2009), and the level of IP<sub>8</sub> is typically less than half of the concentration of IP<sub>7</sub> (Glennon and Shears 1993; Albert et al. 1997). Due to their structural stability, high turnover rate, and high-energy phosphate bonds, PP-IPs have been referred to variously as ‘metabolic messengers’ (Shears 2009), ‘regulators of cell homeostasis’

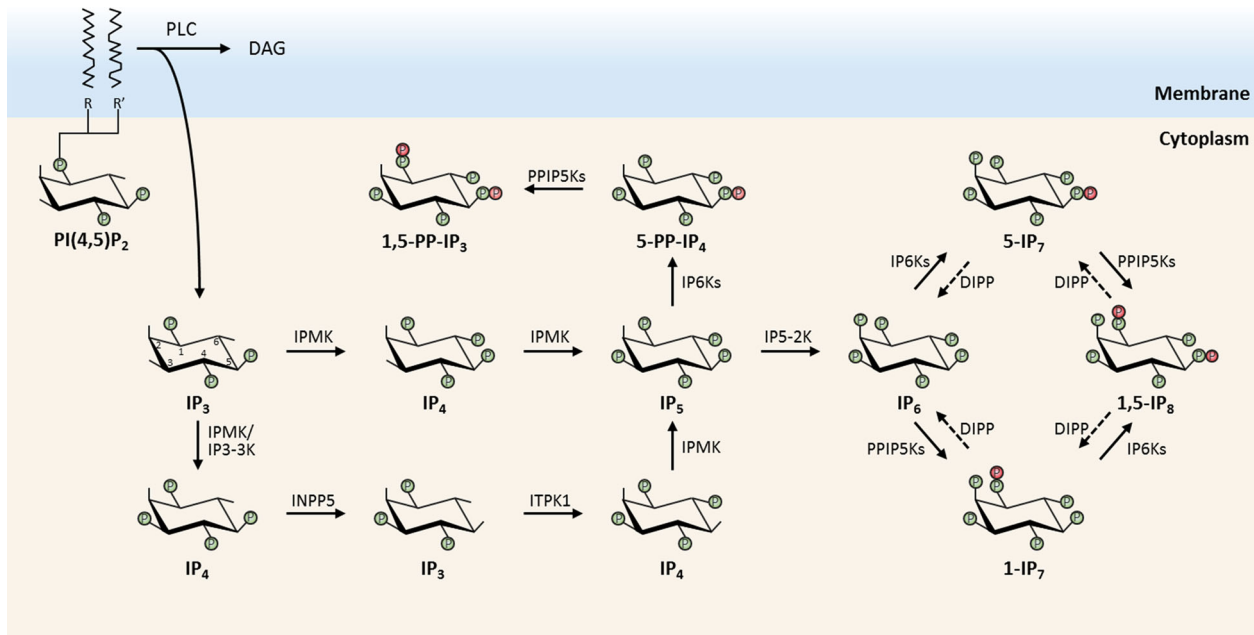
(Wundenberg and Mayr 2012), and ‘mammalian cell signals’ (Chakraborty et al. 2011).

## Biosynthesis of the inositol pyrophosphates

The synthesis of PP-IPs begins with the hydrolysis of PI (4,5)P<sub>2</sub> by phospholipase C (Irvine and Schell 2001). The product of this cleavage, Ins(1,4,5)P<sub>3</sub> (IP<sub>3</sub>), is then released into the cytosol. Inositol polyphosphate multikinase (IPMK) and the IP<sub>3</sub> 3-kinase then cooperate to serially phosphorylate IP<sub>3</sub>, producing IP<sub>4</sub> and IP<sub>5</sub> (Irvine and Schell 2001). IP<sub>5</sub> 2-kinase then phosphorylates the last of the six hydroxyl groups on the inositol ring to generate IP<sub>6</sub> from IP<sub>5</sub>. In mammals, three IP<sub>6</sub>Ks (i.e. IP<sub>6</sub>K1/2/3) form a phosphoanhydride bond by phosphorylating the 5 position of IP<sub>6</sub> to form 5-PP-IP<sub>5</sub> (5-IP<sub>7</sub>) (Saiardi et al. 1999). Two PPIP5Ks (i.e. PPIP5K1/2) can pyrophosphorylate the 1 or 3 position of IP<sub>6</sub> to produce 1-/3-PP-IP<sub>5</sub> (simply designated as 1-IP<sub>7</sub>) (Mulugu et al. 2007; Lin et al. 2009). IP<sub>8</sub> harbors diphosphate groups at both the 5 and 1/3 positions. IP<sub>8</sub> is generated via the combined action of the IP<sub>6</sub>Ks and PPIP5Ks (Wang et al. 2012). The de-phosphorylation of inositol pyrophosphates to IP<sub>6</sub> or IP<sub>5</sub> is catalyzed by the enzyme diphosphoinositol polyphosphate phosphohydrolase (DIPP)

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**Figure 1.** Inositol pyrophosphate biosynthetic pathway. This diagram shows the metabolic pathways of inositol pyrophosphate synthesis in mammalian cells. IP3 3-kinases or IPMK phosphorylates IP<sub>3</sub> to generate IP<sub>4</sub> [Ins(1,3,4,5)P<sub>4</sub> or Ins(1,4,5,6)P<sub>4</sub>]. Subsequent phosphorylation of IP<sub>4</sub> and IP<sub>5</sub> ultimately yields IP<sub>6</sub>. IP6Ks pyrophosphorylate the 5 position, generating 5-PP-IP<sub>4</sub> from IP<sub>5</sub> and 5-PP-IP<sub>5</sub> (5-IP<sub>7</sub>) from IP<sub>6</sub>. PPIP5Ks act upon the 1 or 3 position to produce 1-/3-PP-IP<sub>5</sub> (shown as 1-IP<sub>7</sub>) from IP<sub>6</sub>.

(Safrany and Shears 1998; Safrany et al. 1999; Caffrey et al. 2000; Kilari et al. 2013) (Figure 1). Compared to 5-IP<sub>7</sub>, 1-IP<sub>7</sub> seems to be a better substrate for DIPP (Lonetti et al. 2011; Kilari et al. 2013). This bias may be the reason mammalian cells produce higher levels of 5-IP<sub>7</sub> than 1-IP<sub>7</sub>.

### Dynamic turnover of PP-IPs

According to Shears and colleagues, 50% of the steady-state IP<sub>6</sub> pool and 20% of the steady-state IP<sub>5</sub> pool in mammalian cells are converted to PP-IPs every hour (Menniti et al. 1993). PP-IPs are also turned over rapidly. In primary hepatocytes, the IP<sub>7</sub> pool turns over every 4 min while the IP<sub>6</sub> turns over every 40 min (Glennon and Shears 1993). This presumably allows the levels of the PP-IPs to be tightly regulated in response to various cellular conditions. For example, ovarian cancer cells show a 4-fold increase in IP<sub>7</sub> upon treatment with staurosporine. In addition, several tumor cell lines show cell cycle-dependent modulation of IP<sub>7</sub> levels, and hepatocytes show a 2-fold elevation in IP<sub>7</sub> upon insulin stimulation. In mouse embryonic fibroblasts, serum starvation depletes IP<sub>7</sub> levels and hyperosmotic and thermal stress induce IP<sub>8</sub> levels (Pesesse et al. 2004). Thus, the regulation of the dynamic turnover of PP-IPs that is associated with various physiological stimuli suggests the PP-IPs play important roles in cellular and metabolic signaling.

### The modes of action of the inositol pyrophosphates

Two molecular mechanisms – allosteric non-covalent binding and covalent protein pyrophosphorylation – are thought to account for all of the diverse roles the inositol pyrophosphates play in modulating protein function and directing sequential biological events (Figure 2). The direct binding of an inositol phosphate to a specific protein effector was originally demonstrated in the discovery that the IP<sub>3</sub>-gated Ca<sup>2+</sup> release channel acts as an IP<sub>3</sub> receptor (Mikoshiba et al. 1993). Both 1-IP<sub>7</sub> and 5-IP<sub>7</sub> were found to modulate their targets through allosteric interactions (Wilson et al. 2013). As the inositol pyrophosphates cycle between binding and hydrolysis, their charges may also interfere with other protein-metabolite interactions (Shears 2015). For example, 5-IP<sub>7</sub> interacts with proteins containing pleckstrin homology (PH) domains, and PH domains are known to interact primarily with PI(3,4,5)P<sub>3</sub>. Akt kinase is the most well-studied 5-IP<sub>7</sub>-binding protein. Akt kinase is an important regulator of cellular growth and metabolism that is acutely activated by insulin and growth factors (Manning and Cantley 2007). To fully activate Akt signaling cascade, PI(3,4,5)P<sub>3</sub> binds and recruits Akt to the plasma membrane. 5-IP<sub>7</sub> inhibits Akt by competing with PI(3,4,5)P<sub>3</sub> for binding to Akt's PH domain at an IC<sub>50</sub> of 1 μM (Bhandari et al. 2008; Chakraborty et al. 2010). Using a photocage-based method, 5-IP<sub>7</sub> release was recently found to suppress

## Physiological functions

### IP6K1

- Insulin sensitivity & weight control
- Vesicular trafficking
- Insulin & neurotransmitter release
- Telomere length control
- HIV-VLP release
- DNA repair
- Epigenetic regulation
- Cell spreading & migration
- Spermiogenesis
- Blood clotting
- Psychiatric behavior
- Neuronal migration

### IP6K2

- Cell survival & apoptosis
- Cell migration & metastasis

### IP6K3

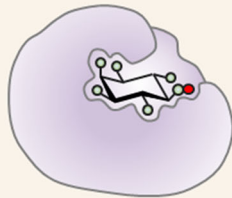
- Metabolic homeostasis & longevity

### PPIP5K1/2

- Cell migration
- Apoptosis
- Viral immunity

## Mode of actions

### 1. Allosteric regulation (binding)



#### PH domain

##### AKT

- Insulin secretion
- Neutrophil migration
- Phagocytic effect

#### Other protein targets

##### CK2

- Mammal apoptosis

##### Synaptotagmin 1

- Synaptic vesicular release

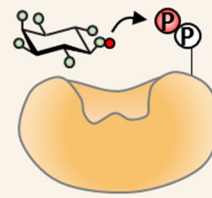
##### Pho81 (CKI)

- Transcriptional regulation

##### Vtc4

- Polyphosphate synthesis

### 2. Protein pyrophosphorylation



#### 5-IP<sub>7</sub>

##### NSR1

- Yeast nucleolar protein

##### GCR1

- Glycolysis

##### RNA polymerase I complex

- Ribosomal RNA synthesis

##### AP-3b1

- Viral particle release

##### Dynein

- Dynein-driven trafficking

#### 1-IP<sub>7</sub>

##### IRF3

- IFN production

**Figure 2.** Proposed modes of action for inositol pyrophosphates. A. Multiple functions of IP6Ks and PPIP5Ks are highlighted. Please see recent review articles for further details (Shah et al. 2017). B. Summary of two mechanisms of action elicited by PP-IPs. The allosteric binding between a PP-IP and a target protein (left) have been demonstrated from many proteins such as Akt (Chakraborty et al. 2010). Pyrophosphorylation of proteins (right) has shown the unique biochemical property of PP-IPs to drive covalent phosphotransfer reactions.

the translocation of Akt from the plasma membrane to the cytoplasm (Pavlovic et al. 2016). The PH domains of stress-activated map kinase-interacting protein 1 (SIN1) and general receptor for phosphoinositides 1 (GRP1) also

bind 5-IP<sub>7</sub> with higher affinity than 1-IP<sub>7</sub> and IP<sub>8</sub> (Gokhale et al. 2013). In addition, inositol pyrophosphates have been found to interact with protein partners that lack PH domains. In response to DNA damage, 5-IP<sub>7</sub>

produced by IP6K2 binds to the TTT complex and modulates its binding to casein kinase 2 (CK2) (Rao et al. 2014). This stabilizes both the DNA-dependent protein kinase catalytic subunit and ATM kinase. This, in turn, increases p53 phosphorylation and activates apoptosis (Rao et al. 2014). 5-IP7 also affects synaptic vesicle fusion during exocytosis through its direct interaction with synaptotagmin1 (Syt1). Syt1 is an essential calcium sensor in presynaptic termini that triggers the membrane fusion events required for neurotransmitter release (Rizo and Rosenmund 2008). Single-molecule imaging studies using reconstituted synaptic vesicles revealed that 5-IP7 is 10-fold more efficient in suppressing vesicle fusion than 1-IP7 or other inositol phosphates. Mechanistically, the dynamic, high-affinity binding of 5-IP7 to the polybasic region of Syt1 appears to hold Syt1 in a nonfunctional configuration (Lee et al. 2016). In budding yeast, 1-IP7 but not 5-IP7 interacts selectively with Pho80-Pho85-Pho81 in the regulation of phosphate homeostasis (Lee et al. 2007). No 1-IP7-specific protein targets in mammals have been discovered yet.

Inositol pyrophosphates also modulate protein function via the pyrophosphorylation of their target protein (Saiardi et al. 2004; Bhandari et al. 2007). Initially, the inositol pyrophosphates were thought to act as high-energy phosphate donors like ATP because of the energy in their phosphoanhydride bonds that is released along with their  $\beta$ -phosphates (Wilson et al. 2013). Indeed, the inositol pyrophosphates can transfer their  $\beta$ -phosphates non-enzymatically to pre-phosphorylated serine residues on nearby acidic amino acids of target proteins in the presence of magnesium. When incubated with cellular extracts prepared from yeast, mouse, and flies,  $^{32}\text{P}$ -labelled IP<sub>7</sub> is capable of transferring  $^{32}\text{P}$  to target proteins. Follow-up mass spectrometry studies revealed a role for CK2 in target protein pyrophosphorylation. CK2 provides phosphoprotein substrates for subsequent pyrophosphorylation because acidic amino acids fit nicely into the consensus motif for CK2. The pyrophosphate bonds formed on serine residues in target proteins appear acid labile, but they are resistant to most protein phosphatases. Further studies will be required to measure the reversibility of protein pyrophosphorylation and isolate any relevant bona fide phosphatases. Remarkably, protein pyrophosphorylation has already been linked to the regulation of diverse biological phenomena ranging from glycolysis (Szigyarto et al. 2011), rRNA synthesis (Thota et al. 2015), viral particle release (Azevedo et al. 2009), and dynein-driven trafficking (Chanduri et al. 2016). Although 5-IP<sub>7</sub>, 1-IP<sub>7</sub>, and even IP<sub>8</sub> show similar capacities for effecting protein pyrophosphorylation *in vitro*, the fact that 1-IP<sub>7</sub>, but not 5-IP<sub>7</sub> pyrophosphorylates and activates interferon regulatory

factor 3 (IRF3) suggests some degree of stereo-selectivity between the various PP-IPs (Pulloor et al. 2014).

## Conclusions

Over the past 20 years, substantial evidence has accumulated implicating PP-IPs and the enzymes that produce them in coordinating a diverse group of signaling and metabolic events. Despite significant advances in our understanding of the physiological functions of inositol pyrophosphates, many questions remain unanswered. For example, can we generalize the mode of interaction between the various PP-IPs and their protein targets? Wu et al. recently took a novel approach with chemically modified, non-hydrolysable IP affinity reagents to carry out a large-scale search for inositol polyphosphate-binding proteins. Using this approach, they identified vacuolar membrane polyphosphate polymerase (Vtc4) as a potential 5-IP<sub>7</sub> binding partner (Wu et al. 2016). Wild et al. further elucidated that Vtc4's positively charged SPX domain binds 5-IP<sub>7</sub>, leading to polyphosphate synthesis in yeast and plant (Wild et al. 2016). Developing innovative technology will be a great challenge for us to identify the endogenous PP-IP interactome in mammals.

The generation and analysis of mouse models harboring tissue-specific deletions of the IP6Ks or PPIP5Ks will surely improve our understanding of the roles inositol pyrophosphates play in the control of mammalian physiology. These genetic approaches, however, should be accompanied with more mechanistic studies of the known functions of the PP-IPs, IP6K1/2/3, and PPIP5Ks using cultured cells and other *in vitro* models. In addition to the more typical methods for analyzing the impact of elevated PP-IPs by overexpressing IP6Ks or PPIP5Ks in mammalian cells over several days, it has recently become possible to synthesize and deliver photocaged PP-IPs into live cells. This and other similar technological advances will allow a more comprehensive dissection of the cellular responses that depend on fine-tuned production and localization of the various PP-IPs.

Recently, SNP variations have been discovered in PP-IP metabolic pathways in disease patients (Crocco et al. 2016). Defining the functional contribution of such genetic variations in PP-IP metabolism will be critical to translate our knowledge into clinical applications. The quantification of inositol poly- and pyrophosphates as potential metabolic biomarkers has so far been possible only by monitoring radiolabeled [ $^3\text{H}$ ]-inositol or [ $^{32}\text{P}$ ]-phosphate via anion exchange HPLC (Azevedo and Saiardi 2006). In the future, we hope to improve the detection of PP-IPs directly from patient cells and tissue samples. It will be possible for us to apply and further

develop the techniques of Saiardi et al. who showed that PP-IPs are efficiently enriched on titanium oxide beads and can be visualized using the fluorescent dye 4',6-diamidino-2-phenylindole (DAPI) (Wilson et al. 2015).

The multifunctionality of PP-IP metabolic pathway form a complex regulatory web when we include signaling actions of IPMK, another promiscuous inositol kinase involved in the production of IP<sub>4</sub>, IP<sub>5</sub>, and PIP<sub>3</sub> (Chakraborty et al. 2011; Kim et al. 2016). For example, IP6Ks and their product, 5-IP<sub>7</sub>, participate in the negative feedback regulation of Akt, whereas IPMK's lipid kinase contributes to the full activation of Akt via PIP<sub>3</sub>. The extensive interface between IP6K2 and IPMK can be also found from the regulation of tumor suppressor p53. In contrast to IPMK that directly binds to p53 and activates its transactivation functions in both apoptosis and cell cycle arrest, IP6K2-mediated 5-IP<sub>7</sub> favors p53-dependent transcription toward apoptosis than cell cycle arrest. It will be thus important to clarify the extent to which signaling and metabolic networks related to the IP6Ks, PPIP5Ks, and IPMK interact or overlap.

As further efforts are made to study PP-IPs in various tissues – both normal and diseased – and as more of their mechanisms of signaling action are revealed, we will eventually discover the full range of the physiological functions of the PP-IPs and the metabolic enzymes that produce them. We strongly believe that this improved understanding of the roles for PP-IPs in mammalian cellular signaling networks will direct the development of novel therapeutic strategies and diagnostic tools for major human diseases like diabetes and cancer.

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We feel sorry for not citing the work of many other PP-IP investigators owing to space constraints. We thank all the members of the Kim lab for discussion and critical comments.

## Disclosure statement

No potential conflict of interest was reported by the authors.

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## References

Albert C, Safrany ST, Bembenek ME, Reddy KM, Reddy K, Falck J, Bröcker M, Shears SB, Mayr GW. 1997. Biological variability in the structures of diphosphoinositol polyphosphates in *Dictyostelium Discoideum* and mammalian cells. *Biochem J*. 327:553–560. doi:10.1042/bj3270553

- Azevedo C, Burton A, Ruiz-Mateos E, Marsh M, Saiardi A. 2009. Inositol pyrophosphate mediated pyrophosphorylation of AP3B1 regulates HIV-1 Gag release. *Proc Natl Acad Sci USA*. 106:21161–21166. doi:10.1073/pnas.0909176106
- Azevedo C, Saiardi S. 2006. Extraction and analysis of soluble inositol polyphosphates from yeast. *Nat Protoc*. 1:2416–2422. doi:10.1038/nprot.2006.337
- Barker CJ, Wright J, Hughes PJ, Kirk CJ, Michell RH. 2004. Complex changes in cellular inositol phosphate complement accompany transit through the cell cycle. *Biochem J*. 380:465–473. doi:10.1042/BJ20031872
- Bhandari R, Juluri KR, Resnick AC, Snyder SH. 2008. Gene deletion of inositol hexakisphosphate kinase 1 reveals inositol pyrophosphate regulation of insulin secretion, growth, and spermiogenesis. *Proc Natl Acad Sci USA*. 105:2349–2353. doi:10.1073/pnas.0712227105
- Bhandari R, Saiardi A, Ahmadibeni Y, Snowman AM, Resnick AC, Kristiansen TZ, Molina H, Pandey A, Werner JK, Juluri KR, et al. 2007. Protein pyrophosphorylation by inositol pyrophosphates is a posttranslational event. *Proc Natl Acad Sci USA*. 104:15305–15310. doi:10.1073/pnas.0707338104
- Caffrey JJ, Safrany ST, Yang X, Shears SB. 2000. Discovery of molecular and catalytic diversity among human diphosphoinositol-polyphosphate phosphohydrolases. An expanding NUDT family. *J Biol Chem*. 275:12730–12736. doi:10.1074/jbc.275.17.12730
- Chakraborty A, Kim S, Snyder SH. 2011. Inositol pyrophosphates as mammalian cell signals. *Sci Signal*. 4:re1. doi:10.1126/scisignal.2001958
- Chakraborty A, Koldobskiy MA, Bello NT, Maxwell M, Potter JJ, Juluri KR, Maag D, Kim S, Huang AS, Dailey MJ, et al. 2010. Inositol pyrophosphates inhibit Akt signaling, thereby regulating insulin sensitivity and weight gain. *Cell*. 143:897–910. doi:10.1016/j.cell.2010.11.032
- Chanduri M, Rai A, Malla AB, Wu M, Fiedler D, Mallik R, Bhandari R. 2016. Inositol hexakisphosphate kinase 1 (IP6K1) activity is required for cytoplasmic dynein-driven transport. *Biochem J*. 473:3031–3047. doi:10.1042/BCJ20160610
- Crocco P, Saiardi A, Wilson MS, Maletta R, Bruni AC, Passarino G, Rose G. 2016. Contribution of polymorphic variation of inositol hexakisphosphate kinase 3 (IP6K3) gene promoter to the susceptibility to late onset Alzheimer's disease. *Biochim Biophys Acta*. 1862:1766–1773.
- Glennon MC, Shears SB. 1993. Turnover of inositol pentakisphosphates, inositol hexakisphosphate and diphosphoinositol polyphosphates in primary cultured hepatocytes. *Biochem J*. 293:583–590. doi:10.1042/bj2930583
- Gokhale NA, Zaremba A, Janoshazi AK, Weaver JD, Shears SB. 2013. PPIP5K1 modulates ligand competition between diphosphoinositol polyphosphates and PtdIns(3,4,5)P<sub>3</sub> for polyphosphoinositide-binding domains. *J Biol Chem*. 288:413–426. doi:10.1042/BJ20121528
- Irvine RF, Schell MJ. 2001. Back in the water: the return of the inositol phosphates. *J Palliat Med*. 2:327–338. doi:10.1038/35073015
- Kilari RS, Weaver JD, Shears SB, Safrany ST. 2013. Understanding inositol pyrophosphate metabolism and function: kinetic characterization of the DIPPs. *FEBS Lett*. 587:3464–3470. doi:10.1016/j.febslet.2013.08.035
- Kim E, Beon J, Lee S, Park J, Kim S. 2016. IPMK: a versatile regulator of nuclear signaling events. *Adv Biol Regul*. 61:25–32. doi:10.1016/j.jbior.2015.11.005

- Lee TS, Lee JY, Kyung JW, Yang Y, Park SJ, Lee S, Pavlovic I, Kong B, Jho YS, Jessen HJ, et al. 2016. Inositol pyrophosphates inhibit synaptotagmin-dependent exocytosis. *Proc Natl Acad Sci USA*. 113:8314–8319. doi:10.1073/pnas.1521600113
- Lee YS, Mulugu S, York JD, O'Shea EK. 2007. Regulation of a Cyclin-CDK-CDK inhibitor complex by inositol pyrophosphates. *Science*. 316:109–112. doi:10.1126/science.1139080
- Lin H, Fridy PC, Ribeiro AA, Choi JH, Barma DB, Vogel G, Falck JR, Shears SB, York JD, Mayr GW. 2009. Structural analysis and detection of biological inositol pyrophosphates reveal that the family of VIP/diphosphoinositol pentakisphosphate kinases are 1/3-kinases. *J Biol Chem*. 284:1863–1872. doi:10.1074/jbc.M805686200
- Lonetti A, Szigyarto Z, Bosch D, Loss O, Azevedo C, Saiardi A. 2011. Identification of an evolutionarily conserved family of inorganic polyphosphate endopolyphosphatases. *J Biol Chem*. 286:31966–31974. doi:10.1074/jbc.M111.266320
- Manning BD, Cantley LC. 2007. AKT/PKB signaling: navigating downstream. *Cell*. 129:1261–1274.
- Menniti FS, Miller NR, Putney JW, Shears SB. 1993. Turnover of inositol polyphosphate pyrophosphates in pancreaticoma cells. *J Biol Chem*. 268:3850–3856.
- Mikoshiha K, Furuichi T, Miyawaki A, Yoshikawa S, Nakade S, Michikawa T, Nakagawa T, Okano H, Kume S, Muto A, et al. 1993. Structure and function of inositol 1,4,5-trisphosphate receptor. *Ann N Y Acad Sci*. 707:178–197.
- Mulugu S, Bai W, Fridy PC, Bastidas RJ, Otto JC, Dollins DE, Haystead TA, Ribeiro AA, York JD. 2007. A conserved family of enzymes that phosphorylate inositol hexakisphosphate. *Science*. 316:106–109. doi:10.1126/science.1139099
- Pavlovic I, Thakor DT, Vargas JR, McKinlay CJ, Hauke S, Anstaett P, Camuña RC, Bigler L, Gasser G, Schultz C, et al. 2016. Cellular delivery and photochemical release of a caged inositol-pyrophosphate induces PH-domain translocation in cellulose. *Nat Comm*. 7:10622. doi:10.1038/ncomms10622
- Pesesse X, Choi K, Zhang T, Shears SB. 2004. Signaling by higher inositol polyphosphates: synthesis of bisdiphosphoinositol tetrakisphosphate ('InsP8') is selectively activated by hyperosmotic stress. *J Biol Chem*. 279:43378–43381. doi:10.1074/jbc.C400286200
- Pulloor NK, Nair S, Kostic AD, Bist P, Weaver JD, Riley AM, Tyagi R, Uchil PD, York JD, Snyder SH, et al. 2014. Human genome-wide RNAi screen identifies an essential role for inositol pyrophosphates in type-I interferon response. *PLoS Pathog*. 10:e1003981. doi:10.1371/journal.ppat.1003981
- Rao F, Chav J, Xu J, Xu R, Vandiver S, Tyagi R, Tokhunts R, Koldobskiy MA, Fu C, Barrow R, et al. 2014. Inositol pyrophosphates mediate the DNA-PK/ATM-p53 cell death pathway by regulating CK2 phosphorylation of Tti1/Tel2. *Mol Cell*. 54:119–132. doi:10.1016/j.molcel.2014.02.020
- Rizo J, Rosenmund C. 2008. Synaptic vesicle fusion. *Nat Struct Mol Biol*. 15:665–674.
- Safrany ST, Ingram SW, Cartwright JL, Falck JR, McLennan AG, Barnes LD, Shears SB. 1999. The diadenosine hexaphosphate hydrolases from *schizosaccharomyces pombe* and *Saccharomyces Cerevisiae* are homologues of the human diphosphoinositol polyphosphate phosphohydrolase. *J Biol Chem*. 274:21735–21740.
- Safrany ST, Shears SB. 1998. Turnover of bis-diphosphoinositol tetrakisphosphate in a smooth muscle cell line is regulated by  $\beta$ 2-Adrenergic receptors through a cAMP-mediated, A-kinase-independent Mechanism. *EMBO J*. 17:1710–1716. doi:10.1093/emboj/17.6.1710
- Saiardi A, Bhandari R, Resnick AC, Snowman AD, Snyder SH. 2004. Phosphorylation of proteins by inositol pyrophosphates. *Science*. 306:2101–2105. doi:10.1126/science.1103344
- Saiardi A, Erdjument-Bromage H, Snowman AD, Tempst P, Snyder SH. 1999. Synthesis of diphosphoinositol pentakisphosphate by a newly identified family of higher inositol polyphosphate kinases. *Curr Biol*. 9:1323–1326. doi:10.1016/S0960-9822(00)80055-X
- Shah A, Ganguli S, Sen J, Bhandari R. 2017. Inositol pyrophosphates: energetic, omnipresent and versatile signalling molecules. *J Indian Inst Sci*. 97:23–40. doi:10.1007/s41745-016-0011-3
- Shears SB. 2009. Diphosphoinositol polyphosphates: metabolic messengers? *Mol Pharmacol*. 76:236–252. doi:10.1124/mol.109.055897
- Shears SB. 2015. Inositol pyrophosphates: why so many phosphates? *Adv Biol Regul*. 57:203–216. doi:10.1016/j.jbior.2014.09.015
- Stephens L, Radenberg T, Thiel U, Vogel G, Khoo KH, Dell A, Jackson TR, Hawkins PT, Mayr GW. 1993. The detection, purification, structural characterization, and metabolism of diphosphoinositol pentakisphosphate(s) and bisdiphosphoinositol tetrakisphosphate(s). *J Biol Chem*. 268:4009–4015.
- Szigyarto Z, Garedew A, Azevedo C, Saiardi A. 2011. Influence of inositol pyrophosphates on cellular energy dynamics. *Science*. 334:802–805. doi:10.1126/science.1211908
- Thota SG, Unnikannan CP, Thampatty SR, Manorama R, Bhandari R. 2015. Inositol pyrophosphates regulate RNA polymerase I-mediated rRNA transcription in *Saccharomyces Cerevisiae*. *Biochem J*. 466:105–114. doi:10.1042/BJ20140798
- Tsui MM, York JD. 2010. Roles of inositol phosphates and inositol pyrophosphates in development, cell signaling and nuclear processes. *Adv Enzyme Regul*. 50:324–337. doi:10.1016/j.advenzreg.2009.12.002.ROLES
- Wang H, Falck JR, Hall TMT, Shears SB. 2012. Structural basis for an inositol pyrophosphate kinase surmounting phosphate crowding. *Nat Chem Biol*. 8:111–116. doi:10.1038/nchembio.733.Structural
- Wild R, Gerasimaite R, Jung JY, Truffault V, Pavlovic I, Schmidt A, Saiardi A, Jessen HJ, Poirier Y, Hothorn M, et al. 2016. Control of eukaryotic phosphate homeostasis by inositol polyphosphate sensor domains. *Science*. 352:986–990. doi:10.1126/science.aad9858
- Wilson MSC, Bulley SJ, Pisani F, Irvine RF, Saiardi A. 2015 A novel method for the purification of inositol phosphates from biological samples reveals that no phytate is present in human plasma or urine. *Open Biol*. 5:150014. doi:10.1098/rsob.150014
- Wilson MSC, Livermore TM, Saiardi A. 2013. Inositol pyrophosphates: between signalling and metabolism. *Biochem J*. 452:369–379. doi:10.1042/BJ20130118
- Wu M, Chong LS, Perlman DH, Resnick AC, Fiedler D. 2016. Inositol polyphosphates intersect with signaling and metabolic networks via two distinct mechanisms. *Proc Natl Acad Sci USA*. 113:E6757–E6765. doi:10.1073/pnas.1606853113
- Wundenberg T, Mayr GW. 2012. Synthesis and biological actions of diphosphoinositol phosphates (inositol pyrophosphates), regulators of cell homeostasis. *Biol Chem*. 393:979–998. doi:10.1515/hsz-2012-0133