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REVIEW

Functions, Mechanisms, and therapeutic applications of the inositol pyrophosphates 5PP-InsP_5 and InsP_8 in mammalian cells

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Abstract

Water-soluble *myo*-inositol phosphates have long been characterized as second messengers. The signaling properties of these compounds are determined by the number and arrangement of phosphate groups on the *myo*-inositol backbone. Recently, higher inositol phosphates with pyrophosphate groups were recognized as signaling molecules. 5-Diphosphoinositol 1,2,3,4,6-pentakisphosphate (5PP-InsP_5) is the most abundant isoform, constituting more than 90% of intracellular inositol pyrophosphates. 5PP-InsP_5 can be further phosphorylated to 1,5-bisdiphosphoinositol 2,3,4,6-tetrakisphosphate (InsP_8). These two molecules, 5PP-InsP_5 and InsP_8, are present in various subcellular compartments, where they participate in regulating diverse cellular processes such as cell death, energy homeostasis, and cytoskeletal dynamics. The synthesis and metabolism of inositol pyrophosphates are subjected to tight regulation, allowing for their highly specific functions. Blocking the 5PP-InsP_5/InsP_8 signaling pathway by inhibiting the biosynthesis of 5PP-InsP_5 demonstrates therapeutic benefits in preclinical studies, and thus holds promise as a therapeutic approach for certain diseases treatment, such as metabolic disorders.

Keywords Inositol pyrophosphate · IP6K · PPIP5K · InsP_7 · InsP_8

Introduction

Water soluble *myo*-inositol phosphates are a group of small molecules, with Ins(1,4,5)P_3 being the most well-studied. Ins(1,4,5)P_3 derives from the hydrolysis of the membrane lipid phosphatidylinositol 4,5-bisphosphate (PtdIns(4,5)P_2). Acting as a "second messenger", Ins(1,4,5)P_3 binds to its receptor, a Ca^{2+} channel in the endoplasmic reticulum. This binding opens the Ca^{2+} channel, leading to the release of Ca^{2+} from the intracellular store, which is necessary for the control of cellular and physiological processes including cell division, cell proliferation, apoptosis, fertilization, development, behavior, learning, and memory. Ins(1,4,5)P_3 is further metabolized to generate a series of inositol phosphate molecules by inositol phosphate kinases (Fig. 1) or phosphatases to recycle these molecules back to inositol.

Inositol polyphosphate multikinase (IPMK) phosphorylates Ins(1,4,5)P_3 to synthesize inositol 1,3,4,5-tetrakisphosphate (Ins(1,3,4,5)P_4) and inositol 1,3,4,5,6-pentakisphosphate (Ins(1,3,4,5,6)P_5) [1]. Both Ins(1,3,4,5)P_4 and Ins(1,3,4,5,6)P_5 are vital for regulating signaling pathways, for example, Ins(1,3,4,5)P_4 antagonizes the PI3K pathway [2], and Ins(1,3,4,5,6)P_5 regulates angiogenesis by modulating HIF1α/VEGF protein levels [3]. IPMK is essential for the synthesis of

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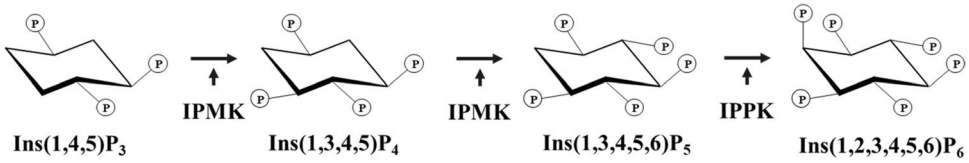

Fig. 1 Metabolic pathway of $Ins(1,4,5)P_3$ to $Ins(1,2,3,4,5,6)P_6$. $Ins(1,4,5)P_3$ is phosphorylated by IPMK to form $Ins(1,3,4,5)P_4$, which can be further phosphorylated by IPMK to form $Ins(1,3,4,5,6)P_5$. IPPK phosphorylates $Ins(1,3,4,5,6)P_5$ to generate $Ins(1,2,3,4,5,6)P_6$. IPMK, inositol polyphosphate multikinase. IPPK, inositol pentakisphosphate 2-kinase

$Ins(1,3,4,5,6)P_5$ but not $Ins(1,3,4,5)P_4$, because deleting IPMK depletes only $Ins(1,3,4,5,6)P_5$ [3, 4]. Knockout of IPMK in Drosophila causes developmental defects in the epidermis [5], and in mice leads to embryonic death [4], indicating that IPMK and $Ins(1,3,4,5,6)P_5$ are essential for life development.

$Ins(1,3,4,5,6)P_5$ is phosphorylated by inositol pentakisphosphate 2-kinase (IPPK) to form inositol-1,2,3,4,5,6-hexakisphosphate ($InsP_6$), which is the most abundant inositol phosphate in nature. $InsP_6$ has a phosphate group attached to each of the inositol's six hydroxyl groups and acts as an important phosphate store in plants. In mammalian cells, $InsP_6$ mediates diverse cellular functions, including DNA reparation, endocytosis, mRNA export, and ion channel regulation [6]. Depleting $InsP_6$ by knocking out IPPK is lethal in mice, highlighting its necessity for in vivo survival [7].

The "fully phosphorylated" $InsP_6$ was once thought to be the highest inositol phosphate. The identification of higher inositol phosphates with more than six phosphate groups indicates that inositol polyphosphates containing diphosphate or pyrophosphate groups exist in cells [8]. The concentrations of inositol pyrophosphates are in the micromolar range in mammalian cells. In hepatocytes, the cellular pools of inositol pyrophosphates are normally turning over at least 10 times every 40 min [9]. Similarly, in pancreatoma cells, 50% of the $InsP_6$ pool is converted to pyrophosphate derivatives within 60 min [10]. The distinctive features of these inositol pyrophosphates, such as highly energic pyrophosphate bonds and rapid turnover, suggest an important role in signaling transduction and metabolism [8]. Indeed, these molecules manifest diverse functions, such as regulation of energy homeostasis [11] and protein stability [12].

5-Diphosphoinositol 1,2,3,4,6-pentakisphosphate (5PP-$InsP_5$ or 5-$InsP_7$) is the first and most extensively studied inositol pyrophosphate. It is soluble in water and can be found in the nucleus, cytosol, and at the cell membrane. In mammalian cells, 5PP-$InsP_5$ is synthesized by a family of three inositol hexakisphosphate kinases (IP6Ks) in its functioning areas. This molecule is involved in various cellular processes, including DNA repair, mRNA processing, vesicle trafficking, and cytoskeleton reorganization [13]. It can be further phosphorylated by diphosphoinositol pentakisphosphate kinases (PPIP5Ks) at the 1-position of the inositol ring to form 1,5-bisdiphosphoinositol 2,3,4,6-tetrakisphosphate ($InsP_8$) (Fig. 2), which is the most phosphorylated and the "final metabolite" of inositol phosphate discovered so far. Although the concentration of $InsP_8$ is only around 10% of that of the total 5PP-$InsP_5$, $InsP_8$ plays critical roles in cellular activities, such as the regulation of ATP synthesis [11].

$InsP_6$ can also be pyrophosphorylated by PPIP5Ks at the 1-position of the inositol ring to form 1-diphosphoinositol 2,3,4,5,6-pentakisphosphate (1PP-$InsP_5$ or 1-$InsP_7$). $Ins(1,3,4,5,6)P_5$ can be pyrophosphorylated by IP6Ks at the 5-position of the inositol ring to generate

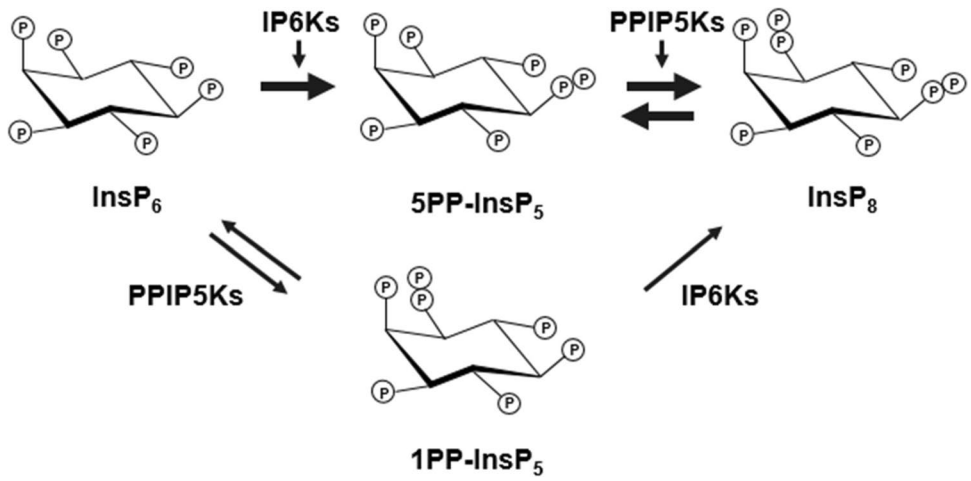

Fig. 2 Metabolic pathways of 5PP-$InsP_5$ and $InsP_8$. $InsP_6$ is mainly phosphorylated by IP6Ks to form 5PP-$InsP_5$, which is phosphorylated by PPIP5Ks to generate $InsP_8$. $InsP_8$ can be dephosphorylated back to 5PP-$InsP_5$ by PPIP5Ks. A minor portion of $InsP_6$ is phosphorylated by PPIP5Ks to form 1PP-$InsP_5$, which can be dephosphorylated back to $InsP_6$ by PPIP5Ks. 1PP-$InsP_5$ can also be phosphorylated by IP6Ks to generate $InsP_8$

5PP-Ins(1,3,4,6)P_4. It is worth noting that both 1PP-InsP_5 and 5PP-Ins(1,3,4,6)P_4 are considered minor inositol pyrophosphates, as their cellular concentrations are far lower than those of 5PP-InsP_5. 1PP-InsP_5 is typically < 2% of total InsP_7 in HCT116 cells [14]. Several functions of 1PP-InsP_5 and 5PP-Ins(1,3,4,6)P_4 have been demonstrated in budding yeast, where 1PP-InsP_5 regulates gene transcription [15–17] and 5PP-Ins(1,3,4,6)P_4 regulates cell death and telomere length [18, 19]. However, their roles in mammalian cells are yet to be determined.

Recently, with the development of a portfolio of detection methods that can assay mass levels of inositol pyrophosphates in cells from diverse species [20–22], new inositol pyrophosphate isomers such as the 3PP-InsP_5 (or 3-InsP_7), 4/6PP-InsP_5 (or 4/6-InsP_7) and 2PP-InsP_5 (2-InsP_7) have been identified in plant, mouse and in human peripheral blood mononuclear cells [23–25]. These findings suggest that inositol pyrophosphate signaling appears more complex than previously thought. The metabolic pathway and bioactive properties of these newly identified isomers, namely 3PP-InsP_5, 4/6PP-InsP_5, and 2PP-InsP_5, are yet to be delineated and are not the focus of this review.

5PP-InsP_5/InsP_8 appears to be the major metabolic pathway of the inositol pyrophosphates in mammalian cells because the combined amount of 5PP-InsP_5 and InsP_8 surpasses 95% of total inositol pyrophosphates. InsP_6 is mainly phosphorylated by IP6Ks to form 5PP-InsP_5, which accounts for more than 90% of the total InsP_7. Although InsP_6 can be phosphorylated by PPIP5Ks to form 1PP-InsP_5, PPIP5Ks prefer to phosphorylate 5PP-InsP_5 in vivo to form InsP_8, which can explain the low cellular levels of 1PP-InsP_5. It should be noted that these molecules also have important roles in fungi and plants, but due to the focus of this review, they will not be discussed. For a comprehensive review of inositol pyrophosphates in plants and fungi, please refer to REF [26, 27].

5PP-InsP_5 does not seem to freely move intracellularly. It is produced by IP6K1, IP6K2, and IP6K3 in compartmentalized subcellular areas where these kinases are located, and it functions at the sites where it is generated. Deletion of individual IP6K in the mouse does not cause noticeable defects. This characteristic makes the IP6K knockout mouse a valuable model for studying the in vivo functions of each IP6K and the 5PP-InsP_5 it produces. However, whether 5PP-InsP_5 is essential for embryonic development is currently unknown because there are no *IP6K1/IP6K2/IP6K3* triple knockout animals available.

InsP_8 is also produced in compartmentalized subcellular areas by PPIP5K1 and PPIP5K2 in mammalian cells. Since InsP_8 is primarily derived from 5PP-InsP_5, it likely operates in the same cellular microenvironments as 5PP-InsP_5 and may participate in similar cellular processes. The role of InsP_8 in embryonic development is currently not known, as there are no *PPIP5K1/PPIP5K2* double KO animals available.

Several inhibitors of IP6K have been developed [28–31], and have demonstrated therapeutic benefits in animal models, such as lowering body weight [32], reducing blood glucose [30], and attenuating myocardial injury [29]. These findings suggest that targeting the 5PP-InsP_5/InsP_8 pathway by blocking 5PP-InsP_5 biosynthesis may represent a viable therapeutic strategy for treating certain diseases. However, adverse effects are recorded in genetically IP6K knockout mice. For example, deletion of IP6K1 leads to neurodevelopmental and male reproductive disorders [33–35], deletion of IP6K2 affects neuronal development [36] and increases the risk of cancer development [37], and deletion of IP6K3 causes neurodevelopmental defects [38, 39]. Therefore, a comprehensive understanding of the functions and mechanisms of 5PP-InsP_5, InsP_8, and their synthetic enzymes is crucial before they can be considered druggable targets.

In this review, we focus on recent advancements in the biology of 5PP-InsP_5, InsP_8, and their synthetic enzymes in mammalian cells. These findings provide new insights into these molecules and may provide novel targets for future therapeutic targets.

Physiological Functions and Mechanisms of 5PP-InsP_5 and InsP_8

5PP-InsP_5 and InsP_8 were identified three decades ago. Myo-[^{3}H]inositol and/or [32]Pi were applied to cells to label newly synthesized inositol phosphates, which were separated by HPLC. The inositol pyrophosphate compounds were eluted from various forms of anion exchange HPLC columns after InsP_6. The detailed structures of these molecules were identified as diphosphoinositol pentakisphosphate (PP-InsP_5 or InsP_7) and bis-diphosphoinositol tetrakisphosphate (InsP_8) [8]. The cytosol contains approximately 85% of 5PP-InsP_5 and InsP_8 [8]. In mammalian cells, the physiological concentration of 5PP-InsP_5 is around 5 μM, while InsP_8 is less than 0.5 μM [40]. Recently, the basal level of 5PP-InsP_5 in rat blood has been determined to be 37.4 ng/mL [20].

Physiological Functions and Mechanisms of 5PP-InsP_5

The functions and molecular mechanisms of 5PP-InsP_5 have been elucidated through models involving genetic knockout of and pharmacological inhibition of IP6Ks (Table 1). Blocking the biosynthesis of 5PP-InsP_5 reduces 90% of total InsP_7 levels, suggesting that 5PP-InsP_5 cannot be compensated by other PP-InsP_5 isomers [28]. Furthermore, genetically knocking out of individual IP6K lowers total 5PP-InsP_5 levels, indicating that 5PP-InsP_5 does not

Table 1 Functions and mechanisms of intracellular 5PP-InsP$_5$

Subcellular areas	Functions	Mechanisms
Plasma membrane	Antagonizes phosphatidylinositol-3,4,5-triphosphate (PtdIns(3,4,5)P$_3$) [49] Regulates synaptic membrane vesicle trafficking [51–53] Regulates membrane proteins activity and stability, such as XPR1 and Na^+/K^+-ATPase [12, 60] Regulates insulin secretion [54–57] Regulates focal adhesion dynamics [33, 39, 42, 62]	Competes with PtdIns(3,4,5)P$_3$ to bind the pleckstrin homology (PH) domain of its target proteins, such as Akt [49] Inhibits synaptotagmin-dependent exocytosis and blocks adaptor protein-mediated synaptic vesicle recycling [51–53] Activates XPR1 by binding to the SPX domain [60] Binds PI3K p85α to promote its interaction with Na^+/K^+-ATPase, which recruits adaptor protein 2 and triggers Na^+/K^+-ATPase endocytosis [12] Regulates the fusion of insulin-containing vesicles with the cell membrane [57], and Ca^{2+} oscillations [58] Binds FAK at its FERM domain to promote FAK dimer formation and phosphorylation [46]
Cytosol	Regulates intracellular ATP levels [60, 63] Regulates mitochondrial biogenesis and function [63] Regulates cytoskeletal reorganization [46] Regulates cytoplasmic dynein-driven vesicle transport [45] Regulates mRNA stability and dynamics of processing-body [69, 70]	Affects GCR1 activity, and inhibits glycolysis [63] Deleting 5PP-InsP$_5$ in mouse embryonic fibroblasts impairs mitochondria function [63], but enhances mitochondrial biogenesis in hepatocytes, adipocytes, and cardiomyocytes by activating the AMPK pathway and reducing the acetylation state of PGC-1α [29] Binds to the Arp2/3 complex and recruits coronin, which promotes disassembly of branched actin networks [46] Promotes dynein complex assembly by enhancing the binding between dynein intermediate chain and p150glued [39, 45] Competes with 5′-capped mRNA for hydrolysis by NUDT3 and thus inhibits NUDT3-mediated mRNA decapping [69, 70]
Nucleus	Promotes p53-mediated apoptosis [72] Affects cell survival and growth [44] Regulates DNA damage repair [76, 77] Regulates genes transcription [78–80] Modulates nucleolus architecture [81]	Binds CK2 to stabilize DNA-PKcs and ATM to stimulate p53 phosphorylation [72] Pyrophosphorylates MYC and recruits the E3 ubiquitin ligase FBW7 to regulate MYC protein stability [44] Promotes the disassembly of the CRL4-CSN to activate CRL4-mediated nucleotide excision repair [76, 77] Induces the disassociation of JMJD2C with chromatin [79] Binds multiple nucleolar proteins [82]

freely diffuse but rather operates within its specific generating area. Additionally, the functions of individual IP6K cannot be compensated by other isoforms [39, 41, 42]. 5PP-InsP$_5$ performs its functions by binding or pyrophosphorylating target proteins, which play crucial roles in nucleotide metabolism, glucose metabolism, ribosome biogenesis, and phosphorylation-based signal transduction pathways [13, 43]. This posttranslational modification of proteins through pyrophosphorylation by 5PP-InsP$_5$ is essential for regulating their functions. [44, 45]. Recent studies have highlighted the importance of 5PP-InsP$_5$-mediated protein pyrophosphorylation in facilitating protein dimer formation, such as focal adhesion kinase (FAK) dimer formation and interferon regulatory factor 3 (IRF3) dimer formation. [46, 47]. While protein pyrophosphorylation was previously believed to be solely a non-enzymatic post-translational modification [48], a recent study has identified the metabolic enzyme UAP1 as a pyrophosphorylase responsible for catalyzing 5PP-InsP$_5$-dependent pyrophosphorylation of S386 on IRF3 [47]. This finding challenges the previous notion and raises the possibility of other instances of enzymatic pyrophosphorylation. Further research is necessary to explore these potentials.

Physiological Functions and Mechanisms of 5PP-InsP$_5$ at the Plasma Membrane

A prominent function of 5PP-InsP$_5$ at the cell membrane is to antagonize phosphatidylinositol-3,4,5-triphosphate (PtdIns(3,4,5)P$_3$). Structurally resembling the inositol head group of PtdIns(3,4,5)P$_3$, 5PP-InsP$_5$ competes with it for binding to the pleckstrin homology (PH) domain of its target proteins [49]. One well-characterized signaling pathway regulated by 5PP-InsP$_5$ is the PDK1-dependent activation of Akt [41]. Binding to the PH domain of Akt, 5PP-InsP$_5$ prevents PtdIns(3,4,5)P$_3$-induced membrane translocation of Akt, and thus Akt remains unphosphorylated [49]. Depleting 5PP-InsP$_5$ strengthens Akt activation [41]. Synthetic methylene-bisphosphonate analogues of 5PP-InsP$_5$, which structurally mimic it but cannot donate its β-phosphoryl groups to protein substrates, can inhibit Akt activation to

the same extent as 5PP-$InsP_5$, indicating that 5PP-$InsP_5$ does not inhibit Akt through pyrophosphorylation [50].

Transporting molecules in or out of cells is a fundamental cellular process. In neuronal cells, 5PP-$InsP_5$ regulates synaptic membrane vesicle trafficking [51–53]. Specifically, 5PP-$InsP_5$ inhibits synaptotagmin-dependent exocytosis by interfering with the fusogenic activity of Ca^{2+} and blocking adaptor protein-mediated synaptic vesicle recycling [52, 53]. In addition, 5PP-$InsP_5$ is required for insulin release in pancreatic β-cells [54–57]. 5PP-$InsP_5$ competes with PtdIns(4,5)P_2 for binding to synaptotagmin-7, Ca^{2+} selectively binds 5PP-$InsP_5$ with high affinity, freeing synaptotagmin-7 to enable fusion of insulin-containing vesicles with the cell membrane [57]. Besides, 5PP-$InsP_5$ regulates Ca^{2+} oscillations, a key element in triggering exocytosis and secretion in β-cells [58]. 5PP-$InsP_5$ also inhibits the release of HIV-1 virus-like particles from HeLa cells by modulating the interaction of adaptor protein complex AP-3 with Kif3A, a motor protein of the kinesin superfamily [59]. Specifically, 5PP-$InsP_5$ pyrophosphorylates the β subunit of AP-3, blocking its interaction with Kif3A [59].

Protein activities and stabilities are crucial for cell functions. 5PP-$InsP_5$ regulates certain plasma membrane proteins' activity and stability [12, 60]. Xenotropic and polytropic retrovirus receptor 1 (XPR1) is an 8-pass transmembrane molecule that mediates phosphate export from the cell. 5PP-$InsP_5$ enhances the activity of XPR1 by binding to its SPX domain [61]. Depleting 5PP-$InsP_5$ leads to an increase in intracellular phosphate by inhibiting XPR1-mediated phosphate export [60]. Pharmacological inhibition of 5PP-$InsP_5$ synthesis in vivo blocks cellular phosphate export and subsequently lowers plasma phosphate levels [31]. Na^+/K^+-ATPase is universally expressed in the plasma membrane of animal cells, and it is essential for cell volume maintenance, signal transduction, and secondary transport of various nutrients. 5PP-$InsP_5$ determines the protein levels of sodium/potassium-transporting ATPase (Na^+/K^+-ATPase) at the cell membrane [12]. Depleting 5PP-$InsP_5$ elicits a two-fold enrichment of Na^+/K^+-ATPase in the plasma membrane. 5PP-$InsP_5$ binds the RhoGAP domain of PI3K p85α to disinhibit its interaction with Na^+/K^+-ATPase, subsequently recruiting the adaptor protein 2 and triggering the clathrin-mediated endocytosis of Na^+/K^+-ATPase and its downstream degradation [12].

Focal adhesions are plasma membrane-associated protein complexes responsible for engaging with the extracellular matrix. The dynamics of these adhesions are regulated by 5PP-$InsP_5$ [33, 39, 42, 62]. Depleting 5PP-$InsP_5$ delays focal adhesion turnover by reducing the phosphorylation levels of focal adhesion kinase (FAK). 5PP-$InsP_5$ binds to the FERM domain of FAK, promoting its dimer formation and leading to FAK phosphorylation [46]. Synthetic analogues of 5PP-$InsP_5$ cannot promote FAK dimer formation, indicating that pyrophosphorylation is required for this process [46]. The antifungal drug itraconazole inhibits angiogenesis by disrupting the 5PP-$InsP_5$-mediated focal adhesion dynamics and cytoskeletal remodeling [46].

Physiological Functions and Mechanisms of 5PP-$InsP_5$ in the Cytosol

5PP-$InsP_5$ participates in regulating intracellular ATP concentration [60, 63]. It affects the activity of the major glycolytic transcription factor GCR1. Depleting 5PP-$InsP_5$ augments glycolysis and elicits approximately a three-fold increase of ATP in mouse embryonic fibroblast cells [63]. Conversely, higher cellular ATP level is associated with increased concentration of 5PP-$InsP_5$ [11, 56]. The mechanisms behind this reciprocal regulation of ATP and 5PP-$InsP_5$ remain unknown, and their physiological and pathophysiological relevance is yet to be demonstrated.

Mitochondria are the primary source of intracellular ATP. 5PP-$InsP_5$ plays a critical role in regulating mitochondria biogenesis and function. In mouse embryonic fibroblast cells, depleting 5PP-$InsP_5$ by deleting IP6K1 reduces oxygen consumption rates, decreases mitochondrial mass, and lowers membrane potential [63]. In contrast, animal studies reveal that depleting 5PP-$InsP_5$ in hepatocytes by knocking out IP6K1 elevates oxygen consumption rates, upregulates mitochondrial oxidative phosphorylation proteins, and increases mitochondrial oxidative capacity [64]. Similarly, in adipocytes, depleting 5PP-$InsP_5$ by knocking out IP6K1 boosts mitochondrial oxygen consumption rates, enhances mitochondria function to augment thermogenic energy expenditure [65, 66], and elevates PGC1-α expression levels under low-temperature conditions [65]. Depleting 5PP-$InsP_5$ also enhances mitochondrial biogenesis and function in cardiomyocytes by activating the AMPK pathway and decreasing the acetylation state of PGC-1α [29]. IP6K2 is also responsible for 5PP-$InsP_5$ production. Deletion of IP6K2 in N2A neuronal cells impairs mitochondrial function and represses the cytochrome c1 subunit of the mitochondrial electron transport chain, complex III. Loss of IP6K2 causes mitochondrial oxidative stress in the cerebellum [67]. IP6K2 is also involved in the attenuation of PINK1-mediated mitochondrial autophagy in the brain in a catalytically independent manner. Deletion of IP6K2 in N2A and PC12 cells results in increased mitochondrial fission and mitophagy. The expression levels of dynamin-related protein 1, PGC1-α, and mitophagy markers (PINK1, Parkin, and LC3-II) are upregulated in the cerebellum of *IP6K2* KO brains [68]. The above evidence indicates that 5PP-$InsP_5$ generated by IP6K1 plays opposite roles than that generated by IP6K2 in regulating mitochondria. It is worth noting that depleting 5PP-$InsP_5$ also depletes $InsP_8$. In both HCT116 cells and HEK293 cells, depleting $InsP_8$

increases mitochondrial mass, elevates oxygen consumption rates, and increases mitochondrial oxidative phosphorylation [11]. The mitochondria are more tubular and less fragmented in $InsP_8$-depleted HCT116 cells [11]. The mechanisms by which 5PP-$InsP_5$ and $InsP_8$ regulate mitochondria have not been delineated.

Cytoskeletal proteins are structural proteins that are critical for regulating cell shape, migration, division, etc. 5PP-$InsP_5$ participates in cytoskeletal reorganization [46]. The Arp2/3 complex generates a dendritic actin network at the leading edge of motile cells to form lamellipodia. 5PP-$InsP_5$ binds to the Arp2/3 complex and recruits coronin, which promotes the disassembly of branched actin networks, and it is required for Arp2/3-mediated actin dynamics in vivo [46]. 5PP-$InsP_5$ is required for cytoplasmic dynein-driven vesicle transport in mammalian cells [45]. 5PP-$InsP_5$ promotes the assembly of the dynein complex by strengthening the binding between the dynein intermediate chain and p150glued [39, 45]. 5PP-$InsP_5$ pyrophosphorylates dynein intermediate chain. Depleting 5PP-$InsP_5$ causes defects in dynein-dependent trafficking pathways, including endosomal sorting, vesicle movement, and Golgi maintenance [45]. Depleting 5PP-$InsP_5$ also affects dynein-mediated cell polarity and neuronal migration [39].

Messenger RNA (mRNA) is essential for protein synthesis within the cytosol. 5PP-$InsP_5$ regulates mRNA stability and dynamics of processing-body, cytoplasmic ribonucleoprotein granules containing translationally repressed mRNAs [69]. 5PP-$InsP_5$ competes with 5′-capped mRNA for hydrolysis by NUDT3 (also named diphosphoinositol polyphosphate phosphatase 1, DIPP1), therefore inhibiting NUDT3-mediated mRNA decapping. Elevating cellular 5PP-$InsP_5$ levels increases amounts of NUDT3 mRNA substrates and raises processing-body abundance [69]. A recent study showed that the IP6K1 protein itself can upregulate the formation of processing-bodies [70]. In that model, IP6K1 acts as a scaffolding protein to promote the interactions of DDX6 and 4E-T with the cap binding protein eIF4E, without requiring IP6K1's product 5PP-$InsP_5$ [70]. Additionally, 5PP-$InsP_5$ is involved in regulating protein activities within the cytosol, for example, 5PP-$InsP_5$ binds to insulin-degrading enzyme (IDE) and promotes IDE-catalyzed cleavage of bradykinin [71].

Physiological Functions of 5PP-$InsP_5$ in the Nucleus

A major function of nuclear 5PP-$InsP_5$ in mammalian cells is to regulate p53-mediated apoptosis. Depleting nuclear 5PP-$InsP_5$ by knocking out IP6K2 impairs p53-mediated apoptosis, instead of favoring cell-cycle arrest [72]. These 5PP-$InsP_5$ depleted cells show some resistance to ionizing radiation and the antiproliferative effects of interferon-β [37]. In contrast, direct microinjection of 5PP-$InsP_5$ induces cell death in SCC22A squamous carcinoma cells [37]. 5PP-$InsP_5$ binds CK2 to enhance its phosphorylation of the Tel2/Tti1/Tti2 complex, thereby stabilizing DNA-PKcs and ATM. This process stimulates p53 phosphorylation at serine 15 to activate the cell death program in HCT116 cells [73]. 5PP-$InsP_5$ also affects cell survival and growth by regulating MYC protein stability. 5PP-$InsP_5$ pyrophosphorylates MYC protein at its PEST domain (enriched in Pro, Glu/Asp, Ser, and Thr residues), recruiting the E3 ubiquitin ligase FBW7. This process causes ubiquitination and degradation of MYC protein [44]. Cigarette smoking disrupts 5PP-$InsP_5$-mediated aged neutrophil death and thus exacerbates chronic obstructive pulmonary disease by elevating the neutrophil-to-lymphocyte ratio [74]. Hypoxic injury increases 5PP-$InsP_5$ production in bone marrow-derived mesenchymal stem cells. The elevated 5PP-$InsP_5$ induces autophagy and mediates hypoxic injury-induced apoptosis [75].

In addition to promoting apoptosis and cell death, nuclear 5PP-$InsP_5$ in mammalian cells also participates in DNA damage response [76, 77]. 5PP-$InsP_5$ mediates DNA damage repair via homologous recombination [76]. The Cullin-RING ubiquitin ligase 4 (CRL4) plays a critical role in nucleotide excision repair, and its basal activity is inhibited by binding to the COP9 signalosome (CSN). Ultra-violet radiation induces the synthesis of 5PP-$InsP_5$ to promote the disassembly of the CRL4-CSN and thus activates CRL4 [77].

5PP-$InsP_5$ may also regulate gene transcription directly or through epigenetic modifications [78–80]. Depletion of 5PP-$InsP_5$ alters the mRNA levels of genes associated with mature neurons, neural progenitor/stem cells, and glial cells, as well as certain genes modulating neuronal differentiation and functioning [80]. It also regulates the Jumonji domain-containing 2C (JMJD2C), a histone lysine demethylase, by inducing the disassociation of JMJD2C with chromatin [79]. Depleting 5PP-$InsP_5$ reduces levels of trimethyl-histone H3 lysine 9 (H3K9me3) and increases levels of acetyl-H3K9, leading to changes in JMJD2C-target gene transcription [79].

The nucleolus is a spherical structure that lies in the cell's nucleus and is the center of ribosomal RNA synthesis and processing [81]. Its architecture is modulated by 5PP-$InsP_5$ as it binds to multiple nucleolar proteins [82]. Elevated levels of 5PP-$InsP_5$ enlarge the outer nucleolar granular region while leaving the inner fibrillar volume of the nucleolus unaffected [82].

Physiological Functions and Mechanisms of $InsP_8$

Experimental evidence demonstrates that $InsP_8$ participates in 5PP-$InsP_5$-related signaling pathways in mammalian cells (Table 2) [11, 60, 83, 84]. In certain signaling pathways, $InsP_8$ displays weaker activities than 5PP-$InsP_5$, and thus converting 5PP-$InsP_5$ to $InsP_8$ may negate the effects of

Table 2 Functions and mechanisms of intracellular $InsP_8$

Subcellular areas	Functions	Mechanisms
Plasma membrane	Regulates cellular phosphate [83] Impacts pancreatic β-cell activity [84]	Binds XPR1 to stimulate phosphate efflux [83] Reduces calcium oscillations and causes granuphilin translocation [58, 84]
Cytosol	Regulates ATP synthesis [11, 63] Inhibits mRNA decapping [87]	Depletion of $InsP_8$ enhances mitochondrial oxidative phosphorylation and glycolysis [11] Competing with 5′-capped mRNA for hydrolysis by NUDT3 [87]
Nucleus	Regulates apoptosis [11]	Antagonizes 5PP-$InsP_5$-mediated P53 signaling pathway [11]

5PP-$InsP_5$ [85]. For example, $InsP_8$ binds the PH domain of Akt, but the affinity of Akt for $InsP_8$ (IC50 > 50 μM) is several folds lower than that for 5PP-$InsP_5$ [85]. Converting 5PP-$InsP_5$ to $InsP_8$ relieves the inhibitory effects of 5PP-$InsP_5$ upon the PtdIns(3,4,5)P_3 signaling pathway, and thus favors the association of PH domain proteins with PtdIns(3,4,5)P_3 [85].

At the plasma membrane, $InsP_8$ functions similarly to 5PP-$InsP_5$, regulating the cellular phosphate levels by affecting both the import and export of phosphate [83, 86]. $InsP_8$ facilitates the efflux of inorganic phosphate from mammalian cells by binding to the N-terminus of xenotropic and polytropic retrovirus receptor 1 (XPR1), which is required for the activity of XPR1-mediated cellular phosphate efflux [83]. Phosphate efflux is also regulated in a dose-dependent manner by liposomal delivery of a metabolically resistant methylene bisphosphonate (PCP) analog of $InsP_8$, indicating the independence of protein pyrophosphorylation [83]. $InsP_8$ also induces cellular phosphate uptake. Delivering $InsP_8$ into HCT116 cells shows it dose-dependently stimulates the rate of phosphate uptake [86]. Intriguingly, both $InsP_8$ and 5PP-$InsP_5$ affect phosphate homeostasis by targeting XPR1 [60, 83]. Whether $InsP_8$ competes with 5PP-$InsP_5$ in regulating XPR1 remains to be determined. In pancreatic β cells, granuphilin is a crucial component of the docking machinery of insulin-containing vesicles to the plasma membrane. Insulin release is triggered by an increase in calcium concentration. $InsP_8$ elicits a direct reduction in calcium oscillations and causes immediate translocation of the C2AB domain of granuphilin from the plasma membrane into the cytosol [84]. 5PP-$InsP_5$ also reduces cytosolic calcium oscillations [58], but 5PP-$InsP_5$ does not cause the translocation of the C2AB domain of granuphilin [84]. The direct targets of 5PP-$InsP_5$ and $InsP_8$ remain to be characterized. The in vivo effects of $InsP_8$ upon insulin release warrant future investigation.

In the cytosol, $InsP_8$ serves as a regulator of ATP production but has weaker activity compared to 5PP-$InsP_5$. Depleting $InsP_8$ by deletion of the PPIP5Ks results in a 35% increase in ATP in HCT116 colon cancer cells by enhancing mitochondrial oxidative phosphorylation and glycolysis [11]. In contrast, depleting 5PP-$InsP_5$ elevates ATP by approximately three-fold in mouse embryonic fibroblast cells [63]. $InsP_8$ also functions similarly to 5PP-$InsP_5$ in regulating insulin-degrading enzyme-dependent cleavage of bradykinin [71]. $InsP_8$ and 5PP-$InsP_5$ are catabolized by NUDT3 (also name DIPP1) in animal cells [87]. Future studies are needed to determine whether $InsP_8$ performs similar functions as 5PP-$InsP_5$ in inhibiting NUDT3-mediated mRNA decapping by competing with 5′-capped mRNA for hydrolysis by NUDT3.

In the nucleus, $InsP_8$ antagonizes the 5PP-$InsP_5$-mediated P53 signaling pathway in HCT116 colon cancer cells. Depleting $InsP_8$ strengthens the P53-dependent growth-inhibited phenotype [11].

More complicated genetic and pharmacological studies are required to specify the functions of 5PP-$InsP_5$ and $InsP_8$.

Regulation of 5PP-$InsP_5$ and $InsP_8$

The intracellular levels of 5PP-$InsP_5$ and $InsP_8$ are primarily regulated by the cellular energy status, as their synthesis is critically dependent on ATP [88]. Increasing the cellular ATP/ADP ratio by either glucose stimulation or direct delivery of ATP leads to a rapid rise in 5PP-$InsP_5$ concentration [11, 56]. $InsP_8$ is more sensitive than 5PP-$InsP_5$ to changes in environmental glucose levels. In low-glucose conditions, $InsP_8$ concentrations decrease even before ATP levels are affected. Restoration of glucose levels quickly rescues $InsP_8$ synthesis [89]. Thus, 5PP-$InsP_5$ and $InsP_8$ may act as indicators of cellular energy status in mammalian cells.

Animal experiments demonstrate that the levels of 5PP-$InsP_5$ are affected by multiple factors such as aging and metabolic disorders. In a mouse model, hepatocytes from 10-month-old mice express higher levels of 5PP-$InsP_5$ than those from 2-month-old [41]. Similarly, bone marrow mesenchymal stem cells isolated from 18-month-old mice display a two-fold increase in 5PP-$InsP_5$ production than those isolated from 2-month-old mice [90]. Furthermore, a 1.5-fold increase of 5PP-$InsP_5$ levels is recorded in the pancreatic β-cells of the obese diabetic ob/ob mice compared to those of controls [54].

$InsP_8$ biosynthesis is tightly regulated by cellular levels of inorganic phosphate. $InsP_8$ levels decrease upon phosphate starvation and subsequently recover during phosphate replenishment. $InsP_8$ is more sensitive than 5PP-$InsP_5$ in reflecting the intracellular inorganic

phosphate levels and has a wider dynamic range of fluctuation [91]. Besides, $InsP_8$ levels are modestly elevated when cells are activated by growth factors. For example, 45–70% increases in $InsP_8$ were observed in EGF-treated DDT1MF-2 cells and PDGF-treated NIH3T3 cells [85, 92]. PDGF and insulin treatment also increase $InsP_8$ levels in L6 myoblast cells [93]. However, the physiological relevance and the mechanism of the growth factor-induced elevation of $InsP_8$ remains to be determined.

$InsP_8$ is the major product of PPIP5Ks and is generated from 5PP-$InsP_5$. The cellular concentration of $InsP_8$ is typically less than 10% of 5PP-$InsP_5$. This indicates that (1) the protein levels of PPIP5Ks are less than 10% of IP6Ks, or (2) the activities of PPIP5Ks are less than 10% of IP6Ks, or (3) 90% of IP6Ks are not surrounded by PPIP5Ks. However, further studies are required to substantiate these conjectures. It is noteworthy that PPIP5Ks are also able to hydrolyze $InsP_8$ back down to 5PP-$InsP_5$, which consequently reduces the pool of $InsP_8$. This process is dependent on the regulatory mechanisms of the phosphatase domain of PPIP5Ks.

Functions of IP6Ks and their Roles in Diseases

Mammalian cells express a family of three IP6Ks that share identical kinase activity domains but differ in their N-terminal and C-terminal sequences [39]. The functions of IP6Ks are non-redundant, with different isoforms performing specific roles within their subcellular region without compensating for each other (Table 3). In both murine and human cells, IP6K1 and IP6K3 are primarily located in the cytosol and at the plasma membrane; however, IP6K1 is also present in the nucleolus. In contrast, IP6K2 is primarily a nuclear protein [39]. The specific N-terminal and C-terminal sequences of individual IP6Ks mediate protein–protein interactions and post-translational modifications, such as phosphorylation [57, 94], which may contribute to the specificity of subcellular distribution and distinct in vivo functions of IP6Ks. While IP6K1 and IP6K2 are ubiquitously expressed, IP6K3 is highly expressed in specific tissues such as the brain and muscle [38, 39, 95–97]. Thus, in most tissues, the cytosol 5PP-$InsP_5$ is mainly produced by IP6K1, and the nuclear

Table 3 Functions, mechanisms, and potential therapeutic applications of IP6Ks

Isoforms	Systems	Functions	Potential therapeutic applications
	Neuronal system	Promotes neuronal cell migration [33], influences presynaptic vesicle release [51], and regulates synaptic vesicle recycling [53]. The null mutation causes brain malformation [33], increases synaptic vesicle exocytosis [52], reduces prepulse and short-term memory formation [102], and impairs locomotor and social behavior [103]	Not yet indicated
IP6K1	Male reproductive system	Promotes chromatoid body formation [35] and male germ cell development [34]. The null mutation causes azoospermia [34, 35, 104]	Birth control
	Pancreatic β-cell	The null mutation reduces insulin secretion	Not yet indicated
	Adipose tissue	The null mutation increases insulin sensitivity, lowers blood glucose, prevents high-fat diet-induced obesity [65, 98], and promotes adipose tissue browning and energy expenditure [65]	Metabolic disorder
	Liver	The null mutation enhances hepatocyte mitochondrial oxidative phosphorylation and protects animals from nonalcoholic fatty liver disease and steatohepatitis [64]	Nonalcoholic fatty liver disease
	Bone	The null mutation prevents trabecular bone loss, increases mesenchymal stem cell yields [106], and promotes osteogenesis and hematopoiesis-supporting activity [106]	Pneumonia
	Lung	The null mutation increases neutrophil bactericidal activity [108], decreases neutrophil accumulation, and reduces inflammatory damage [107]	Anti thrombosis
	Platelet	The null mutation prevents platelet aggregation and increases clotting time [109]	Anti thrombosis
	Cancer	Regulates cancer cell motility, and affects tumor microenvironment [62]	Not yet indicated
IP6K2	Neuronal system	Responsible for neuronal cell death in neurodegenerative diseases. The null mutation causes neurodevelopmental defects [36, 112]	Neurodegenerative diseases
	Cancer	The null mutation predisposes cancer development [37]	Prognostic biomarker
IP6K3	Neuronal system	The null mutation causes neurodevelopmental defects [38, 96]	Not yet indicated
	Muscle	Glucose metabolism in muscle [97, 119]	Not yet indicated

5PP-InsP$_5$ is primarily generated by IP6K2. The in vivo functions of IP6Ks are revealed by utilizing individual *IP6K* KO mice.

Functions and Mechanisms of IP6K1

IP6K1 generates up to 70% of cellular 5PP-InsP$_5$, making it the dominant isoform [41]. The protein expression levels of IP6K1 increase with age [98]. Various factors can regulate the IP6K1 protein expression, such as consumption of lean meat increases IP6K1 in muscle [99], whereas high-intensity exercise decreases IP6K1 in muscle [100]. Additionally, the cold temperature reduces IP6K1 expression in white adipose tissue [65, 66]. IP6K1 activity can also be regulated by phosphorylation [57, 94]. PKD and PKC can phosphorylate IP6K1 at serine 118 and serine 121 respectively, and double-phosphorylation has been shown to enhance its catalytic activity [57].

Global deletion of IP6K1 is viable and does not affect life expectancy in mice, with no significance between *IP6K1* KO mice and their WT littermates. Notably, the *IP6K1* KO mice confer greater resistance to certain diseases such as metabolic disorders [41] but may also result in developmental defects [33–35].

Deletion of IP6K1 causes Neuronal Developmental Defects

IP6K1 plays important roles in neuronal cell migration, it binds to α-actinin, which brings it into proximity with FAK that, together with α-actinin, is part of the focal adhesion complex [33]. IP6K1 generates 5PP-InsP$_5$ in focal adhesions to enhance FAK phosphorylation [33, 46], which in turn augments neuronal migration. Deletion of IP6K1 leads to impaired neuronal migration, brain malformation, and a neonatal death rate of around 40% [33]. P6K1 also influences presynaptic vesicle release and short-term facilitation of glutamatergic synapses in mice hippocampal neurons [51]. It binds to rab3A-interacting-like protein 1, a guanine nucleotide exchange factor for Rab-3A, which localizes to synaptic vesicles to limit the amount of neurotransmitter release [101]. Knocking out IP6K1 augments action potential-driven synaptic vesicle exocytosis at synapses [52]. IP6K1 plays an important role in synaptic vesicle recycling, with its deletion in neurons leading to increased synaptic facilitation and both the exocytosis and endocytosis of synaptic vesicle [53]. Mechanistically, IP6K1 via its product 5PP-InsP$_5$ inhibits synaptic vesicle exocytosis by binding synaptotagmin and inhibiting synaptotagmin-dependent synaptic membrane fusion [52]. The *IP6K1* KO animals exhibit decreased prepulse inhibition, impaired short-term memory formation [102], and abnormal locomotor and social behavior [103].

Deletion of IP6K1 Causes Azoospermia

Deletion of IP6K1 severely disrupts sperm development but does not affect mating behavior. Male *IP6K1* KO mice are completely infertile, while females can become pregnant and produce progenies [34, 35, 104]. IP6K1 is essential for the formation of chromatoid body, a cytoplasmic granule found in round spermatids that is composed of RNA and RNA-binding proteins [35]. Besides, IP6K1 is required for multiple aspects of male germ cell development [34]. Deletion of IP6K1 elicited several aberrations, such as sloughing off of round germ cells; disorientation and malformation of elongating/elongated spermatids; degeneration of acrosomes; defects in germ-Sertoli cell interactions and failure of spermiation. Eventually, sperm cells are not released and are instead phagocytosed by Sertoli cells, leading to an absence of sperm in the epididymis [34, 35]. Evolutionary analyses of single-nucleus transcriptomics of testes from 11 species, including humans and mice, revealed that IP6K1 expression increases towards the end of spermiogenesis in all amniotes except primates. In primates, high IP6K1 expression occurs in early spermiogenesis, before decreasing later, suggesting a shift in the spermatogenic function of IP6K1 during primate evolution [105].

Deletion of IP6K1 Protects Mice from Metabolic Dysfunctions

Deletion of IP6K1 lowers blood glucose levels. The *IP6K1* KO mice display higher activities of Akt and AMPK pathways, which have been proposed to mediate the glucose-lowering effects of IP6K1 deletion [41, 64, 65, 98]. It is worth mentioning that the deletion of IP6K1 also disrupts glucose-mediated first-phase insulin secretion in pancreatic β-cells. IP6K1 may act as a metabolic sensor in pancreatic β-cells. Upon glucose stimulation, which increases the ATP/ADP ratio, IP6K1 kinase activities in β-cells are activated [56, 57]. As a result, the blood insulin levels are lower in *IP6K1* KO mice than in the WT littermates [54, 55].

Whole body deletion of IP6K1 enhances lipolysis [94] and protects animals from high-fat-diet-induced obesity. The *IP6K1* KO mice display reduced fat accumulation and markedly lower blood levels of leptin, a hormone derived from adipocytes that regulates hunger and food intake [41]. The *IP6K1* KO mice consume similar amounts of food as WTs, indicating that deletion of IP6K1 increases leptin sensitivity. Adipose tissue-specific deletion of IP6K1 leads to decreased fat mass and increased adipose tissue browning and enhanced energy expenditure [65]. Hepatocyte-specific *IP6K1* KO mice display improved insulin sensitivity, although not resistant to body weight gain. Deletion of IP6K1 increases hepatocyte mitochondrial oxidative phosphorylation capacity [64]. IP6K1 is

upregulated in human nonalcoholic steatohepatitis livers. Deletion of IP6K1 improves nonalcoholic fatty liver disease and steatohepatitis in animal models [64].

The protein levels of IP6K1 are upregulated in tissues of aged mice, and deletion of IP6K1 protects mice from age-induced weight gain, insulin resistance, and metabolic dysfunction [98]. Knocking out IP6K1 also prevents age-related adipogenesis in animals, as well as increasing mesenchymal stem cell yields from bone marrow while enhancing cell growth and survival. The *IP6K1* KO mesenchymal stem cells exhibit enhanced osteogenesis and hematopoiesis-supporting activity. Furthermore, deletion of IP6K1 also prevents high-fat-diet-caused trabecular bone loss [106].

Deletion of IP6K1 Reduces Inflammatory Damages

Bacterial clearance is enhanced in IP6K1-deficient mice in a bacteria-induced pneumonia model. Knocking out IP6K1 reduces polyphosphate production in platelet, which in turn reduces pulmonary neutrophil accumulation in the pneumonia model and alleviates lung damage in LPS-induced lung inflammation [107]. Neutrophils lacking IP6K1 have greater phagocytic and bactericidal abilities, along with amplified NADPH oxidase-mediated superoxide production. Deletion of IP6K1 in neutrophils activates the Akt signaling pathway, which is responsible for the enhanced bactericidal ability [108]. Inhibiting IP6K1 kinase activity delays neutrophil spontaneous death, which may contribute to the pathogenesis of chronic obstructive pulmonary disease caused by cigarette smoking [74].

Deletion of IP6K1 lowers the Risks of Cardiovascular Disease

Risk factors for cardiovascular disease include obesity, metabolic disorders, diabetes, age, and inflammation. Knocking out of IP6K1 prevents high-fat diet-induced obesity and metabolic disorders [65], lowers blood glucose levels [41], protects mice from age-induced weight gain, insulin resistance, and metabolic dysfunction [98], and attenuates inflammatory reaction [107]. The above evidence indicates that deletion of IP6K1 may reduce the risks of developing cardiovascular disease. Additionally, knocking out IP6K1 could potentially lower the risk of thrombosis. IP6K1 is required for maintaining cellular polyphosphate levels. Deleting IP6K1 reduces polyphosphate levels in platelet, leading to slower platelet aggregation and lengthened plasma clotting time [109]. However, the exact roles of IP6K1 in cardiovascular diseases remain to be demonstrated.

Deletion of IP6K1 alters Tumor Behaviors

Deletion of IP6K1 in cancer cells, such as Hela cervical carcinoma cells and HCT116 human colorectal carcinoma cells, reduces migration, invasion, and anchorage-independent growth. When fed an oral carcinogen, mice lacking IP6K1 show reduced progression from epithelial dysplasia to invasive carcinoma [62]. IP6K1 also regulates the tumor microenvironment [110]. The MC38 mouse colon allograft tumors from *IP6K1* KO host mice contained increased levels of $CD11b^{+}Gr1^{+}$ $IL10^{+}$ cells, but reduced levels of the $CD80^{+}$ IFN-γ M1 macrophages compared to those from WT host mice. The MC38 carcinomas grow faster in the *IP6K1* KO host mice than in WT mice [110].

Deletion of IP6K1 activates the Akt pathway, a major contributor to cancer development. However, the deletion of IP6K1 does not cause spontaneous cancer development in animal models. One possible reason is that deletion of IP6K1 affects other mechanisms that counterbalance cancer development. The roles and mechanisms of IP6K1 in tumor growth and behavior remain to be delineated.

Functions and Mechanisms of IP6K2

IP6K2 is primarily found within the nucleus, where it is responsible for synthesizing 20–40% of intracellular 5PP-$InsP_5$ in mouse fibroblast cells [37, 73]. Whole body deletion of IP6K2 in mice does not cause obvious abnormalities. Both male and female *IP6K2* KO mice can produce progenies, and their lifespan in laboratory animal facilities is similar to that of WT mice.

IP6K2 protein levels can be regulated by phosphorylation and degradation. CK2 selectively phosphorylates IP6K2 at serine residues S347 and S356, which are located within the degradation-specific PEST motif. This enhances the protein's ubiquitination and degradation [40, 111].

Roles and Mechanisms of IP6K2 in Neurological Diseases

Deletion of IP6K2 elicits alterations of the cerebellar Purkinje cell morphology and psychomotor behavior, without requiring IP6K2 kinase activity [36]. Additionally, IP6K2 deletion alters the transcription of genes associated with neuronal function and development, resulting in a neurodevelopmental imbalance in the mammalian gastrointestinal tract [80]. Furthermore, IP6K2 deletion increases glycolysis [68] but reduces mitochondria function, specifically impairing the expression of the cytochrome-c1 subunit of complex III of the electron transport chain in cerebellar neurons [67]. The *IP6K2* KO cells produce less phosphocreatine and ATP, leading to elevated levels of reactive oxygen species and protein oxidative damage [67]. In addition, IP6K2 is involved in the attenuation of

PINK1-mediated mitochondrial autophagy in the brain in a catalytically independent manner. Deletion of IP6K2 upregulates dynamin-related protein 1 and the expression of mitochondrial biogenesis markers (PGC1-α and NRF-1) in the cerebellum [68].

IP6K2 promotes cell death [112], which may be involved in the development of neurodegenerative diseases. The expression levels of IP6K2 were higher in the spinal cord of amyotrophic lateral sclerosis patients [113]. IP6K2 can induce cell death in the anterior horn cells of the spinal cord by binding with phosphorylated TDP-43 in the cytoplasm [114] and inhibiting Akt signaling [113]. It has been observed that IP6K2 can translocate from the nucleus to the cytoplasm to inhibit Akt signaling and trigger cell death in lymphoblast cells from individuals with Huntington's disease [115].

Roles and Mechanisms of IP6K2 in Cancers

Deletion of IP6K2 predisposes animals to carcinogen-induced invasive squamous cell carcinoma formation in the oral cavity and esophagus, although no spontaneous tumors were observed in the *IP6K2* KO mice [37]. When IP6K2 is disrupted in colorectal cancer cells, it selectively impairs p53-mediated apoptosis, favoring cell-cycle arrest instead. IP6K2 interacts with p53 directly, reducing expression of proarrest gene targets such as the cyclin-dependent kinase inhibitor p21 [72]. IP6K2 also generates 5PP-InsP_5 to mediate the DNA-PK/ATM-p53 cell death pathway by enhancing casein kinase 2-mediated phosphorylation of Tti1/Tel2 [73]. Within the nucleus, IP6K2 can bind LKB1, which prevents LKB1 from translocating to the cytosol and blocking cancer cell metastasis by inhibiting the focal adhesion kinase [42]. IP6K2 may also regulate cancer cell behavior by affecting the hedgehog pathway, as its deletion diminishes the hedgehog signaling response [116].

The role of IP6K2 in cancer cell behavior may be tissue specific. IP6K2 predicts favorable clinical outcomes of primary breast cancer [117]. In contrast, in glioma, the elevated IP6K2 level facilitates the proliferation, migration, and invasion of cancer cells [118].

Functions and Mechanisms of IP6K3

The expression of IP6K3 is tissue-specific, with high expression in the brain and muscles [97]. IP6K3 generates less than 25% of intracellular 5PP-InsP_5 in neuronal cells [39]. Global deletion of IP6K3 does not affect embryonic development and leads to normal fertility in mice. The lifespans of *IP6K3* KO mice are comparable to that of WTs.

Roles and Mechanisms of IP6K3 in Neurological Diseases

Deletion of IP6K3 leads to deficits in motor learning and coordination [38]. In cerebellar Purkinje cells, IP6K3 physiologically binds to the cytoskeletal proteins adducin and spectrin. When IP6K3 is deleted, the interactions between adducin and spectrin are disrupted, causing abnormalities in the structure of cerebellar Purkinje cells and decreased numbers of synapses [38]. IP6K3 also plays a critical role in synaptic vesicle recycling. Knocking out IP6K3 decreases synaptic vesicle release, but accelerates AP-3-dependent synaptic vesicle endocytosis. This leads to reduced synaptic facilitation in the *IP6K3* KO mice [38, 53]. IP6K3 is also enriched at the migrating leading edge of cell membranes, where it physiologically interacts with protein dynein intermediate chain 2. These two proteins are mutually recruited to the leading edge of migrating cells. At the cell membrane, IP6K3 promotes cell motility via its effects on the turnover of focal adhesions. Deletion of IP6K3 results in defects in cell motility and neuronal dendritic growth, eventually leading to brain malformations [39].

In humans, analysis of the IP6K3 gene's single nucleotide polymorphism (SNP) in patients with familial and sporadic late-onset Alzheimer's disease reveals that two SNPs in the 5'-flanking promoter region of the IP6K3 gene are associated with sporadic late-onset Alzheimer's disease. One of the SNPs increases IP6K3 promoter activity [96]. Intriguingly, the same SNP of IP6K3 that is associated with increased risk for late-onset Alzheimer's disease, increases the likelihood of longevity. The mechanisms behind this phenomenon are currently not known and warrant further studies [95].

Roles and Mechanisms of IP6K3 in Metabolic Dysfunctions

Systemic administration of dexamethasone elevates blood glucose and upregulates the expression of IP6K3 in the muscles. Consistently, diabetic mice show higher levels of IP6K3 protein in their muscles compared to control mice [97]. While deletion of IP6K3 does not affect muscle mass, it does lead to lower circulating insulin levels and a decrease in fat mass and as well as body weight [97]. Deletion of MyD88 reduces the transcriptional levels of IP6K3 and increases glucose uptake in muscle cells [119], implying that IP6K3 may play a role in regulating blood glucose. However, *IP6K3* KO mice are not protected against diet-induced obesity [97]. The specific roles of IP6K3 in muscle and metabolic dysfunctions have not been elucidated.

Functions and Mechanisms of PPIP5Ks

PPIP5Ks are proteins of large size, with PPIP5K1 and PPIP5K2 having molecular weights of around 160 and 140 kDa, respectively. PPIP5Ks are not only kinase but also

host a phosphatase domain [120]. Experimental data show that PPIP5Ks preferentially phosphorylate 5PP-$InsP_5$ to produce $InsP_8$ in vivo. The concentration of cellular $InsP_8$ is 5 to 10 times higher than that of 1PP-$InsP_5$ [121, 122]. The phosphatase activity of PPIP5K displays positional specificity: only the 1-β-phosphate from either 1PP-$InsP_5$ or $InsP_8$ is hydrolyzed, indicating a futile cycle of 1-kinase/1-phosphatase is catalyzed by the PPIP5Ks [123]. Interestingly, PPIP5Ks prefer to dephosphorylate $InsP_8$ over 1PP-$InsP_5$ as phosphatases, resulting in $InsP_8$ being dephosphorylated about tenfold faster than 1PP-$InsP_5$ [123]. The activities of the kinase and phosphatase domain can be regulated by phosphate, with phosphate inhibiting the $InsP_8$ phosphatase activity while stimulating the 5PP-$InsP_5$ kinase activity [91].

All the aforementioned evidence indicates that the 5PP-$InsP_5$/$InsP_8$ cycle serves as the major metabolic pathway for PPIP5Ks. Based on this, it is plausible to assume that PPIP5Ks function within similar subcellular compartments as IP6Ks (Table 4), given that PPIP5Ks' enzymatic activities rely on IP6K's product. This "futile" cycle of 5PP-$InsP_5$/$InsP_8$ may be essential for the timely and precise modulation of the local concentration of 5PP-$InsP_5$ and $InsP_8$, possibly playing a critical role in signaling on and off.

Functions and Mechanisms of PPIP5K1

PPIP5K1 features a domain that binds to phosphatidylinositols such as PtdIns(4,5)P_2 and, to a greater degree, PtdIns(3,4,5)P_3. The activation of the PI3K pathway leads to the recruitment of PPIP5K1 to the plasma membrane [85, 124], where it metabolizes 5PP-$InsP_5$ to $InsP_8$. The conversion of 5PP-$InsP_5$ to $InsP_8$ relieves the inhibition of Akt by 5PP-$InsP_5$ because $InsP_8$ has a tenfold lower affinity for Akt. PPIP5K1 also possesses a phosphatase domain that can convert $InsP_8$ back to 5PP-$InsP_5$. However, its $InsP_8$ phosphatase activity is inhibited by PtdIns(4,5)P_2 and PtdIns(3,4,5)P_3. These phosphatidylinositols are not phosphatase substrates of PPIP5K1, the inhibition of $InsP_8$ phosphatase activity results from an unusual, functional overlap between the phosphatase domain and the polyphosphoinositide-binding domain [93]. Thus, PPIP5K1 may act as a switch by balancing the amount of 5PP-$InsP_5$ and $InsP_8$ to regulate the inhibitory effect of 5PP-$InsP_5$ on PH-domain-containing proteins.

The plasma membrane localization of PPIP5K1 may also be regulated by 5PP-$InsP_5$, which could potentially compete with PtdIns(3,4,5)P_3 for binding to the polyphosphoinositide-binding domain, a cryptic PH domain. This may prevent the PtdIns(3,4,5)P_3-mediated relocation of PPIP5K1 from the cytoplasm to the plasma membrane. However, additional experimental data is necessary to support this speculation.

PPIP5K1 contains an unusually long and evolutionarily conserved intrinsically disordered region. This region is responsible for the interaction with multiple proteins, involving cellular localization, vesicle-mediated transport, cell division, cellular component biogenesis, actin cytoskeleton organization, phosphatidylinositol metabolic processes, apoptotic DNA fragmentation, and cell migration [125]. It has been reported that deletion of PPIP5K1 leads to decreased motility of HeLa cells in a wound-healing assay [125].

PPIP5K1 overexpression decreases p53 phosphorylation on key residues, including Ser-15, -46, and -392, and thus decreases cell sensitivity to several cytotoxic agents, including etoposide, cisplatin, and sulindac [126]. This impact could be related to the kinase activity of PPIP5K1, since its phosphatase activity converts $InsP_8$ to 5PP-$InsP_5$, enhancing p53-mediated cell death induced by 5PP-$InsP_5$.

Functions of PPIP5K2 and its Roles in Diseases

PPIP5K2 possesses a nuclear localization signal and thus may be responsible for the nuclear $InsP_8$ synthesis. PPIP5K2 facilitates DNA homologous recombination repair, which promotes colorectal carcinoma pathogenesis [127]. PPIP5K2 deficiency has been found to reduce Akt activation in hematopoietic stem cells induced by a high-phosphate diet [128]. Due to the non-productive, substrate-stimulated ATPase activity, PPIP5K2 utilizes approximately 2 ATP molecules to synthesize each molecule of 1PP-$InsP_5$ and 1.2 ATP molecules to synthesize $InsP_8$ [122]. It is worth noting that PPIP5K2 is insensitive to physiological changes in either AMP or ATP/ADP ratios [122]. While the exact mechanisms are not fully understood, PPIP5K2 may play an important role in pancreatic β-cell and participate in type 1 and 2 diabetes in humans [129].

Two mutations in the phosphatase domain (amino acids 363–909) of the PPIP5K2 protein, namely S419A, and N843S, have been linked to keratoconus, a common corneal

Table 4 Functions, mechanisms, and potential therapeutic applications of PPIP5Ks

Isoforms	Functions	Potential therapeutic applications
PPIP5K1	Modulates PtdIns(3,4,5)P_3 signaling pathway activity [85, 124], regulates cell motility [125] and affects p53-dependent cell death [126]	Tumor therapy
PPIP5K2	Facilitates DNA homologous recombination repair [127]. The null mutation inhibits ovarian tumor progression in xenograft models Mutations of PPIP5K2 are associated with keratoconus and hearing loss [130, 131]	Tumor therapy

degenerative disorder that can cause high myopia, irregular astigmatism, and cornea scarring. These mutations may impact the dynamic balance of phosphatase and kinase activities, leading to increased production of $InsP_8$ by the mutant PPIP5K2 in vivo [130].

A mutation in PPIP5K2's arginine 837 residue to histidine is associated with hearing loss in humans. This mutant reduces the phosphatase activity of PPIP5K2 and increases its kinase activity. PPIP5K2 is expressed in the cochlear and vestibular sensory hair cells, supporting cells, and spiral ganglion neurons. Mice homozygous for a targeted deletion of the PPIP5K2 phosphatase domain exhibit degeneration of cochlear outer hair cells and elevated hearing thresholds [131].

Therapeutic Applications of Targeting the 5PP-$InsP_5$/$InsP_8$ Pathway

Several IP6K inhibitors have been characterized and tested in animal models [28, 31, 132–134]. N^2-(*m*-(trifluoromethy)lbenzyl) N^6-(*p*-nitrobenzyl)purine (TNP) is the first characterized IP6K inhibitor [28]. Recently, a more potent orally available selective IP6K inhibitor SC-919 has been developed [31]. Pharmacological inhibition of IP6K kinase activity to block 5PP-$InsP_5$ biosynthesis has shown beneficial effects in the treatment of several diseases (Table 5) [29–31, 135]. Depleting 5PP-$InsP_5$ by administration of TNP did not show long-term damage on spermatogenesis in mice [135], although genetic deletion of IP6K1 causes azoospermia [34, 35].

Blocking 5PP-$InsP_5$ Synthesis Prevents Metabolic Disorders

Pharmacological inhibition of IP6K by administration of TNP prevents high-fat-diet-induced metabolic disorders. TNP administration decelerates the initiation of high-fat-diet-induced obesity and insulin resistance. Inhibiting IP6K activity facilitates weight loss and restores metabolic parameters in obese animals [30]. Additionally, blocking the synthesis of 5PP-$InsP_5$ can protect animals from obesity-induced bone loss [135].

Blocking 5PP-$InsP_5$ Synthesis Protects Cardiomyocytes

In vivo administration of TNP activates the PI3K/Akt/BAD pathway in cardiac tissue, blocking cardiomyocyte apoptosis and reducing myocardial infarction in a mouse model of ischemic-reperfusion injury [29]. TNP also attenuates hypoxia-induced apoptosis of bone marrow-derived mesenchymal stem cells [75] and promotes mesenchymal stem cell engraftment and paracrine effect in infarcted hearts to preserve heart function and decrease fibrosis [136]. Blocking IP6K1 may also prevent coagulation, and thus prevent thrombosis [109].

Table 5 Therapeutic applications of targeting 5PP-$InsP_5$

Pharmacological inhibitors	In vivo effects in animal models
TNP	Lowers blood glucose [30] Prevents high-fat-diet-induced obesity and insulin resistance [30] Protects animals against obesity-induced bone loss [135] Attenuates cardiac injury in ischemic-reperfusion injury model [29] Attenuates hypoxia-induced apoptosis of bone marrow-derived mesenchymal stem cells [75] Promotes mesenchymal stem cells engraftment and paracrine effect [136] Preserves heart function and decreases fibrosis in infarcted hearts [136] Prevents coagulation [109] Reduces pulmonary inflammatory damage caused by bacterial pneumonia [107]
SC-919	Alleviates hyperphosphataemia [31]

Blocking 5PP-$InsP_5$ Synthesis Reduces Inflammatory Damage

Inhibiting IP6K by TNP treatment enhances host bacterial killing and reduces pulmonary neutrophil accumulation, minimizing the lung damage caused by both Gram-positive and Gram-negative bacterial pneumonia [107].

Blocking 5PP-$InsP_5$ Synthesis Alleviates Hyperphosphataemia

Oral administration of SC-919, which inhibits IP6K, has been shown to alleviate hyperphosphatemia, increase ATP levels in the kidneys, and improve kidney function in chronic kidney disease animal models. This in vivo treatment has been found to reduce plasma levels of creatinine, a biomarker for renal dysfunction, and corrects the imbalanced plasma levels of parathyroid hormone and 1,25 dihydroxyvitamin D [31].

Both TNP and SC-919 are pan IP6K inhibitors, targeting IP6K1, IP6K2 and IP6K3 simultaneously. It remains unclear whether the in vivo effects of these IP6K inhibitors are specific to one isoform or all three. Preclinical studies suggest that IP6K1 inhibition may prevent metabolic dysfunctions [41, 65], while pharmacological inhibition of IP6K2 could be useful in treating neurodegenerative diseases [113, 114]. The in vivo functions of IP6K3 remain mysterious, and thus there is insufficient evidence to support it as a useful

therapeutic target for any disease. However, isoform-specific IP6K inhibitors will be necessary to clarify these questions.

Blocking 5PP-$InsP_5$ biosynthesis by targeting IP6Ks in mammalian cells also reduces $InsP_8$ [60], thus we cannot rule out the possibility that the depletion of $InsP_8$ also contributes to the beneficial effects of IP6K inhibitors.

$InsP_8$ might a be Potential Target for Tumor Therapy

PPIP5K2 is found to be associated with the survival risk of cervical cancer [137]. Deleting PPIP5Ks upregulates the expression of p53 and p21, and slows down cell proliferation and G1/S cell-cycle transition [11]. In xenograft models, the knockdown of PPIP5K2 inhibits ovarian tumor progression [138]. The *PPIP5Ks* KO HCT116 cancer cells show a significant reduction in colony formation in soft agar or on a solid surface, as well as a decrease in vivo tumorigenic capacity. Furthermore, the levels of precursors for de novo nucleotide synthesis are lower in the *PPIP5Ks* KO cells, indicating that deletion of PPIP5Ks inhibits de novo nucleotide biosynthesis [89]. Depleting $InsP_8$ by deletion of PPIP5Ks elevates 5PP-$InsP_5$ [11], which may also contribute to the inhibition of cancer development.

It is worth mentioning that the effects of PPIP5Ks deletion can be complicated, as these enzymes possess both kinase and phosphatase activities. It is not yet clear whether it is the kinase activity or the phosphatase activity of PPIP5Ks that is responsible for the observed phenotypes of genetic knockouts. Thus, it is too preliminary to assert that PPIPK5s are druggable targets for the treatment of diseases. Inhibitors of the kinase activity and the phosphatase activity are needed to resolve these issues.

Conclusion and Perspective

5PP-$InsP_5$ and $InsP_8$ are so far the "final metabolites" of inositol pyrophosphate biosynthesis. IP6Ks generate 5PP-$InsP_5$ in their functional locations, while PPIP5Ks act by converting 5PP-$InsP_5$ to $InsP_8$ and $InsP_8$ back to 5PP-$InsP_5$, potentially operating in the same localized regions. Blocking the biosynthesis of $InsP_6$, the precursor of 5PP-$InsP_5$, and lower inositol phosphates causes severe adverse effects, such as embryonic lethality. In contrast, animals with genetic mutations that express lower levels of 5PP-$InsP_5$ or $InsP_8$ are viable and live with normal life span. Currently, the impact on the life of completely depleting 5PP-$InsP_5$ or $InsP_8$ is unknown, as there are no available *IP6K1*/*IP6K2*/*IP6K3* triple knockout animals and *PPIP5K1*/*PPIP5K2* double KO animals. Pharmacological inhibiting 5PP-$InsP_5$ biosynthesis has proved therapeutic benefits, such as preventing metabolic disorder, attenuating infarction, reducing inflammatory injury, and alleviating hyperphosphatemia [29–31, 107, 135]. More specific and effective inhibitors of IP6K1 are currently being developed to treat obesity and related metabolic dysfunctions [139, 140]. Recently, a more selective IP6K2 inhibitor has been developed [141]. These compounds will facilitate the validation of IP6K inhibition in vivo and expedite future drug development.

Although animal models have not shown any abnormal responses to pharmacological inhibitors of IP6K, genetic deletion of IP6K has been found to cause developmental defects and mental disorders in mice [33–35, 39, 103]. Additionally, mutations of PPIP5Ks are associated with diseases [130, 131]. Therefore, it is essential to further investigate the effects and molecular mechanisms of 5PP-$InsP_5$ and $InsP_8$ in different systems in vivo to evaluate their potential as druggable targets. This will be a productive direction for future research.

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Declarations

Competing interest The authors declare that they have no conflicts of interest in this work.

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