Trends in Biochemical Sciences

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Review

The inositol phosphate signalling network in physiology and disease

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Combinatorial substitution of phosphate groups on the inositol ring gives rise to a plethora of inositol phosphates (InsPs) and inositol pyrophosphates (PP-InsPs). These small molecules constitute an elaborate metabolic and signalling network that influences nearly every cellular function. This review delves into the knowledge accumulated over the past decades regarding the biochemical principles and significance of InsP metabolism. We focus on the biological actions of InsPs in mammals, with an emphasis on recent findings regarding specific target proteins. We further discuss the roles of InsP metabolism in contributing to physiological homeostasis and pathological conditions. A deeper understanding of InsPs and their metabolic pathways holds the potential to address unresolved questions and propel advances towards therapeutic applications.

Highlights

Recent advances have revealed an inositol phosphate (InsP) metabolic network more diverse than previously thought.

InsPs and inositol pyrophosphates (PP-InsPs) are responsive to receptor activation cascades and metabolic processes.

InsPs and PP-InsPs act on proteins through multiple mechanisms, ranging from transient binding to serving as a structural cofactor, while PP-InsPs additionally transfer their high-energy β-phosphate to proteins.

The development of mouse knockout models has emphasised the importance of InsP/PP-InsP kinases in the control of fundamental physiological processes and pathophysiological states.

Introduction to InsPs

InsPs (see Glossary) constitute a family of intracellular messengers. The combinatorial substitution of the six hydroxyl groups of inositol with phosphomonoesters gives rise to 63 possible InsPs species [1], most of which have been identified in cells. Further addition of phosphate generates a cohort of diphosphate- (pyrophosphate-) substituted InsPs, called **PP-InsPs**. In the past two decades, the development of assorted InsP kinase knockout (KO) mice has established InsP and PP-InsP signalling paradigms, explaining the contribution of InsPs and PP-InsPs to diverse biological processes and to pathologies that underlie important human diseases [2].

Previous reviews have focused on the canonical 'second messenger' Ins(1,4,5)P_3 and its contribution to wider InsPs and PP-InsPs metabolism. The binding of diverse ligands to cell surface receptors activates phospholipase C that acts on the plasma membrane lipid PtdIns(4,5)P_2, releasing Ins(1,4,5)P_3 that mobilises intracellular calcium. The importance of these processes to mammalian cell physiology is the subject of many textbooks. One could be led to believe that all InsP and InsP_2 species present in mammalian cells arise during the dephosphorylation of Ins(1,4,5)P_3 to inositol and that all InsP_6 and PP-InsP synthesis proceeds from Ins(1,4,5)P_3. Both assumptions are incorrect. The recent application of ^{13}C-NMR technology to InsP signalling proves the contrary: the most abundant lower phosphorylated inositols are the two phosphorylated species Ins2P and Ins(2,3)P_2 that do not originate from Ins(1,4,5)P_3 catabolism [3]. Recent elucidation of the enzymology responsible for the lipid-independent InsP pathway [4] adds a fresh perspective on InsP and PP-InsP signalling. A cytosolic InsP pathway, described in *Dictyostelium* in the early 1990s [5], and even earlier in plants, has been forgotten by most researchers since it is absent in the budding yeast *Saccharomyces cerevisiae*, used extensively as a model to dissect InsP metabolism [1].

As comparative genomics has revealed differential expansion of the InsP kinase families within diverse clades [6], the limitations of a yeast-centric view have become apparent. Here, by reviewing recent discoveries, we navigate the wider complexity of InsP metabolism. We emphasise the

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elegance of a signalling paradigm whose misregulation is the origin of many human diseases. We discuss how recent technological advances aid reinterpretation of the InsP and PP-InsP metabolic network and explain their proposed mechanism of action, physiological roles, and importance in human disease.

This review acknowledges InsP metabolism and signalling that arises from the phosphorylation of *myo*-inositol. Before proceeding, we suggest the reader becomes familiar with Figure 1, which is dedicated to InsP nomenclature.

The InsP metabolic network

It is impossible to provide an all-encompassing network of InsPs and PP-InsPs since diverse organisms differentially use aspects and pathways of this signalling system. Even within the same organism, different cell types are enriched in different components of the InsP network; some human cell lines possess high $InsP_5$ levels while others do not [7]. There are, however, a few unifying elements present across species. $InsP_6$, also known as phytic acid (Figure 1), is the most abundant InsP species in all organisms analysed to date. A second common characteristic is the presence of high levels of inositol mono- and bis-phosphate species. Another conserved feature is that the major anabolic pathways to $InsP_6$ (discussed later) converge at Ins(1,3,4,5,6)P_5 (Figure 1), with the final 2-phosphorylation carried by IPPK (also called IP_5-2K) (Figure 2). However, the identification of organisms whose genomes do not encode IPPK opens the possibility of an alternative route to $InsP_6$ synthesis [6]. Even in mammals, an alternative route to $InsP_6$ synthesis is likely to exist (discussed later). Some of the key aspects of InsP and PP-InsP metabolism are discussed here.

Technologies to study the InsP metabolic network

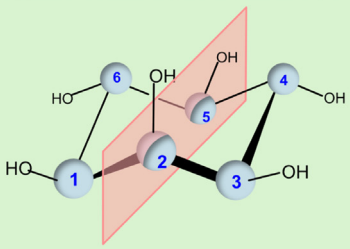
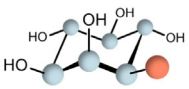
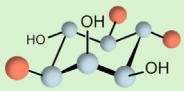
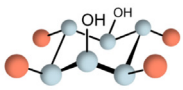
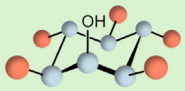
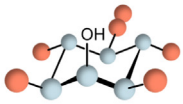
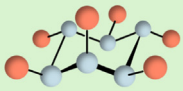
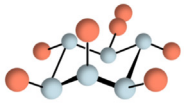
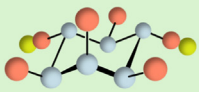
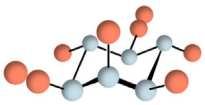
The development of new analytical technologies has revised our understanding of the InsP metabolic network. A lack of intrinsic absorbance or fluorescence of inositol or phosphate has historically hindered the analysis of InsPs. Until recently, the most common method of analysis of the InsP network relied on metabolic labelling of cells or tissues with ^{3}H- or ^{14}C-inositol or ^{32}P-orthophosphate followed by strong anion exchange high-performance liquid chromatography (SAX-HPLC) to resolve radiolabelled InsPs [8]. Metal dye detection (MDD)-HPLC [9], a non-radioactive alternative, was instrumental in discovering the abundant PP-InsPs in amoeba [5], but has not been adopted widely. Polyacrylamide gel electrophoresis (PAGE) and toluidine blue staining are widely used for analysis of $InsP_6$ and PP-InsPs, but the method lacks resolution, especially for lower InsP species [10]. The past 5 years have witnessed the coupling of mass spectrometry (MS) to LC [e.g., hydrophilic interaction chromatography (HILIC)-MS/MS, LC-inductively coupled plasma (ICP)-MS] and to capillary electrophoresis (CE-MS) [7,11,12]. All of these new methods offer opportunities for measurement of InsPs in tissues, organs, and human biopsies, whether in response to diet or physiological intervention [12–14], and all are enabled by the use of TiO_2 to enrich and desalt InsPs [10]. The pre-purification also allows implementation of ^{13}C-NMR analysis [15], technology that does not require chromatography-based InsP separation. ^{13}C-NMR offers direct analysis of InsP metabolism, but, like radiolabelling, demands an isotopic (albeit stable) precursor. Like radiolabelling, ^{13}C-NMR does not report the metabolism of endogenously synthesised inositol (see below). Nevertheless, this recent progress affords opportunities unforeseeable just 5 years ago. For example, MS detection will allow flux and turnover analysis by nonequilibrium labelling with ^{13}C-inositol and/or ^{18}O-water that respectively report on the inositol core and phosphate substituents.

Inositol uptake and synthesis

For simplicity, we describe the InsP and PP-InsP metabolic pathways utilising, primarily, mammalian enzymology (Figure 2 and Table 1). Most eukaryotes can synthesise inositol from the glycolytic

Glossary

Inorganic polyphosphate (polyP): a linear polymer composed of four up to several hundred phosphates linked by high-energy phosphoanhydride bonds.
Inositol phosphates (InsPs): the group of water-soluble InsPs ranges from inositol monophosphate ($InsP_1$) to the fully phosphorylated inositol hexakisphosphate ($InsP_6$).
Inositol pyrophosphates (PP-InsPs): $InsP_7$ or $InsP_8$ harbouring one or two pyrophosphate moieties. These high-energy PP-InsPs are found in all eukaryotic cells, playing emerging roles in diverse signalling functions.
Phosphoinositides (PIs)/phosphatidylinositol phosphates (PtdInsPs): glycerophospholipids found in the cytoplasmic leaflet of the plasma membrane and organelle membranes undergo site-specific phosphorylation and dephosphorylation of the hydroxyl groups on their inositol ring.
Protein pyrophosphorylation: a post-translational modification (PTM) facilitated by PP-InsPs involves transfer of the β-phosphoryl group from PP-InsPs to pre-phosphorylated protein substrates.
SPX domain: a domain approximately 135–400 amino acid residues in length, named after Syg1/Pho81/XPR1 proteins, which functions as an InsP and PP-InsP sensing domain.

Structure	Simplified abbreviation	Abbreviation	Full name
		Ins	*myo*-Inositol
	—	Ins3P	Inositol 3-phosphate
	—	Ins(1,4,5)P_3	Inositol 1,4,5-trisphosphate
	—	Ins(1,3,4,6)P_4	Inositol 1,3,4,6-tetrakisphosphate
	$InsP_5$[2-OH]	Ins(1,3,4,5,6)P_5	Inositol 1,3,4,5,6-pentakisphosphate
	5-PP-$InsP_4$[2-OH]	5-PP-Ins(1,3,4,6)P_4	5-diphosphoinositol 1,3,4,6-tetrakisphosphate
	—	$InsP_6$	Inositol hexakisphosphate
	5-$InsP_7$ 5-PP-$InsP_5$	5-PP-Ins(1,2,3,4,6)P_5	5-diphosphoinositol 1,2,3,4,6-pentakisphosphate
	4/6-$InsP_7$ 4/6-PP-$InsP_5$	4/6-PP-Ins(1,2,3,5,4/6)P_5	4/6-diphosphoinositol 1,2,3,5,4/6-pentakisphosphate
	1,5-$InsP_8$ 1,5-$[PP]_2$-$InsP_4$	1,5-$[PP]_2$-Ins(2,3,4,6)P_4	1,5-bisdiphosphoinositol 2,3,4,6-tetrakisphosphate

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intermediate glucose-6P (Figure 2). InsP synthase (IPS or MIPS) converts glucose-6P to Ins3P, which is then dephosphorylated by inositol-monophosphatase (IMPA) (Table 1) [16]. Intriguingly, MIPS KO cell lines retain a basal level of inositol, made by as-yet-undiscovered enzymes [7]. Cells also acquire inositol from their environment utilising sodium-*myo*-inositol and proton-*myo*-inositol transporters (SMIT and HMIT) [16]. Inositol is an osmolyte in various organs and accumulates to tens of millimolar levels in the brain [17]. In addition to its role as the head group of PtdIns lipids, inositol is also incorporated into sphingolipids such as inositol phosphoceramide (IPC) in yeast and glycosyl inositol phosphoceramide (GIPC) in plants [18]. In *Dictyostelium discoideum* [19] and plants [20], inositol is phosphorylated by *myo*-inositol kinase (MIK). As InsP kinases (Table 1) are unable to phosphorylate inositol in organisms lacking MIK (including mammals), InsP synthesis from exogenously acquired inositol is dependent on flux through PtdIns or sphingolipids.

Synthesis and turnover of $InsP_6$

There are two anabolic pathways to $InsP_6$ (Figure 2). The 'lipid' route in animals involves incorporation of transported inositol into PtdIns, its phosphorylation to PtdIns(4,5)P_2, release of Ins(1,4,5)P_3, conversion to Ins(1,3,4,5,6)P_5 by inositol trisphosphate 3-kinase (IP3K) and inositol polyphosphate multikinase (IPMK), and subsequent transformation to $InsP_6$ by IPPK (Table 1). The 'cytosolic' pathway in mammals originates from Ins3P, which is further phosphorylated by ITPK1 and the subsequent action of IPMK and IPPK [4]. The detection of residual levels of $InsP_6$ in cells lacking IPMK [21] or ITPK1 [4] supports the existence of alternative routes from inositol to $InsP_6$ in mammals.

The InsP network also comprises the action of phosphatases, including lithium-sensitive IMPA1/2 (Table 1) which play a key role in the recovery of inositol from both Ins(1,4,5)P_3 and Ins3P [22]. MINPP1 and homologous phytases are responsible for the dephosphorylation of $InsP_6$ and $InsP_5$s to lower InsPs retaining the phosphate on C2 [3]. The tumour suppressor phosphatase and tensin homolog (PTEN), in addition to its well-documented action on the lipid PtdIns(3,4,5)P_3, also acts on $InsP_5$ [23].

Metabolism of PP-InsPs

At least three, likely more, $InsP_7$ species have been detected in amoebae, mammals, and plants. These include 1-PP-$InsP_5$, 4/6-PP-$InsP_5$, and 5-PP-$InsP_5$ (Figure 1) [24–26]. Inositol hexakisphosphate kinases (IP6Ks) are responsible for 5-PP-$InsP_5$ production in yeast and mammals, while diphosphoinositol pentakisphosphate kinases (PPIP5Ks) generate the 1-PP-$InsP_5$ isomer (Table 1) [27,28]. The enzyme responsible for 4/6-PP-$InsP_5$ synthesis in mammals and plants is unknown, but in *D. discoideum* this isomer is synthesised by IP6K [25]. Conversely, in amoebae, the kinase generating 5-PP-$InsP_5$ remains unknown [25]. Clearly, neither the inositol isomer specificity nor the endogenous substrate pool of an enzyme can be inferred by simple

Figure 1. Structures of selected inositol phosphates (InsPs) and nomenclature guidelines. Of nine possible isomers, the *myo*-isomer [referred to inositol (Ins) in the main text] constitutes the carbon skeleton of the InsPs present in mammals, yeast, plants, and the vast majority of organisms. The examples shown here provide a guide to understand the structure–nomenclature relationship used to describe all InsPs and inositol pyrophosphates (PP-InsPs). The positions (locants) of phosphate (red sphere), pyrophosphate (double red spheres), or hydroxyl (OH) substituents on the cyclohexane ring of *myo*-inositol are numbered according to the D-notation [94]. Hydride H atoms, present on all carbon atoms (grey spheres) are not shown. The characteristic feature of *myo*-inositol is a single axial hydroxyl group on carbon given the locant 2 (C2). The other hydroxyl groups take equatorial positions. This generates a plane of symmetry between C2 and C5 (indicated by the mirror) such that substitution of carbons outside the plane of symmetry (C1, C3, C4, or C6) gives rise to enantiomers. Of the molecules shown, optically inactive mesocompounds are: Ins(1,3,4,6)P_4, Ins(1,3,4,5,6)P_5, 5-PP-Ins(1,3,4,6)P_4, and 5-PP-Ins(1,2,3,4,6)P_5. Substitutions of β-phosphate on undetermined enantiomeric positions, such as C4 or C6, are indicated as 4/6 (yellow spheres). Hydroxyl substituents are conventionally not numbered but in certain instances (e.g., $InsP_5$ isomers, the $InsP_5$ derivative PP-$InsP_4$), their numbering offers a convenient shorthand notation for the naming of isomers.

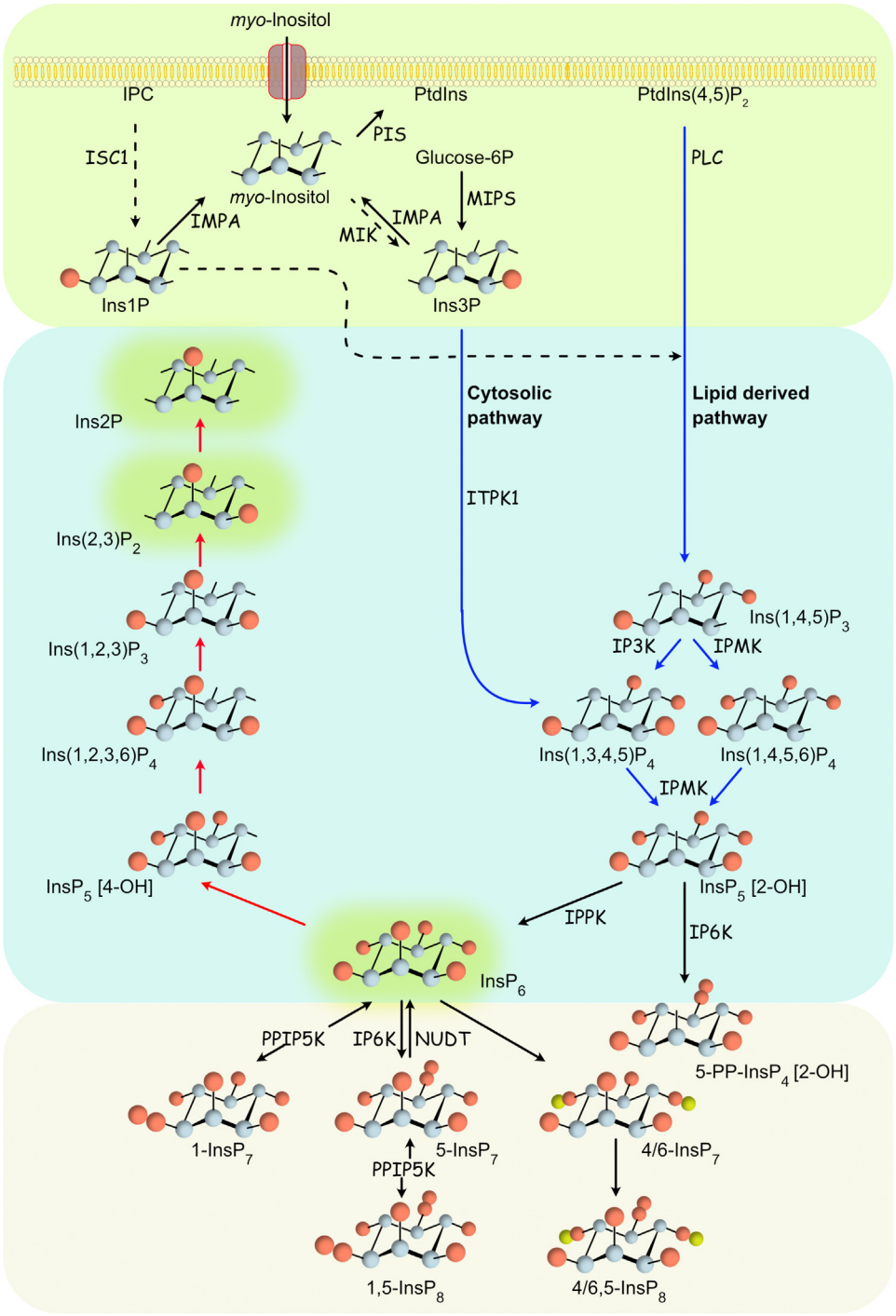

Figure 2. Simplified inositol phosphate (InsP) metabolic network. Here, we represent the contributions of named enzymes to a simplified 'mammalian centric' InsP network. The three coloured backgrounds represent empirical partitioning of the InsP and inositol pyrophosphate (PP-InsP) metabolic network: the origin and initial fate of inositol (green), the major metabolic pathways of InsPs (blue), and interconversions of PP-InsPs (beige). Not all stereoisomers or enantiomers of InsPs are shown, and uncertainties remain; for example, particularly around the order of phosphorylation of

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sequence homology. This is particularly evident in plants where the synthesis of 5-PP-$InsP_5$ is performed by enzymes (ITPK1/2) of a different class than IP6Ks [29]. In mammals, 1,5-$InsP_8$ is made predominantly from 5-$InsP_7$ by PPIP5K [28]. While PP-InsPs derived from $InsP_6$ are the best characterised, $InsP_5$ can give rise to PP-$InsP_4$ by the action of IP6Ks (Figure 2). Similarly, PP-InsPs derived from $InsP_2$ and $InsP_3$ could exist in yeast [30]. The recent identification of a virus-encoded InsP kinase that catalyses the synthesis of PP-InsPs from *myo*- and *scyllo*-InsPs raises the possibility of novel biological species [31].

The β-phosphate in PP-InsPs is attacked by Nudix hydrolases (NUDT3, NUDT4, NUDT10, and NUDT11) (Table 1), members of a family of broad substrate phosphatases acting on PP-InsPs, diadenosine metabolites, and inorganic polyphosphate [32,33]. PPIP5Ks represent a unique class of enzymes as they display both a kinase and a phosphatase domain [28], with the phosphatase domain acting on the kinase-generated 1-PP-$InsP_5$. In fission yeast, the phosphatase domain of the PPIP5K homolog (Asp1) is an important regulator of PP-InsP metabolism [34]. Siw14 in yeast and its orthologs in plants represent another class of phosphatases acting on the pyrophosphate moiety [35].

While the metabolic network may appear intimidating, the few rules highlighted earlier will help the reader comprehend these pathways in the context of their organism of choice. Importantly, additional enzymology awaits discovery as several dozen InsPs and PP-InsPs in mammalian cells are governed by only 11 kinases and 12 phosphatases (Table 1). As a comparison, the just seven **phosphatidylinositol phosphates (PtdInsPs)** are managed by 22 kinases and 26 phosphatases [36].

Dynamic control of cellular InsP levels

On activation by a GPCR ligand such as acetylcholine, hydrolysis of PtdIns(4,5)P_2 yields a 50–100-fold rise in intracellular Ins(1,4,5)P_3 levels within a matter of seconds to trigger Ca^{2+} release by $InsP_3$ receptors (IP3Rs) in the endoplasmic reticulum [37]. Downregulation of this signal is brought about by rapid dephosphorylation of Ins(1,4,5)P_3 to Ins by InsP phosphatases, including INPP5A, INPP1, and IMPA (Table 1) [22]. An alternative metabolic fate of Ins(1,4,5)P_3 is its phosphorylation by IP3Ks to generate Ins(1,3,4,5)P_4. This family of enzymes, absent from yeast and plants, is the only family of InsP kinases with a recognisable modulatory domain, the calmodulin (CaM) binding domain, and thus its activity is upregulated by calcium [38]. The levels of Ins(1,3,4,5)P_4 that may rise in the order of minutes after GPCR activation may be considered a 'signal' in its own right [39]. By contrast, $InsP_5$ and $InsP_6$ are considered 'metabolic sluggards' [40]. They are responsive on much longer timescales to conditions such as phosphate starvation [24,41] that impact nucleotide homeostasis. A convincing demonstration of rapid, activated metabolism of PP-InsPs is yet to be described, although treatment with fluoride strongly upregulates the levels of PP-InsPs in mammalian cell lines by inhibiting NUDTs [also called

Ins3P by ITPK1 and the points of intersection of cytoplasmic and lipid-derived pathways. The anabolic cytoplasmic and lipid-derived pathways are represented by blue arrows, while the principal intermediates arising from dephosphorylation of $InsP_6$ by MINPP1 are depicted by red arrows. The yellow highlight indicates the most abundant InsPs present in mammalian cells. Structural organisation of the represented InsPs and their abbreviated names follow the guidelines in Figure 1 (except that OH groups are not indicated). Participating kinases and phosphatases are abbreviated as in Table 1; due to space constraints, we do not spell out the different isoforms (e.g., IP6K stands for IP6K1/2/3). Additional represented enzymes are phospholipase C (PLC), *myo*-inositol phosphate synthetase (MIPS), and phosphatidylinositol synthase (PIS). Enzymology represented by broken lines is absent in mammals, but is important to appreciate the varied nature of this metabolic network. In organisms such as fungi and trypanosomes, the lipid-derived pathway can originate from inositol phosphoceramide (IPC) by the action of inositol phosphosphingolipid phospholipase C (ISC1). Organisms such as amoebae and plants possess *myo*-inositol kinase (MIK), which is able to phosphorylate inositol and initiate the cytosolic route of InsP synthesis from imported inositol.

Table 1. InsP kinases and phosphatases[a,b]

Kinases	*Homo sapiens*	Main reaction catalysed	*Saccharomyces cerevisiae*
Inositol-trisphosphate 3-kinase	IP3KA IP3KB IP3KC	Ins(1,4,5)P_3 to Ins(1,3,4,5)P_4	–
Inositol polyphosphate multikinase	IPMK	Ins(1,4,5)P_3 to Ins(1,3,4,5,6)P_5	Arg82 (Ipk2)
Inositol hexakisphosphate kinase	IP6K1 IP6K2 IP6K3	InsP_6 to 5-InsP_7	Kcs1
Inositol-tetrakisphosphate 1-kinase	ITPK1	Ins(1,3,4)P_3 to Ins(1,3,4,6)P_4	–
Inositol-pentakisphosphate 2-kinase	IPPK	InsP_5 [2-OH] to InsP_6	Ipk1
Diphosphoinositol-pentakisphosphate kinase	PPIP5K1 PPIP5K2 (kinase domain)	5-InsP_7 to 1,5-InsP_8	Vip1 (kinase domain)
Phosphatases	*Homo sapiens*	Main reaction catalysed	*Saccharomyces cerevisiae*
Inositol monophosphatase	IMPA1 IMPA2	Ins1P to Ins	Inm1 Inm2
Inositol polyphosphate-1-phosphatase	INPP1	Ins(1,4)P to Ins4P	–
Inositol polyphosphate-5-phosphatase A	INPP5A	Ins(1,4,5)P_3 to Ins(1,4)P_2	–
PTEN	PTEN	Ins(1,3,4,5,6)P_5 to Ins(1,4,5,6)P_4	–
Multiple inositol-polyphosphate phosphatase	MINPP1	InsP_6 to Ins(1,2)P_2	–
Diphosphoinositol polyphosphate phosphohydrolase	NUDT3 NUDT4 NUDT10 NUDT11	5-InsP_7 to InsP_6	Ddp1
Diphosphoinositol-pentakisphosphate kinase	PPIP5K1 PPIP5K2 (phosphatase domain)	1,5-InsP_8 to 5-InsP_7	Vip1 (phosphatase domain)
Inositol phosphatase Siw14	–	–	Siw14 (Oca3)

[a]The table was constructed by consulting the following databases: *S. cerevisiae*, https://www.yeastgenome.org/; *H. sapiens*, https://www.uniprot.org/.
[b]Shown here is the primary, but not exclusive, enzymatic reaction catalysed by the mammalian enzyme.

diphosphoinositol polyphosphate phosphohydrolases (DIPPs)] [33]. Using this metabolic trap, it has been calculated that every hour ~30–50% of the entire cellular pools of InsP_5 and InsP_6 cycle through PP-InsPs [42]. While a recent report claims that GPCR activation led to PP-InsP_5 production [43], this may be a cell-specific phenomenon since an earlier measurement of PP-InsPs after GPCR activation revealed no change in PP-InsP levels [44].

Modes of protein regulation by InsPs

InsPs can influence specific target proteins to control cellular signalling through various molecular actions that are summarised below (Figure 3, Key figure).

Transient regulatory binding of InsPs with proteins

Akin to the 'classical' Ins(1,4,5)P_3-gated Ca^{2+} channel IP3Rs, many proteins undergo regulation by allosteric binding with specific InsPs (Table 2). 5-InsP_7 binding to the PH domain of the protein kinase AKT competitively inhibits its recruitment to PtdIns(3,4,5)P_3 at the plasma membrane and hinders AKT activation [45]. Binding with 5-InsP_7 deactivates the calcium sensor synaptotagmin (SYT1), to suppress synaptic vesicle exocytosis and neurotransmitter release in neuronal presynaptic terminals [46]. A role for 5-InsP_7-mediated upregulation of insulin exocytosis in pancreatic β cells [47] has been attributed to its binding another calcium sensor, SYT7 [43]. Sequestration of 5-InsP_7 by calcium releases 5-InsP_7 bound SYT7 to instead bind PtdIns(4,5)P_2 on the plasma membrane, where it facilitates the fusion of insulin-containing vesicles. The **SPX domain** (named for yeast Syg1, Pho81, and human XPR1) has emerged as a canonical InsP binding domain present in several proteins involved in phosphate homeostasis [48]. In mammals, the only documented SPX domain containing protein is xenotropic and polytropic retrovirus receptor 1 (XPR1), a phosphate exporter that is allosterically upregulated on high-affinity binding with 1,5-InsP_8 [49].

InsP as a stable cofactor

An important feature of InsPs is their ability to stably bind to proteins and contribute to their structural integrity. InsPs have been detected in several protein structures, suggesting their involvement in numerous cellular processes (Table 2), including RNA editing and growth signalling [50,51]. For example, InsP_6 is largely buried within the core of the RNA editing enzyme adenosine deaminase acting on RNA 2 (ADAR2), thereby stabilising the protein fold [50], analogous to the incorporation of metal ions essential for the folding of metalloproteins.

InsP as an intermolecular bridge

InsPs can serve as essential components in multisubunit complexes, acting as an intermolecular 'electrostatic glue' by binding positively charged surfaces on proteins. This binding can contribute to stabilisation of the protein complex and modulation of its activity. For instance, HIV-1 recruits InsP_6 into virions by coordination with lysine residues in its immature capsid hexamers, to promote viral assembly and replication [21,52].

Protein pyrophosphorylation by PP-InsPs

A regulatory mechanism unique to PP-InsPs is the transfer of their β-phosphate to pre-phosphorylated Ser residues to generate pyrophosphorylated Ser [53,54]. Sequences undergoing pyrophosphorylation are typically intrinsically disordered and rich in Ser, Glu, and Asp residues. A newly developed MS workflow revealed endogenous **protein pyrophosphorylation** on more than 70 human proteins, of which nucleolar proteins NOLC1 and TCOF1 were extensively pyrophosphorylated [55]. Perturbation of 5-InsP_7 synthesis led to reduced pyrophosphorylation of these proteins, along with a decrease in rRNA synthesis, supporting a role for nucleolar protein pyrophosphorylation in ribosome biogenesis. Other cellular functions attributed to 5-InsP_7-mediated pyrophosphorylation include the regulation of protein stability, glycolysis, and vesicle trafficking (Table 2). While CK2 is primarily responsible for pre-phosphorylation of the Ser residues that subsequently undergo pyrophosphorylation, phosphotransfer from PP-InsP to the protein *per se* is thought to be enzyme independent. The metabolic enzyme UDP-N-acetylglucosamine pyrophosphorylase 1 (UAP1) has been reported to catalyse pyrophosphorylation of the immune signalling factor interferon regulatory factor 3 (IRF3) [56], but it remains to be seen whether UAP1

Key figure

Schematic overview of inositol phosphate (InsP) metabolism-mediated biological actions

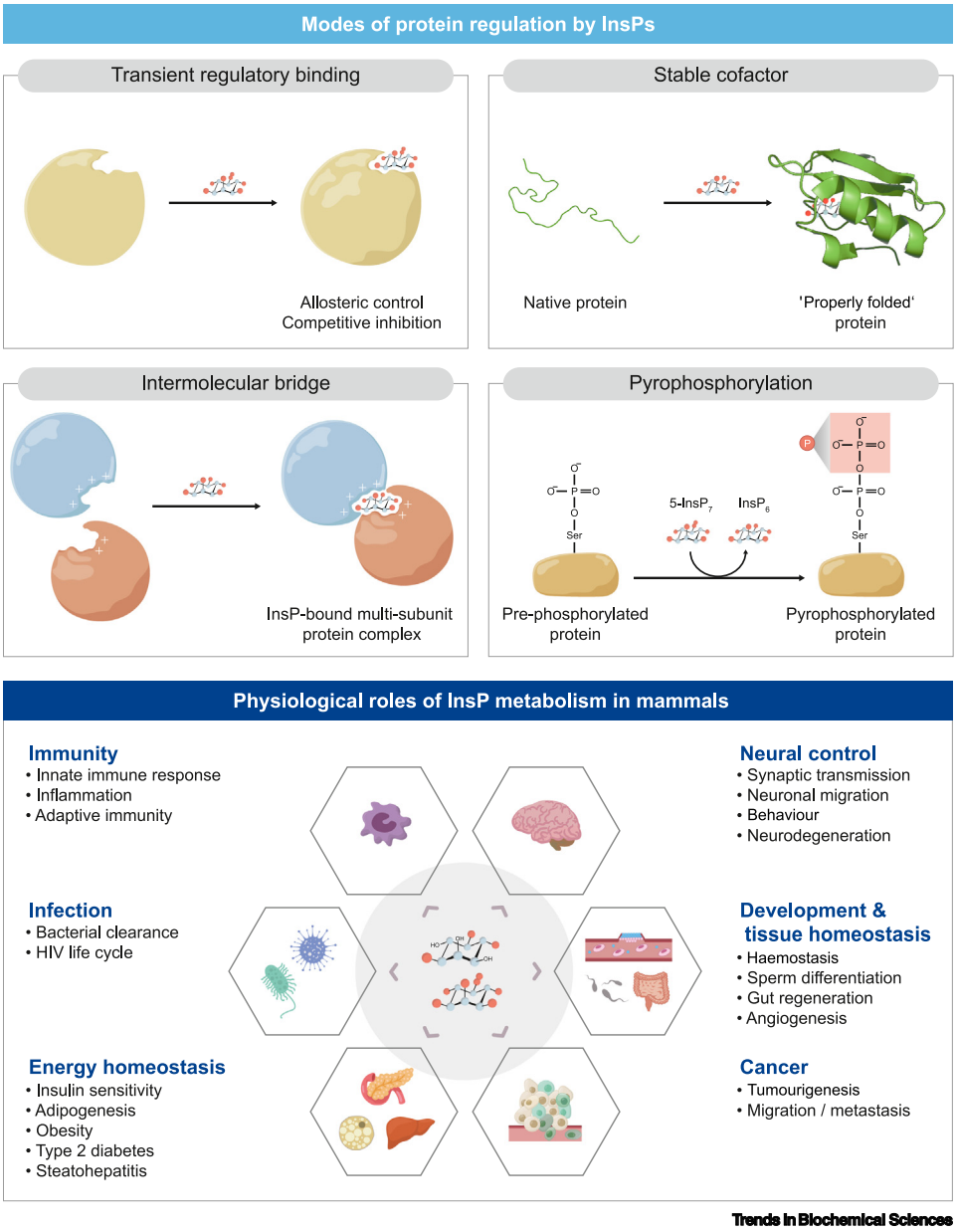

Figure 3. Summary of four major molecular mechanisms of action elicited by InsPs (top panel). InsPs can modulate the characteristics of target proteins (e.g., activity, function, localisation, stability), serving as a transiently binding regulator, a stable cofactor, or an intermolecular bridge. In particular, inositol pyrophosphates (PP-InsPs) can mediate protein pyrophosphorylation. In mammals, InsP metabolism regulates various biological events in the control of homeostasis and in pathological conditions (bottom panel).

Table 2. List of InsP–protein interactions and their functional significance[a,b]

Transient regulatory InsP–protein binding			
Target protein	Ligand	Function	Refs
SPX domain proteins*,**	5-InsP$_7$ 1-InsP$_7$ 1,5-InsP$_8$	Control of phosphate homeostasis via phosphate transporters (e.g., XPR1), PHR transcription factors, and polyphosphate polymerase VTC complex	[48,49]
AKT	5-InsP$_7$	Inhibition of AKT via competition with PIP$_3$ for binding at the PH domain of AKT	[45]
SYT1, SYT7		Inhibition of SYT function in vesicle fusion	[43,46]
CK2		Allosteric activation of CK2	[83]
PI3K p85α		Increased binding between PI3K p85α and Na^+/K^+-ATPase-α1	[95]
Pho81*	1-InsP$_7$	Pho81-dependent Pho80-Pho85 inactivation	[96]
BTK	InsP$_6$	Allosteric activation of BTK by stimulating its dimerisation	[97]
CK2		Activation of CK2 via competitive binding at its substrate recognition site	[98]
DNA-PK		Stimulation of nonhomologous end joining via direct InsP$_6$–Ku70/80 interaction	[99]
MLKL		Direct activation of MLKL via displacement of its autoinhibitory region	[84]
ITK	Ins(1,3,4,5)P$_4$	Activation of ITK by promotion of ITK PH domain binding to PIP$_3$	[72]
InsP as a stable cofactor			
Target protein	Ligand	Function	Refs
ADAR2, ADAT1	InsP$_6$	Essential for RNA-editing deaminase activity	[50]
mTORC2		Predicted to control folding and/or activity	[51]
PDS5		Critical for PDS5 folding to control cohesion dynamics	[100]
TRPA1		Predicted to control folding and/or activity	[101]
TIR1**		TIR1 function in auxin perception	[102]
COI1**	InsP$_5$ [2-OH]	COI1-Jaz coreceptor complex for jasmonate perception	[103]
InsP as an intermolecular bridge			
Target protein	Ligand	Function	Refs
HIV capsid protein CA	InsP$_6$	Assembly and maturation of the HIV capsid lattice	[21,52]
IntS4, IntS9, IntS11		Integrator function in snRNA 3′-end processing and mRNA transcription attenuation	[104]
NAA10, NAA15		Formation of N-terminal acetyltransferase (Nat) complex	[105]
Cullin, CSN2	InsP$_6$, 5-InsP$_7$	Assembly and disassembly of the CRL–CSN complex	[81]
HDAC3, DAD	Ins(1,4,5,6)P$_4$	Assembly of the SMRT–HDAC3 repression complex	[106]
Protein pyrophosphorylation			
Target protein	Substrate	Function	Refs
AP3B1	5-InsP$_7$	Stimulation of HIV-1 Gag release via Kif3A	[107]
Dynein IC		Control of vesicle transport via increased dynein–dynactin interaction	[108]
MYC		Promotion of MYC ubiquitination and degradation	[109]
RNA Pol I subunits		Activation of transcriptional elongation	[110]
Gcr1*		Transcriptional suppression of glycolytic genes	[111]
IRF3		Activation of type I interferon responses	[56]
NOLC1, TCOF1, UBF1		Possible regulation of rDNA transcription	[55]
Nsr1*, Srp40*		Unknown	[54]

can catalyse the pyrophosphorylation of other protein substrates. Specific depyrophosphatases, which can convert pyrophospho-Ser back to phospho-Ser, also await discovery.

In summary, InsPs influence specific target proteins to control cellular signalling. Noncovalent interactions between InsPs and proteins can occur either transiently or stably. PP-InsPs also serve as donors for covalent protein modification. Despite the increasing number of proteins directly targeted by InsPs, our understanding of the biochemical principles underlying the mechanistic actions of InsPs on target protein structure and function remains insufficient. Further investigation is required to elucidate the upstream signalling pathways governing InsP–protein interactions.

Biological actions of InsPs in physiology and disease

To investigate the cellular actions of InsPs, their levels have been manipulated through overexpression or deletion of InsP kinases responsible for their biosynthesis. These approaches impact not just the substrate and product of the manipulated InsP kinase, but also groups of InsPs that lie upstream or downstream in the metabolic pathway. In addition, InsP kinases may perform functions independent of their catalytic activity. The scope of cellular processes controlled by InsPs is remarkably broad, encompassing cell growth, energy metabolism, vesicular trafficking, and immune signalling (Figure 3). The identification of specific InsP–target protein interactions, and their mechanisms of action, lays the foundation for the understanding of InsP-regulated cellular events.

The *in vivo* relevance of InsP metabolism in mammals has been revealed by studies of mouse models selectively deleted for specific InsP kinases. For InsP kinases with multiple paralogs (e.g., IP6K1/2/3), individual gene KO mice grow to adulthood, but for those kinases with a single mammalian isoform (e.g., IPMK, IPPK), whole-body KO mice exhibit embryonic lethality, underscoring the vital role of InsP metabolism [57,58]. Consequently, cell-type-specific KO studies in mice have yielded significant insight into the physiological roles of InsPs (Table 3). Targeting specific InsP kinases in mice can also ameliorate or aggravate diseases such as obesity, bacterial infection, or cancer. Some of the key functions of InsP metabolism that emerge from studies in mice are described here.

Energy homeostasis and metabolic syndrome

The significant impact of InsP metabolism on energy homeostasis was first highlighted through whole-body deletion of IP6K1 in mice [45,59]. Loss of IP6K1 and the consequent decrease in 5-InsP$_7$ leads to hyperactivation of AKT and downstream insulin signalling events. Consequently, IP6K1 KO mice fed a high-fat diet are shielded from obesity, hyperinsulinemia, hyperglycaemia, hepatic steatosis, and insulin resistance [45]. Conditional deletion of IP6K1 in adipocytes enhances thermogenic energy expenditure in mice, providing protection against high-fat diet-induced obesity [60]. Whole-body and adipocyte-specific deletion of IP6K1 in mice also increases plasma adiponectin levels by preventing 5-InsP-stimulated adiponectin degradation, thereby ameliorating myocardial ischaemia-reperfusion injury [61].

Behaviour and neurological function

Lithium, the most prescribed mood-stabilising drug for bipolar disorder, targets neuronal InsP signalling by inhibiting IMPA1 and blocking the recycling of Ins(1,4,5)P$_3$ [22,62]. IP6K1 KO mice

Notes to Table 2:
[a]*Yeast and **plant exclusive proteins. Mammals possess only one SPX domain-containing protein, whereas > 10 are found in yeasts and > 20 in plants.
[b]Abbreviations: ADAT, adenosine deaminase acting on tRNA; BTK, Bruton's tyrosine kinase; CK, casein kinase; DAD, deacetylase activating domain; DNA-PK, DNA-dependent protein kinase; HDAC, histone deacetylase; mTORC2, mechanistic target of rapamycin complex 2; PDS5, precocious dissociation of sisters 5; TRPA1, transient receptor potential ankyrin 1.

Table 3. Biological actions of InsP metabolism in physiology and disease[a]

Function	Model system	Phenotype	Refs
Embryo development	IPPK KO mice	Early embryonic lethality	[57]
	IPMK KO mice	Early embryonic lethality Embryonic growth retardation Neural tube defect	[58]
	ITPK1 hypomorphic mice	Embryonic growth retardation Neural tube defect Defective skeletal development	[112]
Energy homeostasis and metabolic syndrome	IP6K1 KO mice, adipocyte IP6K1 KO mice, hepatocyte IP6K1 KO mice	Protection of mice from obesity Insulin hypersensitivity Increased fat breakdown and impaired adipogenesis Decreased hepatic steatosis Elevated thermogenic activity Reduced myocardial ischaemia-reperfusion injury	[45,59–61,113]
	β cell IP6K1 KO mice	Reduced insulin secretion and glucose clearance elicited by muscarinic stimulation	[43]
	$IP6K1^{S118D/S121D}$ knock-in mice	Augmented insulin release, congenital hyperinsulinaemia, and obesity	[43]
	IP6K3 KO mice	Enhanced glucose tolerance, reduced circulating insulin, deceased fat mass, lowered body weight, increased lifespan	[114]
	Hepatocyte IPMK KO mice	Impaired glucose homeostasis and insulin sensitivity/exacerbated NASH progression	[115,116]
Behaviour and neural control	IP6K1 KO mice	Increased excitatory synaptic vesicle release Impaired synaptic endocytosis Defective neuronal migration Dysregulated locomotor activity and social behaviour	[63,66,67]
	IP6K2 KO mice	Disrupted cerebellar disposition and psychomotor behaviour	[64]
	IP6K3 KO mice	Abnormalities in Purkinje cell structure and synapse number Deficits in motor learning and coordination	[65]
	Excitatory neuron IPMK KO mice	Enhanced LTP, spatial memory deficit, accelerated fear memory extinction, increased vascularisation	[69,117]
	IP3KA KO mice	Impaired LTP	[68]
Infection and immunity	IP6K1 KO mice	Enhanced bacterial killing Reduced neutrophil-mediated pulmonary damage in bacterial pneumonia	[75,118]
	B lymphocyte IPMK KO mice	Defective B cell responses against T cell-independent antigens	[74]
	Treg lymphocyte IPMK KO mice	Diminished effector Treg cell differentiation Reduced Treg immune function (e.g., exacerbated colitis, augmented antitumour immunity)	[73]
	Macrophage IPMK KO mice	Protection of mice against polymicrobial sepsis and lipopolysaccharide-induced systemic inflammation	[119]
	Hepatocyte IPMK KO mice	Increased liver inflammation Reduced hepatic tissue regeneration	[120]
	IP3KB KO mice, IP3KC KO mice	Impaired development of T cell and B cell	[121,122]
Haemostasis	IP6K1 KO mice	Reduced platelet aggregation Increased plasma clotting time	[76]

Table 3. (continued)

Function	Model system	Phenotype	Refs
Gut	Intestine epithelial cell IPMK KO mice	Reduced intestine tissue regeneration Impaired development of Paneth cell and tuft cell	[123,124]
Kidney	IP6K1 KO mice	Reduced renal Na^+ excretion on a high-salt diet	[95]
	Kidney epithelial cell IP6K1/IP6K2 double-KO mice	Lowered phosphate uptake into proximal renal brush border membranes Kidney dysfunction (e.g., increased bone resorption, diuresis, albuminuria, hypercalciuria)	[125]
Reproduction and fertility	IP6K1 KO mice	Defective germ cell differentiation and development	[59,77–79]
Sensory system	PPIP5K2 phosphatase domain KO mice	Degeneration of cochlear outer hair cells Progressive hearing loss Abnormal corneal curvature and thinning	[87,88]
Cancer	IP6K1 KO mice	Resistance to 4NQO-induced carcinogenesis	[85]
	IP6K2 KO mice	Increased tumorigenesis in response to 4NQO exposure	[86]

[a]Abbreviations: LTP, long-term potentiation; NASH, non-alcoholic steatohepatitis; 4NQO, 4-nitroquinoline oxide.

exhibit differences in amphetamine-induced locomotion and display autism-like social behaviour, including reduced exploration and engagement [63]. IP6K2 and IP6K3 KO mice exhibit notable impairment in motor coordination, with defects in cerebellar synaptic structure and function [64,65]. Recent discoveries on the influence of 5-InsP on synaptic vesicle cycling [46,66,67] and synaptic transmission [68,69] will spur investigations on spatial and temporal regulation of InsP metabolism in the brain. Elevated levels of $InsP_7$ have been detected in tissue samples from amyotrophic lateral sclerosis patients and lymphoblasts from Huntington's disease patients [70,71]. Understanding the proapoptotic signalling actions of PP-InsPs underlying neuronal cell death may offer new ways to tackle the onset and progression of neurodegenerative diseases.

Infection and immunity

$Ins(1,3,4,5)P_4$ promotes binding of the PH domain of IL-2-inducible tyrosine kinase (ITK) to $PtdIns(3,4,5)P_3$, which is crucial for ITK activation during T cell development [72]. Deletion of IPMK impairs the function of regulatory T cells, as these cells rely on IPMK-generated $Ins(1,3,4,5)P_4$ for T cell receptor-triggered calcium influx that drives their differentiation [73]. Loss of $InsP_6$ arising from conditional deletion of IPMK in B cells reduces the activation of the tyrosine kinase BTK and cellular proliferation in response to antigens [74]. Deletion of IP6K1 in mice results in decreased accumulation of neutrophils and mitigation of lung damage on bacterial pneumonia [75]. IP6K1 supports the production of **inorganic polyphosphate (polyP)** in platelets [76], which is required for the formation of infection-induced neutrophil–platelet aggregates and the accumulation of neutrophils in alveolar spaces during bacterial pneumonia [75].

Reproduction and fertility

Deletion of IP6K1 in mice leads to male sterility without affecting the female reproductive system [59]. IP6K1 KO males show a failure of spermiogenesis, with few advanced spermatids in seminiferous tubules and no sperm in the epididymis. IP6K1 deletion disrupts the chromatoid body, a spermatid-specific ribonucleoprotein granule involved in translational repression [77]. Premature synthesis of key spermatid proteins transition nuclear protein 2 (TNP2) and protamine 2 (PRM2) results in abnormal nuclear DNA condensation in elongating IP6K1 KO spermatids. In addition, interactions between germ cells and Sertoli cells are disrupted in IP6K1 KO testes, pointing to a role for IP6K1 in maintaining cell junction integrity [78,79].

Cell growth, survival, and migration, DNA repair, and cancer

Cell line-based studies have uncovered diverse functions of InsPs in pathways relevant to cancer, including DNA repair, cell proliferation, and migration. As previously mentioned, 5-InsP$_7$ downregulates AKT [45], a kinase known to stimulate cell growth and proliferation. 5-InsP$_7$ also contributes to DNA repair pathways including homologous recombination [80] and nucleotide excision repair (NER) [81]. IP6K1 forms a complex with the COP9 signalosome (CSN), a multiprotein complex that keeps the ubiquitin E3 ligase CRL4 inactive. Exposure to UV light triggers 5-InsP$_7$ synthesis, which disrupts this complex and activates CRL4-dependent NER. Depletion of IP6K2 in cancer cells protects against apoptosis by inhibiting p53-mediated induction of cell cycle arrest genes [82]. 5-InsP$_7$ stimulates CK2-mediated phosphorylation of the TTT co-chaperone complex, activating phosphorylation of p53 by the protein kinases DNA-PK and ATM, to promote cell death [83]. InsP$_6$ interacts with and activates mixed lineage kinase domain-like pseudokinase (MLKL), leading to necroptosis, a form of programmed cell death combining necrosis and apoptosis [84]. 5-InsP$_7$ also regulates cell migration, with IP6K1 deletion reducing focal adhesion kinase (FAK) activity [85]. KO of IP6K1 in mice protects against chemical carcinogenesis [85], while the loss of IP6K2 sensitises mice to chemical tumorigenesis and increases spontaneous cancer occurrence [86]. Comprehensive studies using mouse tumour models are necessary to advance the above findings towards possible therapeutic applications of the targeting of specific InsPs in different types of cancer.

Developmental disorders

Whole-genome sequencing in familial keratoconus patients identified mutations in the phosphatase domain of PPIP5K2 [87]. Keratoconus is an eye disorder characterised by progressive thinning and conical protrusion of the cornea, resulting in blurry vision, nearsightedness, and light sensitivity. A mouse model carrying a PPIP5K2 phosphatase domain deletion exhibits corneal irregularities and thinning akin to keratoconus. Mutations in PPIP5K2 have also been associated with non-syndromic recessive inherited hearing loss (NSRHL) [88]. A missense mutation in the phosphatase domain of PPIP5K2 has been identified in NSRHL families. Mice lacking the PPIP5K2 phosphatase domain display cochlear outer hair cell degeneration and increased hearing thresholds. MINPP1 has been proposed as a candidate gene for pontocerebellar hypoplasia, a neurodevelopmental disorder characterised by atrophy and hypoplasia of the pons and cerebellum [89]. Further research is necessary to elucidate the molecular mechanisms underlying the role of altered InsP metabolism in these developmental disorders.

Concluding remarks

The rewriting of InsP metabolic pathways in the contemporary literature offers fresh perspectives on the contribution of this canonical signalling system to physiology. InsPs and PP-InsPs employ a variety of molecular mechanisms to perform a diverse array of cellular and physiological functions. Recent studies have uncovered critical roles for these molecules and their cognate enzymes in the aetiology and progression of various diseases, spurring the discovery of specific InsP kinase inhibitors that may see therapeutic applications in the future [90]. While historically, receptor activation was seen as the driver of InsP metabolism, recently cellular energetics and phosphate homeostasis have been identified as modulators and targets of InsP signalling [41,49]. We must apply modern analytical approaches to characterise the enantiomeric complexity, flux, and turnover of cellular InsPs and PP-InsPs. Metabolically diverse InsP pools are likely to localise in different cellular compartments receptive to distinct InsP fluxes, and are thus likely to assume diverse signalling roles (see Outstanding questions). The localisation, at least in part, of IPMK and IP6K1 within the nucleus [55,91], combined with the pancellular action of their products, points to the existence of InsP movement between cellular compartments. Defining InsP transport by newly developed microscopy approaches will be transformative in identifying the versatility of InsP

Outstanding questions

Cell biology/biochemistry

- What mechanisms do cells have to compartmentalise individual InsPs and PP-InsPs? Do these include a combination of localised synthesis, intracellular transport, and concentration-driven diffusion?
- Are InsPs and PP-InsPs actively transported between subcellular organelles? If yes, what players are involved in this exchange?
- In the milieu of multiple InsPs and PP-InsPs, how are InsP and/or PP-InsP ligands of cognate binding proteins isolated from the crowd?
- In cases where multiple InsPs/PP-InsPs can bind a specific protein, how can we identify the *bone fide in vivo* ligand?
- How can we distinguish whether an InsP kinase/phosphatase KO phenotype is dependent on a specific InsP or a pleiotropic effect on the entire pathway?

Physiology

- How much of InsP and PP-InsP metabolism and function is isolated from cell surface receptor activation?
- What are the potential therapeutic applications of specific InsP kinase inhibitors?
- How do InsPs and PP-InsPs in different tissues and organs respond to treatment with InsP kinase inhibitors?
- Are tissue InsPs and PP-InsPs responsive to diet, and what is the sensitivity of different organs?

Evolutionary biology

- What are the origins and biological functions of non-*myo*-InsP isomers?
- Can we harness the differences in InsP pathways and the structural diversity of InsP kinases for therapeutic opportunities against fungal and protozoan pathogens?

signalling. Looking forward, breakthroughs could arise from the development of InsP-specific microscopy sensors or fluorescence probes. While the similarity in structures between InsPs might make this endeavour difficult, success in this direction will generate paradigm-shifting discoveries. Besides probes and sensors, multi-isotope imaging MS (MIMS) [92] and imaging Raman microspectroscopy potentially offer noninvasive opportunities, the latter at least for abundant $InsP_6$ [93]. With all new discoveries, it is of paramount importance that the methodologies and interpretations arising therefrom are validated in other laboratories.

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Declaration of interests

The authors declare that they have no conflicts of interest.

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