The inositol pyrophosphate pathway in health and diseases

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Abstract

Inositol pyrophosphates (IPPs) are present in organisms ranging from plants, slime moulds and fungi to mammals. Distinct classes of kinases generate different forms of energetic diphosphate-containing IPPs from inositol phosphates (IPs). Conversely, polyphosphate phosphohydrolase enzymes dephosphorylate IPPs to regenerate the respective IPs. IPPs and/or their metabolizing enzymes regulate various cell biological processes by modulating many proteins *via* diverse mechanisms. In the last decade, extensive research has been conducted in mammalian systems, particularly in knockout mouse models of relevant enzymes. Results obtained from these studies suggest impacts of the IPP pathway on organ development, especially of brain and testis. Conversely, deletion of specific enzymes in the pathway protects mice from various diseases such as diet-induced obesity (DIO), type-2 diabetes (T2D), fatty liver, bacterial infection, thromboembolism, cancer metastasis and aging. Furthermore, pharmacological inhibition of the same class of enzymes in mice validates the therapeutic importance of this pathway in cardio-metabolic diseases. This review critically analyses these findings and summarizes the significance of the IPP pathway in mammalian health and diseases. It also evaluates benefits and risks of targeting this pathway in disease therapies. Finally, future directions of mammalian IPP research are discussed.

I. INTRODUCTION

Myo-inositol and its various soluble and lipid-associated inositol phosphate (IP) derivatives play important biological functions in some bacteria, and in fungi, higher plants, and mammals (Michell, 1975; Holub, 1986). In the early 20th century, the inositol hexakisphosphate or phytic acid [I(1,2,3,4,5,6)P6 or IP6] was identified as the principal phosphate-storage molecule in plants (Plimmer & Page, 1913; Vucenik & Shamsuddin, 2003). In the mid-1980s, the discovery of inositol trisphosphate [I(1,4,5)P3 or IP3]-mediated release of intracellular calcium accelerated interest in the biological functions of IPs (Streb *et al.,* 1983; Berridge, Lipp & Bootman, 2000). IP derivatives with energetic di(β)phosphates

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of IP6 were identified in the early 1990s (Europe-Finner, Gammon & Newell, 1991; Stephens *et al.,* 1991; Oliver *et al.,* 1992; Wong *et al.,* 1992). These molecules were termed 'inositol pyrophosphates (IPPs)', to distinguish them from the monoester-based IPs (Glennon & Shears, 1993; Menniti *et al.,* 1993; Stephens *et al.,* 1993). Due to a rapid turnover rate, this class of molecules was predicted to function as signalling modulators (Glennon & Shears, 1993).

IPP nomenclature has been discussed elsewhere (Barker *et al.,* 2009; Saiardi, 2012; Wundenberg & Mayr, 2012; Shears, 2015). Four well-characterized mammalian IPPs, 5-diphosphoinositol (1,3,4,6)-tetrakisphosphate (5PP-IP4), 1-diphosphoinositol (2,3,4,5,6) pentakisphosphate (1PP-IP5 or 1-IP7), 5-diphosphoinositol (1,2,3,4,6) pentakisphosphate (5PP-IP5 or 5-IP7) and 1,5-bisdiphosphoinositol (2,3,4,6) tetrakisphosphate (1,5PP2-IP4 or 1,5-IP8) are discussed here. When 5-IP7 and 1-IP7 are indistinguishable, the term IP7 is used. PPP-IP5, PP-IP3, (PP)2-IP3 and (PP)2-IP2 (Draskovic *et al.,* 2008; Wundenberg & Mayr, 2012; Thota & Bhandari, 2015) are not characterized in mammals and thus, are omitted from further discussion.

Studies in lower organisms establish that IPPs regulate various biological processes. A slime mould-specific IP7 regulates chemotaxis (Luo *et al.,* 2003). In fungi, the pathway modulates telomere length (Saiardi *et al.,* 2005; York *et al.,* 2005), DNA hyper-recombination (Luo *et al.,* 2002), vacuole biogenesis (Saiardi *et al.,* 2000), endocytosis and call wall integrity (Saiardi *et al.,* 2002), ribosomal RNA (rRNA) transcription (Thota *et al.,* 2015), environmental stress response (Worley, Luo & Capaldi, 2013), adaptation to the host environment and pathogenicity (Lev *et al.,* 2015), virulence (Li *et al.,* 2016), phosphate homeostasis (Lee *et al.,* 2007) and cell death (Onnebo & Saiardi, 2009). In fruit flies *Drosophila melanogaster*, it is associated with insulin signalling (Williams *et al.,* 2015). In zebrafish *Danio rerio*, the IPP pathway acts as an effector of the hedgehog signalling pathway, which modulates development of craniofacial structures and neural crest cells (Sarmah & Wente, 2010).

A major question is 'what is the significance of the IPP pathway in mammalian health?' To address this, extensive research has been carried out in mammalian systems, including detection of IPPs (Menniti *et al.,* 1993; Stephens *et al.,* 1993), and cloning and characterization of relevant enzymes (Nagase *et al.,* 1996; Voglmaier *et al.,* 1996; Saiardi *et al.,* 1999, 2001; Yang, Safrany & Shears, 1999; Caffrey *et al.,* 2000; Choi *et al.,* 2007; Fridy *et al.,* 2007). Impacts of the IPP pathway on mammalian cells were also discovered (Barker *et al.,* 2009; Chakraborty, Kim & Snyder, 2011; Wundenberg & Mayr, 2012; Wilson, Livermore & Saiardi, 2013; Thota & Bhandari, 2015) and generation of knockout (KO) mouse models established the *in vivo* significance of the IPP pathway (Bhandari *et al.,* 2008; Morrison *et al.,* 2009; Rao *et al.,* 2014; Fu *et al.,* 2015; Moritoh *et al.,* 2016; Zhu *et al.,* 2016). The beneficial effects of pharmacological inhibition of this pathway in various rodent disease models demonstrated its therapeutic significance. This review encompasses impacts of the IPP pathway in health and diseases.

II. IPP-METABOLIZING ENZYMES IN MAMMALS

The intricacies of the IPP metabolic pathway have been discussed elsewhere (Irvine & Schell, 2001; Shears, 2004; Bennett *et al.,* 2006; Barker *et al.,* 2009; Chakraborty *et al.,* 2011; Wilson *et al.,* 2013; Thomas & Potter, 2014; Thota & Bhandari, 2015; Shears *et al.,* 2016; Shah *et al.,* 2017) and thus are only briefly presented here (Figs 1 and 2). In mammals, hormone- or growth factor-stimulated enzyme phospholipase C (PLC) cleaves the phosphoinositide phosphatidylinositol (4,5)-bisphosphate (PIP2) to generate diacylglycerol (DAG) and IP3. Inositol polyphosphate multikinase (IPMK) and IP3 3-kinase (IP3K) phosphorylate IP3 to generate I(1,3,4,5)P4 (IP4) (Takazawa *et al.,* 1990; Saiardi *et al.,* 1999) that is further phosphorylated to I(1,3,4,5,6)P5 (IP5) by IPMK. Alternatively, the inositol polyphosphate 5-phosphatase [INPP5; primarily type I, although type II and SH2-domain containing inositol phosphatase-1 (SHIP1) also possess this activity] dephosphorylates IP4 to I(1,3,4)P3 (IP3*) (Erneux *et al.,* 1998). Inositol tetrakisphosphate 1-kinase (ITPK1) synthesizes I(1,3,4,6)P4 (IP4*) from IP3*, which is converted to IP5 by IPMK (Fig. 1). It is not known to what extent these enzymes contribute *in vivo* to IP5 level.

Inositol pentakisphosphate 2-kinase (IP5K) converts IP5 to IP6. A family of three inositol hexakisphosphate kinases (IP6Ks) synthesizes 5PP-IP4, 5-IP7 and 1,5-IP8 from IP5, IP6 and 1-IP7 respectively. IP6Ks can also dephosphorylate IP6 to I(2,3,4,5,6)P5 (IP5*) at a reduced ATP/ADP ratio (Wundenberg *et al.,* 2014). Diphosphoinositol pentakisphosphate (PPIP5) kinases (PPIP5Ks) are a distinct family of enzymes which primarily produce 1,5-IP8 from 5-IP7 and to a lesser extent 1-IP7 from IP6 (Shears *et al.,* 2016). Diphosphoinositol polyphosphate phosphohydrolases (DIPPs) hydrolyse IPPs to their respective IPs (Fig. 2). The genes involved in IPP metabolism are not only evolutionarily conserved, but also are amplified in mammals, which indicates advanced isoform-selective functions in higher organisms.

(1) Inositol hexakisphosphate kinase (IP6K)

The three human IP6K (E.C.2.7.4.21) proteins range from 410 to 441 amino acids (Saiardi *et al.,* 1999, 2001; Thomas & Potter, 2014). Critical amino acids in and around the catalytic domain are largely conserved in IP kinases including IP6K1/2/3 (Fig. 3, cyan – catalytic) (Saiardi *et al.,* 2001; Holmes & Jogl, 2006), although isoform-specific structural details are lacking. Substantial homology is also observed in some other regions (Fig. 3, cyan). Conversely, distinct isoform-specific sequences also exist (Fig. 3, white). These regions are involved in protein–protein interactions and post-translational modifications. These events cause specificity of IP6K's functions *in vivo* by regulating activity, stability, subcellular distribution and target-protein identification of the enzymes (Barker *et al.,* 2009; Chakraborty *et al.,* 2011,*b*; Ghoshal *et al.,* 2016) (see Sections IV–VI).

IP6Ks also differ in their tissue expression patterns. *IP6K1* is abundant in the majority of murine tissues, with highest expression in brain and testis (Saiardi *et al.,* 1999; Moritoh *et al.,* 2016). Conversely, in humans, *IP6K1* and *IP6K2* are equally abundant, although *IP6K2* is slightly higher in mammary gland, thymus, colon, adipose tissue, testis, prostate and smooth muscle. *IP6K3* is minimally expressed in murine tissues with exceptions in heart, skeletal muscle and brain (Fu *et al.,* 2015; Moritoh *et al.,* 2016). However, in humans, *IP6K3*

is expressed at similar levels to *IP6K1/2* in the thyroid, whereas it is the primary form in the heart. *IP6K3* is the major form in murine and human skeletal muscles (Moritoh *et al.,* 2016). However, unlike mouse, the human samples in that study were pooled from subjects of different ages, which means that age-dependent variations could not be ruled out. The above comparisons were based on messenger RNA (mRNA) expression, and should be confirmed at the levels of protein and isoform-specific activities. Thus, despite their fairly ubiquitous nature, differential isoform-specific expression patterns of IP6Ks are observed in mammalian tissues.

(2) Diphosphoinositol pentakisphosphate kinase (PPIP5K)

Soon after the discovery of the 1-IP7 synthesizing enzyme VIP1 in *Saccharomyces cerevisiae*, (Mulugu *et al.,* 2007; Lin *et al.,* 2009; Wang *et al.,* 2012), its mammalian orthologs *PPIP5K1* and *PPIP5K2* were cloned (Choi *et al.,* 2007; Fridy *et al.,* 2007). Compared to IP6Ks, PPIP5Ks (E.C. 2.7.4.24) are relatively large proteins (PPIP5K1: 160 kDa; PPIP5K2: 140 kDa) (Choi *et al.,* 2007; Fridy *et al.,* 2007). The kinase domain of PPIP5Ks resides at the N-terminus (Fig. 4) (Shears *et al.,* 2016). The substrate-binding pocket of the human PPIP5K2-kinase domain is composed of two near-parallel grooves that accommodate the inositol with six phosphate/pyrophosphate groups (Wang *et al.,* 2012). PPIP5Ks also host a phosphatase domain (Fig. 4) (Fridy *et al.,* 2007). Although the amino-acid sequence of the phosphatase domain resembles the phytase subgroup of histidine acid phosphatases, PPIP5Ks hydrolyse the 1-β-phosphate of 1-IP7 and 1,5-IP8 (Wang *et al.,* 2015). *PPIP5K1* is widely expressed, although higher expression levels are observed in skeletal muscle, heart and brain (Fridy *et al.,* 2007).

(3) Diphosphoinositol polyphosphate phosphohydrolase (DIPP)

The IPP-hydrolysing DIPP (E.C. 3.6.1.52) enzymes belong to the *NUDT* gene family (McLennan, 2006). Nudix enzymes hydrolyse a wide range of organic pyrophosphates, including nucleoside di- and triphosphates, dinucleoside and diphosphoinositol polyphosphates, inorganic polyphosphates (polyP), nucleotide sugars and RNA caps, with varying degrees of substrate specificity (Yang *et al.,* 1999; Fisher *et al.,* 2002; McLennan, 2006; Lonetti *et al.,* 2011). DIPP enzymes range from 164 to 181 amino acids (Leslie, McLennan & Safrany, 2002). In mammals, *NUDT3* and *NUDT4* encode *DIPP1* and *DIPP2* respectively, of which, the latter has two alternatively spliced isoforms, *DIPP2α* and *DIPP2β* (Safrany *et al.,* 1998; Caffrey *et al.,* 2000; Caffrey & Shears, 2001; Fisher *et al.,* 2002). DIPP2α and DIPP2β differ by one amino acid, yet, it causes a significant difference in their catalytic constant (*Kcat*) values on IPPs (Caffrey *et al.,* 2000). The products of *NUDT10* and *NUDT11* are DIPP3α and DIPP3β, which also differ by a single amino acid (Fisher *et al.,* 2002; Hidaka *et al.,* 2002; Leslie *et al.,* 2002). In general, DIPPs have lower substrate concentration at half maximum velocity (*Km*) values for diphosphoinositol polyphosphates (0.004–0.088 μM) than for diadenosine polyphosphates (5.9–43 μM) (McLennan, 2006). However, substrate preference and utilization vary with divalent ions or redox conditions *in vitro* suggesting possible switching in their catalytic activities *in vivo* (Leslie *et al.,* 2002; Hua *et al.,* 2003). The ranking of *Kcat* values of DIPPs are in the order 1-IP7 > 5-IP7 = 1,5,-IP8, and DIPP1 is 10- to 60-fold more active than DIPP2α/β and DIPP3α/β (Kilari *et al.,* 2013). *DIPP1* expression is higher in brain, heart, pancreas and liver (Safrany *et al.,* 1998).

DIPP2 is expressed in the heart, and to a lower extent in skeletal muscle, pancreas, and kidney (Caffrey *et al.,* 2000; Hidaka *et al.,* 2002). *DIPP3α* expression is greater in the brain and liver (Leslie *et al.,* 2002). *DIPP3β* expression is higher in brain, pancreas and testis (Hidaka *et al.,* 2002; Leslie *et al.,* 2002). The significance of DIPP *in vivo* is yet to be understood.

III. REGULATION OF IPPS

Intracellular IPPs are measured using a standard technique of labelling cells with [3H]myo-inositol followed by high-performance liquid chromatography (HPLC)-based separation [Azevedo & Saiardi, 2006 and references therein]. In mammalian cells, about 50% of IP6 and 20% of IP5 are converted to pyrophosphates per hour (Menniti *et al.,* 1993). The IP7 pool can turn over around 10 times in every 40 min (Glennon & Shears, 1993). 5-IP7 and IP6 concentrations are 0.5–5 and 10–100 μM, respectively (Albert *et al.,* 1997; Barker *et al.,* 2004; Illies *et al.,* 2007; Lin *et al.,* 2009; Wundenberg & Mayr, 2012). Thus, intracellular 5-IP7 concentration is about 1–5% of IP6 levels. 1-IP7 comprises 2–10% of total intracellular IP7 levels (Padmanabhan *et al.,* 2009; Gu *et al.,* 2016; Shears *et al.,* 2016). 1,5-IP8 levels may be undetectable under basal conditions, although it can reach up to 10–20% of total IP7, depending on cell type and conditions [see Gu *et al.* (2016), Thota & Bhandari (2015), Wundenberg & Mayr (2012) and references therein].

Intracellular IP7 levels respond to diverse conditions such as metabolic, apoptotic and aging. In mouse embryonic fibroblasts (MEFs) or human hepatocellular carcinoma (HepG2) cells, overnight serum starvation decreases IP7 levels, which are restored by insulin-like growth factor-1 (IGF-1) or insulin (Chakraborty *et al.,* 2010). Insulin-induced adipogenesis in 3T3L1 cells also enhances the IP7 level (Chakraborty *et al.,* 2010). Cellular ATP/ADP ratio is a major determinant of IP6K activity (due to its higher *Km* toward ATP), which may explain differential levels of IP7 in anabolic *versus* catabolic conditions (Wundenberg *et al.,* 2014). For the same reason, inhibitors of phosphoinositide 3-kinase (PI3K) (wortmannin and LY294002) or insulin receptor tyrosine kinase [hydroxy-2-naphthalenylmethylphosphonic acid (HNMPA)] that reduce the ATP/ADP ratio, also diminish IP7 levels in an insulin-secreting HIT-T15 cell line. The PI4K and tyrosine phosphatase inhibitor phenylarsine oxide (PAO) and the PLC inhibitor 1-[6-((17β–3-methoxyestra-1,3,5(10)-trien-17-yl)amino)hexyl]-1H-pyrrole −2,5-dione (U73122) also reduce the ATP/ADP ratio and IP7 levels. However, they also directly inhibit IP6K1 (Rajasekaran *et al.,* 2017).

Apoptosis inducers, such as the anticancer drug cisplatin, the heat shock protein 90 (HSP90) inhibitor novobiocin, the broadspectrum kinase inhibitor staurosporine and the cytokine interferon-β (IFNβ) increase IP7 concentrations in various mammalian cell lines (see Section VI.4a) (Morrison *et al.,* 2001; Nagata *et al.,* 2005; Chakraborty *et al.,* 2008, 2011). Acute or prolonged treatments of certain stress/apoptosis inducers differentially influence IP7 levels. For example, overnight treatment of the endoplasmic reticulum (ER) Ca^{2+}-ATPase inhibitor thapsigargin, which induces ER stress and apoptosis (Janssen *et al.,* 2009) increases IP7 levels in the smooth muscle cell line DDT$_1$- MF2 (Safrany, 2004), although its short-term (40 min) treatment has the opposite effect in hepatocytes (Glennon & Shears, 1993). Similarly, an apoptotic dose of the casein kinase-2 (CK2) inhibitor 4,5,6,7-

tetrabromobenzotriazole (TBB, 50 μM, 16 h) enhances IP7 level in IP6K2-overexpressing osteosarcoma (U2OS) cells (Chakraborty *et al.,* 2011), whereas a lower dose (10 μM, 30 min) reduces IP7 levels and ATP/ADP ratio in IP6K1-predominated HIT-T15 cells (Rajasekaran *et al.,* 2017).

IP7 levels also alter in diseases. Pancreatic islets of leptin-deficient obese and hyperinsulinemic ob/ob mice display ~50% higher 5-IP7 levels than lean mice (Section VI. 3a) (Illies *et al.,* 2007). IP7 is higher in older (10-month old) primary hepatocytes than younger (2-month old) controls (Section VI.3i) (Chakraborty *et al.,* 2010). Aged (18-month old) murine bone marrow mesenchymal stem cells (BM-MSCs) also exhibit an approximately twofold increase in IP7, compared to young (2-month old) cells (Section VII. 1d) (Zhang *et al.,* 2014). IP7 level is higher in lymphoblasts of Huntington's Disease patients (Nagata *et al.,* 2011). The chemotactic bacterial peptide formyl-met-leu-phe (fMLP) depletes IP7 in neutrophils (human promyelocytic leukemia cell line HL60) (Prasad *et al.,* 2011), whereas nicotine-mediated reduction of IP7 decelerates apoptosis in aged neutrophils (Section VI.3h) (Xu *et al.,* 2013). The cytokine oncostatin-M (OSM), which alleviates cardiac ischaemic/reperfusion (I/R) injury by inhibiting cardiomyocyte apoptosis, also reduces IP7 levels. This study measured IP7 levels in the border zone of myocardial infarction (MI) by injecting [3H] myoinositol in the MI region (Section VII.1c) (Sun *et al.,* 2015).

Intracellular 1,5-IP8 level is also sensitive to diverse factors. Experiments were conducted in Syrian hamster vas deferens smooth muscle DDT$_1$-MF2 cells. Acute heat shock at 42°C increases 1,5-IP8 levels in these cells, although it decreases IP7 level (Choi, Mollapour & Shears, 2005). Hyperosmotic-stress-inducing agents such as sorbitol and sucrose significantly raise 1,5-IP8 levels (but slightly lower IP7 levels) in the above cell line (Safrany, 2004). Endothelial growth factor (EGF) treatment of DDT$_1$-MF2 cells causes a modest increase in 1,5-IP8 without altering IP7 levels (Pesesse *et al.,* 2004), whereas platelet-derived growth factor (PDGF) treatment does not influence IPP levels in NIH3T3 cells (Gokhale *et al.,* 2013). The compound genistein, but not herbimycin decreases 1,5-IP8 level (Safrany, 2004). These compounds are broad-spectrum tyrosine kinase inhibitors, yet they also target oestrogen receptor, DNA topoisomerase, α-glucosidase, and HSP90, often more potently than tyrosine kinases, which presumably explains their differential effects. The ATP synthase inhibitor oligomycin, which increases cellular AMP levels, reduces 1,5-IP8 levels without directly affecting levels of PPIP5Ks (Choi *et al.,* 2008). The AMP-mimetic aminoimidazol-carboxamide-ribonucleoside (AICAR) decreases 1,5-IP8 levels *via* an adenosine monophosphate-activated protein kinase (AMPK)-independent mechanism (Choi *et al.,* 2008). Activation of the cyclic AMP– protein kinase A (cAMP– PKA) pathway by 3-isobutyl-1-methylxanthine (IBMX) or the beta-adrenergic receptor (β-AR) agonist isoproterenol reduces IP8 level (Safrany & Shears, 1998).

IV. REGULATION OF IPP-METABOLIZING ENZYMES

IPP-metabolizing enzymes are regulated at various levels by diverse factors. Cold exposure reduces *IP6K1* expression in white adipose tissue (WAT) (Zhu *et al.,* 2016). Protein kinases PKA/PKC specifically phosphorylate IP6K1 at serine residues 118 and 121, which modulate

its interaction with the lipolytic regulator perilipin-1 (PLIN1) (Figs 3A and 5A and Section VI.3j) (Ghoshal *et al.,* 2016). IP6K2 is also regulated by phosphorylation. CK2 selectively phosphorylates IP6K2 at serine residues S347 and S356 within the degradation-specific PEST motif, which enhances its ubiquitination and degradation (Figs 3B and 5B and Section VI.4a) (Chakraborty *et al.,* 2011). IP6K1 is also phosphorylated at serine 151 (Dephoure *et al.,* 2008), although the functional significance of this is currently unknown. A number of other modifications of unidentified function are also specific to IP6K1 (A. Chakraborty, unpublished observation). Conversely, catalytic activity of IP6K is regulated by protein interaction. DNA damage binding protein-1 (DDB1) and HSP90 inhibit IP6K1 and IP6K2, respectively (Figs 3B, 5C, D and Section VI.4a) (Chakraborty *et al.,* 2008; Rao *et al.,* 2014).

PPIP5Ks contain a polyphosphoinositide binding domain (PBD), within their phosphatase domain, which mediates stimulus-dependent translocation of cytosolic PPIP5K1 to the plasma membrane (Fig. 4 and Section VI.2b) (Gokhale, Zaremba & Shears, 2011). PPIP5Ks also contain an intrinsically disordered domain (IDR) at the C-terminus, which mediates interactions with proteins of vesicular trafficking, cytoskeleton, and lipid metabolism, although the functional significance of these interactions is unknown (Machkalyan *et al.,* 2016; Shears *et al.,* 2016). PPIP5K2 contains a penta-arginine (CPA) nuclear-localization sequence in the IDR domain (Fig. 4) (Yong *et al.,* 2015). Overexpressed green fluorescent protein (GFP)-tagged CPA domain is nuclear, and phosphorylation of a serine residue adjacent to the domain regulates its nuclear localization (Yong *et al.,* 2015). The impacts of phosphorylation of DIPP3*a* at multiple C-terminal residues are unknown (Gauci *et al.,* 2009).

V. MECHANISMS BY WHICH IPP-METABOLIZING ENZYMES REGULATE CELLULAR PROCESSES

IPPs and their metabolizing enzymes regulate various cellular processes by modulating proteins *via* diverse mechanisms (Fig. 6). IPPs can regulate proteins by binding or by pyrophosphorylation. The mechanism of action of 5-IP7 is the best characterized. Most, but not all, actions of IPPs depend on the catalytic activity of their metabolizing enzymes. IP6Ks can interact directly with proteins to facilitate 5-IP7-mediated effects, although this is not always essential. Protein interaction is involved in scaffolding actions of IP6Ks, which do not require their catalytic activity. The physiological significance of these mechanisms is discussed in Section VI.

(1) Interaction of IPPs with proteins

(a) Pleckstrin homology (PH)-domain interaction—PH-domains in proteins bind phosphoinositides (Lemmon, 2007). Growth factor-stimulated receptor tyrosine kinases promote class I PI3K-mediated conversion of membrane-bound PIP2 to phosphatidylinositol (3,4,5)-trisphosphate (PIP3). The protein kinase B (PKB or Akt) and phosphoinositide-dependent kinase-1 (PDK1) bind to PIP3 *via* their PH-domains. PDK1 then phosphorylates Akt at the threonine 308 residue, which activates the kinase. Studies have established that 5-IP7 reduces the phosphorylation, membrane translocation and activation of Akt (Luo *et al.,* 2003; Chakraborty *et al.,* 2010). 5-IP7 inhibits PDK1-mediated phosphorylation of Akt with

half-maximal inhibitory concentration (IC_{50}) values of ~20–200 nM *in vitro* (Chakraborty *et al.,* 2010; Wu *et al.,* 2013). A stable version of 5-IP7, 5PCP-IP5 has an IC_{50} of 129 nM (Wu *et al.,* 2013). 1-IP7 and its analog 1PCP-IP5 also potently inhibit Akt phosphorylation (IC_{50} of ~55 and 105 nM, respectively) (Wu *et al.,* 2014).

PDK1-mediated phosphorylation of Akt is increased by PIP3 (Calleja *et al.,* 2007). In the presence of 1 μM PIP3, 5-IP7 prevents phosphorylation of Akt (IC_{50} ~1 μM), whereas IP6 shows a 10- to 15-fold lower efficiency. This inhibitory effect is observed when 5-IP7 and Akt are pre-incubated prior to PIP3 addition. When PIP3 is added before 5-IP7, the pyrophosphate is less effective (Downes *et al.,* 2005; Chakraborty *et al.,* 2010). Accordingly, IC_{50} values for 5-IP7 and 1-IP7 analogs (5PCP-IP5 and 1PCP-IP5, respectively) on Akt phosphorylation are 129 and 105 nM, respectively, in the absence of PIP3, but increase to 4.5 and 3.6 μM if PIP3 is added simultaneously with the analogs (Wu *et al.,* 2014). 5-IP7, IP6, 1-IP7 and 1,5-IP8 bind the Akt-PH-domain with IC_{50} values of 7, 14, 45 and >50 μM, respectively (Gokhale *et al.,* 2013). Thus, binding affinities of IPPs are higher than their inhibitory potencies, presumably due to differences in assay conditions.

In vivo, Akt activity is higher in *IP6K1*-deleted or IP6K-inhibited mice (Chakraborty *et al.,* 2010; Prasad *et al.,* 2011; Zhang *et al.,* 2014; Sun *et al.,* 2015; Zhu *et al.,* 2016; Ghoshal *et al.,* 2016). Furthermore, photouncaging-induced enhancement in intracellular 5-IP7 rapidly excludes the PH-domain of Akt from the plasma membrane (Pavlovic *et al.,* 2016), whereas IP6 is ineffective (H. J. Jessen, personal communication). Although the precise molecular and structural details of this mechanism are yet to be understood, it is conceivable that ligand-induced conformational alterations of the PH-domain of Akt may contribute to its differential regulation by phosphoinositides and IPPs. In fact, the inactive (PH-in) conformation of Akt is inaccessible to PDK1. PIP3 binding converts Akt to the PDK1-accesible PH-out conformation (Calleja *et al.,* 2007). 5-IP7 appears to stabilize PH-in. IPPs also interact with other mammalian PH-domain-containing proteins such as PI3-kinase enhancer (PIKE), T-lymphoma invasion and metastasis-inducing protein (TIAM), general receptor for phosphoinositides-1 (GRP1) and stress-activated protein kinase-interaction protein-1 (SIN1) *in vitro* (Luo *et al.,* 2003; Gokhale *et al.,* 2013), although the *in vivo* significance of these interactions is not known.

(b)   C2-domain interaction—C2-domains, comprising about 130 residues, are found in various eukaryotic proteins. In classical PKCs, C2-domains are responsible for Ca^{2+}-dependent membrane binding (Corbalan-Garcia & Gomez-Fernandez, 2014). The other class of C2-domain, containing the Ca^{2+}-sensor protein synaptotagmin (Syt), regulates vesicle fusion and neurotransmitter release in the pre-synaptic terminal (Chapman, 2008). IPs such as IP4 and IP6 are known binders of the C2B domain of Syt (Mikoshiba *et al.,* 1999; Yang *et al.,* 2012). A recent study demonstrated that 5-IP7 displays a 45-fold higher binding affinity to Syt1 over IP6 (Lee *et al.,* 2016). Molecular details of 5-IP7 and C2B domain interaction are not yet known.

(c)   Other interactions that require catalytic activity and target-binding of IPP-metabolizing enzymes—IP6Ks interact with diverse proteins or protein complexes in an isoform-specific manner, followed by its catalytic activity-dependent modulation of the

target proteins. For example, IP6K2/5-IP7 regulates the interaction of CK2 with telomere length regulation protein 2 (Tel2)-interacting proteins 1 and 2 (Tti1 and Tti2) complex (TTT) (Rao *et al.,* 2014), tumour suppressor protein p53 (Koldobskiy *et al.,* 2010) and liver kinase B1 (LKB1) (Rao *et al.,* 2015), IP6K1/5-IP7 modulates Cullin-ring finger ligase-4 (CRL4) complex (Rao *et al.,* 2014), *α*-actinin (Fu *et al.,* 2017) and jumonji domain-containing 2C protein (JMJD2C) (Burton *et al.,* 2013), whereas IP6K1/IP6 controls AMPK (Zhu *et al.,* 2016). Details are described in Section VI.

(2) IPP-mediated pyrophosphorylation of target proteins

Protein phosphorylation is a common post-translational event, which regulates every aspect of cell growth, metabolism and survival (Manning *et al.,* 2002; Ubersax & Ferrell, 2007; Derouiche, Cousin & Mijakovic, 2012). A distinct post-translational modification, termed 'protein pyrophosphorylation', by IPPs further modulates protein phosphorylation-mediated regulation of cellular functions (Wilson *et al.,* 2013; Thota & Bhandari, 2015). Because of steric hindrance caused by the negatively charged bulky phosphate groups, the phosphoanhydride bond of IPP has a high energy of hydrolysis (Stephens *et al.,* 1993; Laussmann *et al.,* 1996; Hand & Honek, 2007; Wilson *et al.,* 2013; Shears, 2017). Therefore, transfer of *β*-phosphate from 5-IP7 or 1,5-IP8 is considered thermodynamically favourable (Hand & Honek, 2007). In fact, the *β*-phosphate of 5-IP7 is transferred to ADP by IP6K1 *in vitro* (Voglmaier *et al.,* 1996). This information suggests that IPPs are phosphorylating agents (Voglmaier *et al.,* 1996). Indeed, IPPs donate their *β*-phosphate to the prephosphorylated serine residue in a non-enzymatic fashion (Saiardi *et al.,* 2004; Bhandari *et al.,* 2007). All IPPs are capable of pyrophosphorylation (Thota & Bhandari, 2015). The pyrophosphates in proteins are acid labile, but are resistant to protein phosphatases (Bhandari *et al.,* 2007). Longer incubations of synthetic pyrophosphopeptides with alkaline phosphatases can remove the *β*-phosphate group on pyrophosphoserine-containing peptides (Yates & Fiedler, 2015), indicating that protein pyrophosphorylation is a reversible process (Thota & Bhandari, 2015). CK2 primarily acts as the priming kinase for the pyrophosphorylation event. However, direct *in vivo* evidence of pyrophosphorylation is lacking.

(3) Maintenance of intracellular inorganic polyphosphate (polyP) levels by IPPs

PolyPs are linear polymers of orthophosphates that are linked by phosphoanhydride bonds, as observed in ATP. These polymers, with various chain lengths, are found in all organisms (Rao, Gomez-Garcia & Kornberg, 2009). The idea that IP6K/5-IP7 regulates cellular polyP levels in mammals arose from studies in yeast, in which deletion of the *KCS1* (*IP6K1* ortholog), but not the *PPIP5K* ortholog, reduces polyP levels. Moreover, complementation of active but not inactive mouse *IP6K1* restores polyP levels in *KCS1*-null yeast (Auesukaree *et al.,* 2005; Lonetti *et al.,* 2011). PolyPs are critical in coagulation of platelets. Accordingly, *IP6K1*-KO platelets display a reduction in polyP levels and in platelet coagulation (Section VI.3d) (Ghosh *et al.,* 2013).

(4) Modulation of targets *via* protein–protein interactions

The above mechanisms imply that IPP-metabolizing enzymes require their catalytic activity to modulate protein targets. However, phylogenetic analysis predicts that IP6Ks evolved

prior to the upstream IP-kinases, which generate substrates for IP6Ks (Bennett *et al.,* 2006). What was IP6K doing in the absence of its substrates? Perhaps it was involved in scaffolding functions, which do not require involvement of IPPs. A number of studies suggest that IP6Ks can function as a scaffold to modulate protein targets, for which their catalytic activities are not required. Binding of IP6K1 to the guanine nucleotide exchange factor for Rab3A (GRAB) (Luo *et al.,* 2001), the protein kinase glycogen synthase kinase (GSK3) (Chakraborty *et al.,* 2014) and the lipolytic regulator PLIN1 (Ghoshal *et al.,* 2016), interaction of IP6K2 with TNF receptor-associated factor-2 (TRAF2) (Morrison *et al.,* 2007) and binding of IP6K3 to spectrin and adducin (Fu *et al.,* 2015) are examples of such functions. Details of these interactions and their functional significance are described in Section VI.

VI.   FUNCTIONS OF IPP-METABOLIZING ENZYMES IN MAMMALS

Pleiotropic effects of the IPP pathway modulate various cellular processes *via* diverse mechanisms, thus affecting the animal's phenotype. Below, the functional significance of IPP enzymes is discussed. The mechanisms (where known) associated with each function are also mentioned.

(1) DIPP

(a)   Extracelluar signal-regulated kinase (ERK) signalling—The Ras family of GTP-binding proteins transduces signals from several receptor tyrosine kinases, activating mitogen-activated protein kinases (MAPKs) like ERK. Ectopic expression of murine DIPP1 inhibits the ERK1/2 pathway. However, phosphohydrolase activity-deficient DIPP1 blocks ERK1/2 signalling with a greater efficiency, indicating that phosphohydrolase activity is not required by this process (Chu *et al.,* 2004). The exact mechanism is not known.

(b)   Cell migration—The $5'$7-methyloguanosine cap and $3'$-terminal poly(A) tract regulate the synthesis, translation, and degradation of mRNA. DIPP1 possesses mRNA-decapping activity, which modulates migration of the breast cancer cell line MCF-7. A reduction in DIPP1 level promotes migration of these cells, due to enhanced filopodial extension. The phenotype is reversed by complementation with the wild type, but not by the DIPP1 mutant that lacks mRNA-decapping activity. The impact of the inositol pyrophosphatase activity of DIPP1 is unknown (Grudzien-Nogalska *et al.,* 2016).

(2) PPIP5K

(a)   Type-1 interferon response—The retinoic acid-inducible gene-I (RIG-I) receptor facilitates type-I interferon production. A human genome-wide RNA interference (RNAi) screen identified PPIP5Ks to be positive regulators of this process, which is involved in cellular infection by Sendai and influenza A viruses (Pulloor *et al.,* 2014). Overexpression of catalytically active IP5K, IP6Ks and PPIP5Ks induces interferon induction. Conversely, silencing of IP5K and PPIP5Ks but not IP6Ks has the opposite effect, indicating involvement of 1-IP7 in this process. PPIP5Ks mediate phosphorylation and activation of the interferon regulatory transcription factor 3 (IRF3) that promotes expression of type-1 interferon. The addition of purified IPPs to a cell-free reconstituted RIG-I signalling assay

also showed 1-IP7 to be essential for IRF3 activation. 1-IP7 is thought to act by pyrophosphorylation, since its action was not mimicked by a synthetic phosphonoacetate analog, although no experimental evidence was presented (Pulloor *et al.,* 2014). Intracellular 1-IP7 level is low and at times undetectable (Gu *et al.,* 2016), so determining the concentration and regulation of 1-IP7 in primary macrophages and/or other relevant cells will be critical to evaluate the physiological relevance of this interesting finding.

(b)   PIP3-mediated mammalian target of rapamycin complex-2 (mTORC2) signalling—In L6 myotubes, knockdown of PPIP5K1 impairs insulin-induced phosphorylation of S473 but not of T308 of Akt (Gokhale *et al.,* 2013). The mTORC2 complex phosphorylates Akt at S473 and PPIP5K is proposed to activate this pathway. PPIP5K substrates (IP6/5-IP7) but not products (1-IP7/1,5-IP8) bind to the PH-domain of the mTORC2 regulator SIN1 *in vitro* (Section V.1a). In unstimulated cells, the PH-domain of SIN1 binds to and suppresses mTORC2 activity. Upon PI3K activation, SIN1 binds to PIP3 at the membrane, which activates mTORC2 (Yuan & Guan, 2015). Similar stimulation also promotes the binding of PPIP5K1 to PIP3 (Gokhale *et al.,* 2011). Presumably, the catalytic activity of PPIP5K1 activates mTORC2 by a local reduction in IP6/5-IP7 level, which leads to increased SIN1–PIP3 and reduced SIN1–mTORC2 binding. PPIP5K1 activity seems specifically to regulate SIN1–PIP3 but not Akt–PIP3 binding, although the reason is unknown. Influences of various kinase, phosphatase and PBD mutants of PPIP5K1 on Akt phosphorylation should be assessed to determine the precise cellular mechanism involved. It is clear, however, that the IPP pathway regulates PI3K signalling at various levels.

(c)   Cell growth and metabolism—Clustered regularly interspaced short palindromic repeats and its associated protein 9 (CRISPR-Cas9)-based genetic deletion of both *PPIP5K1* and *PPIP5K2* revealed impacts of 1,5-IP8 depletion on various phenotypes of the HCT116 colon cancer cell line. *PPIP5K* deletion abolishes 1,5-IP8, which causes a threefold increase in 5-IP7 levels in these cells. *PPIP6K*-KO cells display growth-inhibited and hypermetabolic phenotypes (Gu *et al.,* 2017). Phenotypes of *PPIP5K*-KO cells are discussed and compared with *IP6K1*-or *IP6K2*-deleted cells in Sections VI.3 and 4.

(3)   IP6K1

(a)   Vesicular trafficking—Vesicular trafficking includes endocytosis and exocytosis, by which vesicles transport materials among different cellular compartments and between a cell and its environment. Various studies demonstrate that IP6K1 exerts both stimulatory and inhibitory effects on vesicular trafficking.

IP6K1 stimulates insulin exocytosis. Pancreatic β-cells express *IP6K1* and *IP6K2* and maintain relatively high levels (~6 μM) of 5-IP7, which is further increased in hyperinsulinemic ob/ob mice (Section III) (Illies *et al.,* 2007). Exogenous IPPs (5-IP7, 1-IP7, 3-IP7 and 4-IP7) enhance exocytosis in mouse β-cells. Overexpression of catalytically active IP6K1 or IP6K2 enhances exocytosis, whereas silencing of *IP6K1* but not *IP6K2* inhibits the process. Depletion of *IP6K1* or *IP6K2* in β-cells causes ~50 or 25% reduction in 5-IP7 levels, respectively. Presumably, an optimal concentration of IP6K1- but not IP6K2-

generated 5-IP7 is critical for insulin exocytosis (Illies *et al.,* 2007). A reduced serum insulin level in *IP6K1*-KO but not in *IP6K2*-KO mice supports this possibility (Bhandari *et al.,* 2008; Morrison *et al.,* 2009). The precise mechanism by which IP6K1/5-IP7 mediates insulin release is not known.

IP6K1 regulates neuroexocytosis *via* both catalytic activity-dependent and independent mechanisms. The nucleotide exchange factor GRAB inhibits exocytosis *via* its action on the GTPase Rab3A. Both catalytically active and inactive versions of IP6K1 interact (Section V. 4) and compete with GRAB for binding to Rab3A, which stimulates dopamine or growth hormone release in PC12 cells (Luo *et al.,* 2001). Conversely, IP6K1-generated 5-IP7 reduces Ca^{2+}-mediated neuroexocytosis in PC12 and in primary hippocampal neuronal cells by interacting with the C2-domain of Syt1 (Section V.1b) (Lee *et al.,* 2016). Overexpression of IP6K1 suppresses, whereas its depletion promotes, exocytosis. 5-IP7 inhibits vesicle fusion *in vitro*, at a 10-fold higher affinity than IP6 or 1-IP7 (Lee *et al.,* 2016). 5-IP7-dependent reduction of synaptic vesicle fusion was abolished by increasing Ca^{2+} levels, suggesting competition between them. Ca^{2+}-bound Syt directs vesicular fusion *via* macromolecular interactions of lipid membranes and soluble N-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) proteins. IP6K1/5-IP7-dependent inhibition of synaptic vesicle fusion was abolished by increasing Ca^{2+} levels or by expressing the Ca^{2+}-binding C2B domain of Syt1. Thus, binding of 5-IP7 to Syt1 interferes with the fusogenic activity of Ca^{2+}. Whether 5-IP7 regulates other C2 domain-containing proteins, is not known.

Two independent studies suggest that 5-IP7 reduces or facilitates trafficking by pyrophoshorylation (Section V.2) of distinct cytoskeletal proteins. Long-range intracellular transport occurs on the microtubule cytoskeleton and is driven by two classes of motor proteins: kinesins and cytoplasmic dynein (Vallee, McKenney & Ori-McKenney, 2012). In MEFs, 5-IP7 inhibits kinesin-induced exocytosis of viral particles (Azevedo *et al.,* 2009). Interaction of AP3β1, a clathrin-associated protein complex with the kinesin family motor protein Kif3A is required for release of viral particles. Catalytically active IP6K1 or IP6K2 reduces AP3β1– Kif3A interaction and subsequent release of virus particles (Azevedo *et al.,* 2009). Direct *in vitro* and indirect *in vivo* (back phosphorylation) studies demonstrated that 5-IP7 pyrophosphorylates AP3β1, which may regulate this process.

On the other hand, 5-IP7 facilitates dynein-mediated trafficking. One study employed the transferrin protein as a model to show that IP6K1-deleted MEFs display defective dynein-dependent trafficking pathways, including endosomal sorting, vesicle movement, and Golgi maintenance. Complementation of catalytically active IP6K1 reverses the phenotype. Accordingly, phagosomal motility is reduced in *IP6K1*-KO macrophages (see Section VI.3h for neutrophil phagocytosis). The interaction between dynein intermediate chain (DIC) and the p150^{Glued} subunit of dynactin is critical for the transport, which is reduced in *IP6K1*-KO cells. Direct *in vitro* and indirect *in vivo* (back phosphorylation) pyrophosphorylation, together with mutation studies, suggests that 5-IP7-mediated pyrophosphorylation of the serine 51 residue of DIC regulates its interaction with p150^{Glued} and presumably the transport process (Chanduri *et al.,* 2016). Acquiring further evidence such as *in vivo* detection of pyrophosphorylation and impacts of pyrophosphorylation-deficient mutants on

trafficking are critical to determine the molecular details of the mechanisms involved. IP6K1/5-IP7 regulates vesicle trafficking *via* diverse mechanisms, which can diminish or stimulate exocytosis depending on the cell/tissue and/or physiological context. The mechanisms and physiological relevance of insulin and neurotransmitter release are distinct, which explains differential role of IP6K1 in these processes. The activity-independent stimulatory and activity-dependent inhibitory actions of IP6K1 on neurotransmitter release require further study. Transgenic mouse models of active and inactive IP6K1 are necessary to distinguish these functions *in vivo*.

(b)   Chromatin remodelling—Eukaryotic DNA is packaged in a complex manner, which hinders transcription and DNA repair. Coordinated remodelling of chromatin at specific sites is critical to initiate such processes. Various modifications including DNA and histone methylation regulate chromatin remodelling, and are modulated by IP6K1. DNA methylation leads to long-term gene repression (Cedar & Bergman, 2009; Rose & Klose, 2014). Nuclear-localized IP6K1 enhances DNA methylation in a catalytic activity-dependent manner, which inhibits expression of the inositol biosynthetic gene *mIno1* (Yu, Ye & Greenberg, 2016).

Conversely, histone methylation can either activate or inhibit the transcription process. For histone H3, methylation of the lysine 4 residue activates, whereas similar modification at the lysine 9 residue blocks the transcription process (Kouzarides, 2002; Rice *et al.,* 2003). The interaction of IP6K1 with the histone lysine demethylase JMJD2C dissociates the demethylase from the chromatin and causes a corresponding increase in trimethyl-histone H3 lysine 9 (H3K9me3) levels. Accordingly, *IP6K1*-KO MEFs display increased association of JMJD2C with the chromatin and reduced levels of H3K9me3, which is reversed by expression of catalytically active but not inactive IP6K1. Thus, IP6K1 inhibits transcription of the JMJD2C target gene, the E3 ubiquitin ligase *MDM2* (Burton *et al.,* 2013). The molecular mechanism by which 5-IP7 regulates DNA or histone methylation is not understood.

(c)   DNA damage and repair—*IP6K1*-KO MEFs show decreased viability and reduced recovery after induction of DNA damage. Although homologous recombination repair is initiated, it is not completed in knockout cells. Expression of catalytically active but not inactive IP6K1 restores repair in these cells, implying a requirement for 5-IP7 in this process (Jadav *et al.,* 2013), although the precise mechanism is not known.

A response to DNA damage (DDR)-induced stress is regulated by various pathways including the ubiquitin proteasome system. Cullin complex (CRL4) is a multi-protein complex primarily composed of DDB1, DDB2 (XPE), cullin-4A (Cul4A), regulator of Cullins-1 E3 (Roc1-E3) and the Cop9 de-ubiquitination signalosome (CSN) (Bergink, Jaspers & Vermeulen, 2007; Scrima *et al.,* 2011). Following ultraviolet (UV) radiation (and other DNA-damaging agents), CSN is dissociated from the complex, leading to activation of the E3 ligase, which ubiquitinates several components of the nucleotide excision repair (NER) machinery, which is required to initiate NER. In MEF cells, IP6K1 forms a ternary complex with CSN and CRL4, in which both IP6K1 and CRL4 are inactive under basal conditions. DDB1 present in the complex inhibits IP6K1 (Section IV). UV dissociates

IP6K1 from the complex, which activates the kinase. IP6K1-generated 5-IP7 disrupts the CSN–CRL4 complex, which activates CRL4-mediated NER. Accordingly, *IP6K1*-KO MEFs display enhanced UV-induced CRL4 activation, NER and reduced apoptosis. Thus, IP6K1 mediates UV-induced disassembly of the CRL4–CSN complex, which regulates NER and apoptosis (Rao *et al.,* 2014). The molecular details of the process by which 5-IP7 dissociates the signalosome–Cullin complex are unclear.

(d)   Haemostasis—Haemostasis is a physiological response to prevent blood loss following vascular injury. In aberrant haemostasis, the timing of blood clotting is altered. Slow blood clotting leads to diathesis, which is a condition of susceptibility to bleeding. Conversely, abnormally fast clotting leads to thromboembolism, which can cause fatal pathological conditions such as stroke, pulmonary embolism, deep vein thrombosis, and myocardial infarction. 5-IP7 is a key player in maintaining haemostasis (Ghosh *et al.,* 2013). *IP6K1*-KO mice display a significant delay in platelet aggregation, lengthening plasma clotting time (Ghosh *et al.,* 2013) and hence a longer tail bleeding time. As a result, *IP6K1*-KO mice are protected from ADP-induced pulmonary thromboembolism. Mechanistic studies reveal that levels of polyP, a critical clotting factor, are reduced in the platelets of *IP6K1*-KO mice. Addition of polyP but not 5-IP7 rescues clotting in sera derived from *IP6K1*-KO mice, indicating that 5-IP7 modulates the process *in vivo* indirectly by regulating polyP levels (Ghosh *et al.,* 2013) (Section V.3).

(e)   Spermatogenesis—*IP6K1*-KO male mice are sterile, with few advanced spermatids in the testes, and no sperm in the epididymis (Bhandari *et al.,* 2008). *IP6K1* is highly expressed in round spermatids and is enriched in a perinuclear ribonucleoprotein complex called the chromatoid body. Deletion of *IP6K1* impairs formation of the chromatoid body, which causes defects in elongation and condensation of sperm nuclei that subsequently block differentiation of spermatids. During spermiogenesis, nucleosomal histones are replaced by transition proteins 1 and 2 (TNP1 and TNP2) and protamines (PRM1 and PRM2). Premature expression of these proteins is associated with defective spermatid formation and infertility in mice. Juvenile *IP6K1*-KO mice display premature synthesis of TNP2 and PRM2 in round spermatids, which correlates with this phenotype (Malla & Bhandari, 2017). Thus, IP6K1 mediates the post-transcriptional regulatory machinery of testicular germ cells *via* a hitherto unknown mechanism. However, pharmacological inhibtion of the relevant IPP pathway does not cause sterility (Ghoshal *et al.,* 2016) (Section VII.2). The molecular details by which IP6K1 regulates *TNP2* and *PRM2* are not known.

(f)   Cell migration-mediated neuronal development and cancer metastasis— Cell migration is critical in many physiological and pathological motility processes such as development, immunity and cancer metastasis. Two independent studies demonstrated that IP6K1 regulates cell migration. *IP6K1*-KO MEFs display impaired cell spreading and migration. The knockout cells also display reduced tyrosine phosphorylation and activity of the focal adhesion kinase (FAK), which diminishes cell migration (Jadav *et al.,* 2016). Expression of catalytically active IP6K1 reverses migration defects in these cells suggesting involvement of 5-IP7 in this process. Actin cytoskeleton remodelling and cell migration support the ability of cancer cells to achieve their oncogenic potential. Accordingly, cancer

cells like HeLa and HCT116 with depleted *IP6K1* levels, display reduced migration, invasion, and anchorage-independent growth (Jadav *et al.,* 2016). Subcutaneous injections of control and *IP6K1*-depleted HCT116 cells in nude mice form similar-sized tumours, yet, the invasive property of *IP6K1*-depleted cells is significantly less. Accordingly, *IP6K1*-KO mice fed the oral carcinogen 4-nitroquinoline-1-oxide (4-NQO) show reduced progression of invasive carcinoma (Jadav *et al.,* 2016). This is discussed further in Sections VI.4b,c in the context of IP6K2.

IP6K1/5-IP7-mediated cell migration is also crucial for brain development. *IP6K1*-KO mice display abnormal brain development due to impaired neuronal migration. IP6K1 localizes to focal adhesion and interacts with the microfilament protein α-actinin. Experiments in MEF and HeLa cells revealed that IP6K1 binds α-actinin, which promotes its tyrosine phosphorylation by FAK, intracellular localization and function. These events are critical for stress fibre formation, cell migration and spreading. Catalytic activity of IP6K1 is not required for binding, but is indispensable in FAK-mediated phosphorylation of α-actinin. Accordingly, 5-IP7 promotes FAK autophosphorylation-mediated activation *in vitro* (Fu *et al.,* 2017), which demonstrates the mechanism by which IP6K1/5-IP7 promotes cell migration (Jadav *et al.,* 2016; Fu *et al.,* 2017). The molecular details of 5-IP7-mediated FAK phosphorylation are unknown.

(g)   Behavioural responses—*IP6K1*-KO mice display certain behavioural alterations such as reduced locomotor activity in novel environments and impaired social interactions (Chakraborty *et al.,* 2014). Behavioural responses are results of complex biological actions and thus, may arise due to multiple mechanistic changes in the knockout mice. One such change is altered Akt/GSK3 signalling in the brain. IP6K1 activates the protein kinase GSK3 by: (*i*) 5-IP7-mediated inhibition of its negative regulator Akt (Chakraborty *et al.,* 2010); and (*ii*) by direct protein interaction (Sections V.1a and 4) (Chakraborty *et al.,* 2014). Consequently, *IP6K1*-KO mice display reduced GSK3 activity in diverse brain regions. However, the extent to which the catalytic or protein-interaction activity of IP6K1 contributes *in vivo* remains unclear. Defective brain development due to impaired neuronal migration (Section VI.3f) may also cause these alterations. Generation of brain-specific transgenic mice with catalytically active and inactive versions of IP6K1 may provide answers, but the above studies at least suggest that IP6K1 is essential for brain development. Conversely, targeting IP6K1 in the brain may have beneficial effects in psychiatric and neurodegenerative diseases, where abnormalities in insulin signalling are demonstrated (Chakraborty *et al.,* 2014).

(h)   Neutrophil functions—Neutrophils are critical players in innate immunity and host defence. However, excessive accumulation or hyper-responsiveness of neutrophils can be detrimental to the host, so neutrophils are under stringent regulation. IPPs are major regulators of neutrophil function in infection and inflammation (Luo & Mondal, 2015). PIP3-induced Akt activation plays a critical role in regulating neutrophil chemotaxis, phagocytosis, and nicotinamide adenine dinucleotide phosphate (NADPH) oxidase-mediated superoxide production. IP6K1 regulates neutrophil function through 5-IP7-mediated Akt inhibition (Section V.1a). The chemoattractant fMLP stimulates Akt activity in neutrophils

by reducing 5-IP7 levels (Section III). Accordingly, fMLP-induced Akt activation is significantly higher in *IP6K1*-KO neutrophils. Moreover, *IP6K1*-KO neutrophils display enhanced Akt-mediated transient superoxide production and bactericidal activity. However, unlike HCT116 or neuron cells, *IP6K1*-KO neutrophils do not display alterations in cell adhesion or migration (Prasad *et al.,* 2011).

Abnormal accumulation of neutrophils in the lungs generates reactive oxygen species (ROS), which augments pathological phenotypes in chronic obstructive pulmonary disease (COPD) (Barnes, 2003). A 5-IP7-mediated decrease in Akt activity promotes spontaneous neutrophil death, which protects mice from nicotine-induced lung inflammation. Cigarette smoke extract or nicotine reduces 5-IP7 in aging neutrophils, which delays spontaneous neutrophil death and thus contributes to the pathogenesis of COPD. Accordingly, deletion of *IP6K1* augments cigarette smoke-induced neutrophil accumulation and lung damage (Xu *et al.,* 2013). Thus, 5-IP7-mediated Akt inhibition and neutrophil death exert differential effects on immunity and COPD. *IP6K1*-KO macrophages display reduced phagosomal motility (Chanduri *et al.,* 2016), which may reduce phagocytosis. Genrally, neutrophils respond to acute, whereas macrophages function during chronic inflammation. Thus, IP6K1 may differentially regulate inflammation.

(i)   Insulin signalling—Insulin induces 5-IP7, which in turn inhibits the hormone's stimulatory action on Akt (Sections III and V.1a) (Manning, 2010; Chakraborty *et al.,* 2011) implying that *IP6K1* deletion will enhance insulin sensitivity. Indeed, mild insulin sensitivity is observed in young, chow-fed *IP6K1*-KO mice (Bhandari *et al.,* 2008), and significant sensitivity in older (10-month old) knockouts (Chakraborty *et al.,* 2010). In gastrocnemius muscle, epididymal WAT (EWAT) and primary hepatocytes of the older knockouts, insulin-induced Akt activity is markedly increased. These results, together with higher IP7 level in older wild-type hepatocytes compared to younger controls (Section III), suggest that age-induced insulin resistance is caused, at least in part, by 5-IP7-mediated Akt inhibition (Chakraborty *et al.,* 2010). IP6K1 regulates insulin signalling in the adipose tissue, which in turn modulates global insulin sensitivity. As a result, both global (*IP6K1*-KO) and adipocyte-specific *IP6K1*-KO (AdKO) mice are also protected from high fat diet (HFD)-induced insulin resistance (Chakraborty *et al.,* 2010; Zhu *et al.,* 2016). HFD-fed *AdKO* mice display higher Akt activity in the EWAT, liver and gastrocnemius muscle. Thus, adipocyte-specific *IP6K1* deletion alters expression/secretion of certain adipokines, enhancing insulin sensitivity in other tissues. Indeed, the plasma level of the insulin-sensitizing adipokine adiponectin (ADIPOQ) is higher in HFD-fed AdKO mice (Zhu *et al.,* 2016). Serum insulin level is lower in HFD-fed AdKO mice compared to the corresponding wild-type, which suggests that adipocyte-specific *IP6K1* deletion-mediated insulin hypersensitivity reduces insulin secretion indirectly (Zhu *et al.,* 2016). Thus, IP6K1 regulates insulin secretion and signalling *via* pleiotropic mechanisms, which can be targeted in type-2 diabetes (T2D) (Chakraborty *et al.,* 2011; Boucher, Kleinridders & Kahn, 2014; MacKenzie & Elliott, 2014; Zhang, Liu & Liu, 2017).

(j)   Energy metabolism—IPPs are proposed to be metabolic messengers (Shears, 2009). Yet, the suggestion that IP6K1 inhibits energy expenditure (EE) emerged from the

observation that *IP6K1*-KO mice are protected from HFD-induced weight gain despite eating the same amount of food (Chakraborty *et al.,* 2010; Zhu *et al.,* 2017). Furthermore, cold exposure, which enhances adipose tissue browning-mediated thermogenesis, reduces *IP6K1* expression. These results suggest that *IP6K1* deletion reduces weight gain by enhancing EE in adipocytes. Protection of AdKO mice from HFD-induced weight gain further supports this possibility (Zhu *et al.,* 2016). Stored energy is expended by coupled respiration that generates ATP and/or by uncoupling protein-1 (UCP1)-mediated uncoupled respiration that releases heat. Brown adipocytes in brown adipose tissue (BAT) or beige adipocytes in WAT expend energy primarily by thermogenesis. Therefore, enhanced browning of WAT facilitates thermogenesis-mediated fat loss in rodents and humans (van Dam *et al.,* 2015; Sidossis & Kajimura, 2015, and references therein). Indeed, both *IP6K1*-KO and AdKO mice expend more energy, at least in part, due to enhanced thermogenic EE (Chakraborty *et al.,* 2010; Zhu *et al.,* 2016, 2017). Stromal vascular fractions isolated from WAT display higher beige adipogenesis, further evidenced by enhanced expression of the EE machinery. Accordingly, mitochondrial oxygen consumption rate (OCR) is higher in AdKO beige adipocytes, whereas glycolysis is unaltered.

The protein kinase AMPK enhances EE (Zhu *et al.,* 2016, and references therein). *IP6K1* deletion augments EE by stimulating AMPK *via* a novel mechanism. IP6 stimulates LKB1-mediated stimulatory phosphorylation of the AMPKα (catalytic) subunit at the threonine 172 residue, whereas IP7 is ineffective. Catalytically active IP6K1 interferes with IP6-mediated AMPK activation. The amino acid residues 146–205 in IP6K1 are required for its interaction with AMPKα (Fig. 3A). Presumably, IP6K1 interferes with IP6-mediated AMPKα activation by converting IP6 to 5-IP7 (or IP5*) (Fig. 2). Thus, dynamic alterations in IP6/5-IP7 ratio in a microenvironment also regulate protein targets (Zhu *et al.,* 2016). However, the molecular mechanism by which IP6 enhances AMPK phosphorylation is not understood. IP6K1 may also indirectly inhibit intracellular AMPK. Similar to IP6K2 (Rao *et al.,* 2015; Section VI.4c), IP6K1-generated 5-IP7 may promote nuclear localization of LKB1, which is known to perturb AMPK activation (Xie *et al.,* 2009). Nevertheless, IP6K1-mediated inhibition of AMPK reduces adipocyte EE, promoting weight gain (Zhu *et al.,* 2016). AMPK also displays insulin-sensitizing effects, which may contribute to insulin hypersensitivity in *IP6K1*-deleted mice. Although AMPK and Akt display synergistic effects on insulin sensitivity, they have differential roles in cell proliferation and survival. In general, Akt stimulates but AMPK inhibits these processes. Thus, in certain tissues/conditions, if IP6K1 inhibits one kinase, it may activate the other.

Moreover, IP6K1 regulates EE by modulating lipolysis *via* protein–protein interaction, which does not require catalytic activity of IP6K1 (Ghoshal *et al.,* 2016). In lipolysis, lipases hydrolyse stored triglycerides (TAGs) that release free fatty acids (FFA) and glycerol. These products are utilized at various stages of the EE pathway. PLIN and comparative gene identification-58 (CGI-58) proteins regulate lipolysis by modulating activities of various lipases. PLIN1 binds and perturbs the stimulatory action of CGI-58 on adipose triglyceride lipase (ATGL) and thus, inhibits basal lipolysis (Ghoshal *et al.,* 2016). Basal lipolysis is enhanced in *IP6K1*-KO mice. IP6K1 is a major PLIN1-interacting protein in the adipose tissue. Furthermore, PKA/PKC phosphorylation of IP6K1 at serine residues 118 and 121 (Fig. 3A) facilitates its binding to PLIN1, which modulates lipolysis (Section IV) (Ghoshal

et al., 2016). Thus, IP6K1 regulates lipolysis and thermogenesis to promote fat accumulation.

IP6K1 also inhibits EE in MEFs, albeit *via* reducing glycolysis (Szijgyarto *et al.,* 2011). Under basal conditions, *IP6K1*-KO MEFs display higher ATP levels due to increased glycolysis, but mitochondrial activity is compromised. The mechanism of this phenotype is known in *S. cerevisiae*, which exhibit similar phenotypes in the absence of the IP6K ortholog *KCS1*. The interaction between glycolytic genes transcriptional activators 1 and 2 (GCR1 and GCR2) is essential for transcription of the glycolytic genes. The *KCS1*-KO strain displays increased GCR1–GCR2 interaction, which stimulates expression of glycolytic genes such as *GAPDH* and *PGK1*. *In vitro* pyrophosphorylation and mutation studies suggest that 5-IP7 pyrophosphorylates (Section V.2) GCR1 and destabilizes its interaction with GCR2, which subsequently reduces glycolysis (Szijgyarto *et al.,* 2011).

Experiments in HCT116 cells suggest that the IPP 1,5-IP8 plays a role in regulating ATP level, although the precise mechanism by which 1,5-IP8 regulates this process is unknown. In these cells, *PPIP5K* deletion eliminates 1,5-IP8 (but increases 5-IP7) and elevates ATP levels. *PPIP5K*-KO cells accumulate more ATP due to increased EE caused by enhanced glycolysis and oxidative phosphorylation. Overexpressed active but not kinase-inactive *PPIP5K1* restores 1,5-IP8 level and returns ATP to normal levels, whereas reduction of 5-IP7 does not (Gu *et al.,* 2017). IP6K1 may also regulate EE partly *via* 1,5-IP8. Although the IPP pathway inhibits EE, the precise mechanism may vary depending on the specific enzyme and cell type/conditions as energy metabolism varies among cell types, conditions and proliferative nature (Bereiter-Hahn, Munnich & Woiteneck, 1998).

(k) Bone marrow-derived mesenchymal stem cell (BM-MSC) fitness and skeletal involution—During skeletal aging, oxidative stress reduces bone mineral density and increases marrow adiposity due to impaired osteogenesis and enhanced adipogenesis of bone marrow-resident MSCs. For this reason, obese and/or diabetic patients have an increased risk of fracture. Therefore, targeting pathways that prevent these dysfunctions have therapeutic potential in bone diseases. *IP6K1*-KO mice display an increased yield of BM-MSCs. Moreover, *IP6K1*-KO MSCs exhibit enhanced growth, survival, osteogenesis and haematopoiesis. Conversely, they show reduced adipogenesis, which further explains reduced adipose mass in these animals (Chakraborty *et al.,* 2010). Unlike adipocytes (Section VI.3j), *IP6K1*-KO MSCs display unaltered OCR, yet a reduced ROS level. Oxidative stress induces p53-mediated growth arrest and apoptosis in murine MSCs. Accordingly, p53 protein level is reduced and its E3 ubiquitin ligase mouse double minute-2 homolog (MDM2) is increased in *IP6K1*-KO MSCs. IP6K1 inhibits the transcription of *MDM2* by inhibiting the demethylase JMJD2C in MEFs (Section VI.3b), although this mechanism has not been tested in MSCs. In summary, IP6K1-mediated regulation of MSCs promotes fat accumulation but reduce bone formation (Boregowda *et al.,* 2017). The therapeutic significance of this finding in osteoporosis is discussed in Section VII.1b.

IP6K1, which is abundant in brain and testis (Section II.1), is essential for development of these organs. Conversely, its role in metabolic and other tissues is regulatory, representing a possible therapeutic targeted in diet- or age-induced diseases.

(4) IP6K2

(a) Apoptosis—IP7 level is increased during apoptosis (Section III). Several studies suggest an essential role of IP6K2-generated 5-IP7 in this process. An antisense screening study in OVCAR3 cells first identified IP6K2 as a mediator of IFNβ-induced apoptosis (Morrison *et al.,* 2001). In addition to OVCAR3 cells, IP6K2 also sensitizes various other cells including HeLa, HEK293, PC12, Jurkat and HL60 to apoptotic actions of cisplatin, etoposide, hydrogen peroxide, staurosporine, hypoxia, γ-irradiation and mutant huntingtin expression (Morrison *et al.,* 2002; Nagata *et al.,* 2005, 2011). Consequently, *IP6K2*-KO mice display a better survival rate following total body irradiation (8–10 Gy; 1 Gy = 1J radiation/kg) (Morrison *et al.,* 2002, 2009).

Catalytically active IP6K2 regulates the transcriptional function of p53 (Koldobskiy *et al.,* 2010). In HEK293 cells, the chaperone HSP90 binds IP6K2 at the amino acid region 132–143, which inhibits the kinase (Fig. 3B). Disruption of IP6K2–HSP90 binding promotes the activation and nuclear localization of IP6K2 (Chakraborty *et al.,* 2008; Koldobskiy *et al.,* 2010). Nuclear IP6K2 binds p53 *via* amino acids 1–67 (Fig. 3B), which suppresses p53-mediated transcription of the cell cycle arrest regulator *p21*. As a result, DNA repair is hampered, and the cells undergo apoptosis. Conversely, IP6K2 enhances the expression of one of p53's apoptotic targets, phorbol-12-myristate-13-acetate-induced protein 1 (PMAIP1 or NOXA). Thus, IP6K2 reduces the cell cycle arrest functions of p53, whereas it enhances its apoptotic actions (Koldobskiy *et al.,* 2010). Subsequent studies provided further details about the mechanism involved (Rao *et al.,* 2014). The phosphoinositide-3-kinase-related kinases (PIKKs) DNA dependent protein kinase (DNA-PK) and ataxia–telangiectasia-mutated protein kinase (ATM) phosphorylate and activate p53. The TTT co-chaperone family of proteins stabilises PIKKs. The C-terminus of IP6K2 (amino acids 212–426, Fig. 3B) binds to the TTT complex through direct interactions with Tti1, which facilitates 5-IP7's binding-mediated activation of CK2. 5-IP7 binds more potently than IP6 (IC_{50} of 5-IP7 ~ 0.4 μM; IC_{50} of IP6 ~14 μM) to the lysine-rich cluster of CK2α, which facilitates access of CK2's substrate to the kinase. Activated CK2 stimulates phosphorylation of the TTT complex, thereby enhancing the ability of DNA-PK/ATM to activate p53. Thus, in *IP6K2*-KO HCT116 cells, DNA damage (etoposide)-mediated induction of p53 is reduced, attenuating expression of its apoptotic targets *PUMA* and *NOXA*. Even a reduced level of p53 is sufficient to elevate p21 in *IP6K2*-KO cells (Rao *et al.,* 2014). Studies in *PPIP5K*-KO HCT116 cells further demonstrate the impacts of IPPs on cell proliferation. *PPIP5K* deletion increases levels of 5-IP7 (opposite effect to *IP6K2*-KO), decreases 1,5-IP8 (similar to *IP6K2*-KO), enhances p53 (opposite to *IP6K2*-KO) and p21 (similar to *IP6K2*-KO) and reduces proliferation (similar to *IP6K2*-KO) (Gu *et al.,* 2017). Thus, although *IP6K2* or *PPIP5K* deletion reduces cell proliferation, the mechanisms cannot be attributed solely to differences in IPPs. IP6K2 and PPIP5K may have distinct targets in this process.

Catalytic activity-independent apoptotic action of IP6K2 has also been reported. IP6K2 interacts with TRAF2, which interferes with transforming growth factor beta-activated kinase-1 (TAK1) phosphorylation, nuclear factor kappa B (NF-κB) signalling, and apoptosis. Overexpression of the TRAF2-binding deficient IP6K2 (S347A/S359A) (Fig. 3B) mutant in OVCAR3 cells enhances NF-κB signalling, which partly impairs tumour necrosis

factor alpha (TNFα)-induced apoptosis (Morrison *et al.,* 2007). On the other hand, CK2, which mediates cell survival, degrades IP6K2 by phosphorylating it at residues S347 and S356 (Fig. 3B) (Section IV) (Chakraborty *et al.,* 2011). Further studies are required to determine the precise effects of these IP6K2 residues in stressor-mediated apoptosis. Although the apoptotic function of IP6K2 is established, to what extent its subcellular distribution regulates this process remains unclear. Besides a nuclear role (Morrison *et al.,* 2005; Koldobskiy *et al.,* 2010), apoptotic functions of mitochondrial (Nagata *et al.,* 2005) and cytosolic (Nagata *et al.,* 2011, 2016; Moriya *et al.,* 2017) IP6K2 have also been reported, which require elucidation.

(b) Chemical-induced carcinogenesis—*IP6K2*-KO mice display normal embryogenesis, development, growth, blood parameters, serum insulin level and fertility (Morrison *et al.,* 2009). Although these mice do not develop spontaneous tumours, they do show accelerated development of aerodigestive tract carcinoma when exposed to the UV-mimetic chemical carcinogen 4-NQO (Morrison *et al.,* 2009). Enhanced susceptibility of *IP6K2*-KO mice to this chemical carcinogen is associated with upregulation of the transcription termination factor 1 (TTF1) and the twist family of basic helix-loop-helix transcription factor neighbour protein (TWISTNB) that may have implications in cancer (Morrison *et al.,* 2009). Conversely, downregulation of putative tumor suppressor genes like *DUSP16* and *EXT2* are observed in *IP6K2*-KO mice. IP6K2 promotes the apoptosis actions of p53 (Koldobskiy *et al.,* 2010; Rao *et al.,* 2014). Transgenic mice with a dominant negative p53 mutation are also susceptible to 4-NQO-induced oral cancer (Zhang *et al.,* 2006). Therefore, susceptibility of *IP6K2*-KO mice to 4-NQO-induced cancer may involve p53 activity. IP6K2, but not IP6K1, exhibits apoptotic functions, which may partly explain why *IP6K1* deletion protects, whereas *IP6K2* deletion augments chemical carcinogenesis.

(c) Cell migration and cancer metastasis—IP6K2 enhances cell–matrix adhesion, but reduces cell–cell adhesion (Rao *et al.,* 2015). *IP6K2*-KO HCT116 cells exhibit delayed growth, reduced cell–matrix adhesion, spreading and tyrosine phosphorylation of FAK (see Section VI.3f). Conversely, cell–cell adhesion and level of the adhesion-promoting protein zona occludin-1 (Zo-1) are increased in knockout cells. As a result, these cells display reduced anchorage-independent growth and migration. *In vitro* tumour invasion, monitored by tumour growth in a collagen invasion assay, is greatly reduced by *IP6K2* deletion. Moreover, subcutaneous xenograft tumours grown from *IP6K2*-KO HCT116 cells are reduced in size. Knockout xenografts, when implanted into the caecum of nude mice, display reduced growth and diminished metastasis to liver. Overexpression of catalytically active but not inactive IP6K2 restores adhesion and associated features in the knockout cells. A complex mechanism is suggested. The tumour suppressor LKB1 inhibits cell migration and metastasis *via* activation of cytosolic tyrosine phosphatases that dephosphorylate FAK. The catalytic activity of IP6K2 is not required for binding, but is critical for nuclear sequestration of LKB1, which inhibits the phosphatases. Accordingly, *IP6K2*-KO cells accumulate cytosolic LKB1 and display higher tyrosine phosphatase activity, which facilitates FAK dephosphorylation and cell migration. However, direct evidence of enhanced LKB1 phosphorylation-dependent activation of phosphatases in *IP6K2*-KO cells is lacking. Nevertheless, results obtained in two different studies indicate that IP6K1- or IP6K2-

generated 5-IP7 promotes cell migration *via*: (*i*) direct stimulation of FAK autophosphorylation (Fu *et al.,* 2017); and/or (*ii*) LKB1–tyrosine phosphatase-mediated indirect regulation of FAK (Rao *et al.,* 2015).

Based on the studies discussed here and in Sections VI.1b and 3f, it is clear that deletion of *IP6K1* or *IP6K2* reduces cell migration, whereas a reduction of DIPP1 level has the opposite effects (Grudzien-Nogalska *et al.,* 2016). Conversely, deletion of *IP6K2* but not *IP6K1* reduces tumour volume. This phenotypic variation may arise due to: (*i*) distinct methods of disruption of these genes; (*ii*) differential expression patterns of these isoforms; or (*iii*) additional, yet to be identified mechanisms. Understandably, 5-IP7 levels in these two systems are different. *IP6K2*-KO HCT116 cells display an almost total loss, whereas *IP6K1*-depleted HCT116 cells show a modest decrease in IPP levels (Koldobskiy *et al.,* 2010; Jadav *et al.,* 2016). Perhaps cell migration but not tumour growth is susceptible to a smaller decrease in IPP levels (Jadav *et al.,* 2016). Tissue-specific *IP6K*-KO mice or isoform-selective inhibitors may allow the roles of these isoforms in the initiation, progression and metastasis of cancer to be clarified.

(5)   IP6K3

(a)   Synapse formation and motor function—Although *IP6K3*-KO mice do not display gross abnormalities in brain structure, their motor learning and coordination are disturbed, which suggests Purkinje cell dysfunction. *IP6K3* is highly expressed in Purkinje cells. Moreover, *IP6K3*-KO mice display alterations in Purkinje cell structure with withered dendritic trees. The cell size and spine density of mutant Purkinje cells are also decreased in these knockouts. Both inhibitory symmetric and excitatory asymmetric synapses in the cerebellar molecular layer of *IP6K3*-KO mice are reduced. *IP6K3*-KO cerebella display reduced levels of the γ-aminobutyric acid (GABA)-synthesizing enzyme glutamic acid decarboxylase (GAD65), which is a marker of GABAnergic inhibitory neurons. Whether IP6K3 directly influences GAD65, is not known. The shape of Purkinje cell dendritic spines is determined by the arrangements and attachments of their cytoskeletal proteins such as adducin and spectrin. IP6K3 binds to the calponin homology (CH) domain of adducin and spectrin, whose mutual interactions are perturbed in *IP6K3*-KO mice. Catalytic activity of IP6K3 is not required to maintain β-adducin–β2-spectrin binding (Fu *et al.,* 2015).

(b)   Metabolism and aging—*IP6K3* is highly expressed in both mouse and human myotubes and muscle tissues (Moritoh *et al.,* 2016). Furthermore, *IP6K3* expression is elevated in skeletal muscle under diabetic, fasting, and disuse conditions. Chow-fed, aged (1.5-year old) *IP6K3*-KO mice display reduced body mass and accumulate less fat, although they eat a similar amount of food. Moreover, aged *IP6K3*-KO mice exhibit lower blood glucose and reduced circulating insulin levels. These mice also exhibit increased plasma lactate levels, suggesting that glycolysis is increased in the knockouts. *IP6K3*-KO mice (15-weeks old) also display enhanced glucose removal during glucose- and insulin-tolerance tests. The knockouts exhibit reduced expression of the muscle pyruvate dehydrogenase kinase-4 (PDK4) enzyme. PDK4 inhibits pyruvate to acetyl coenzyme A (acetyl-CoA) conversion and thus inhibits glucose oxidation. Thus, reduced PDK4 expression may also increase glucose oxidation, which may reduce lipogenesis-mediated fat accumulation in

these mice. Published results (Chakraborty *et al.,* 2010) and recent experiments (S. Ghoshal & A. Chakraborty, unpublished data) suggest that *IP6K1*-KO mice are also protected from age-induced weight gain and insulin resistance. However, unlike *IP6K1*-KO, *IP6K3*-KO mice are not protected against diet-induced obesity (DIO) (Moritoh *et al.,* 2016).

IP6K3-KO mice exhibit an increased survival rate, although the lifespan experiment was not conducted to completion (Moritoh *et al.,* 2016). Phosphorylation of the mTORC1 effector S6 ribosomal protein is reduced in heart but not in skeletal muscle of *IP6K3*-KO mice, although stimulatory phosphorylation of the direct mTORC1 target S6 kinase-1 (S6K1) and the mTORC2 target Akt are unchanged (Moritoh *et al.,* 2016). Although mTOR activity is associated with aging (Kennedy & Lamming, 2016), it is not clear whether the same mechanism is operative in *IP6K3*-KO mice. Although the impact of IP6K1 on mTOR pathway in the heart is unknown, in various *IP6K1*-KO cells and in the skeletal muscle of IP6K1-KO HFD-fed mice, it is upregulated along with the Akt pathway (Chakraborty *et al.,* 2010). Further studies are needed to distinguish tissue-specific functions of IP6K1 and IP6K3 in metabolism and aging.

VII.   PHARMACOLOGICAL TARGETING OF IP6KS

(1)   Characterization of the pan-IP6K inhibitor TNP

The above studies indicate that pharmacological targeting of the IPP pathway may have beneficial effects in diseases (Chakraborty *et al.,* 2011; MacKenzie & Elliott, 2014; Shears, 2016). The compound N2-(m-trifluorobenzyl)N6-(p-nitrobenzyl)purine (TNP) was originally discovered as an IP3–3K inhibitor (Chang *et al.,* 2002), but was later found to be more potent on IP6Ks. TNP is fairly specific to IP6Ks; it does not inhibit PPIP5Ks or ~70 other kinases (Padmanabhan *et al.,* 2009). TNP reduces 5-IP7 level in various cells (Padmanabhan *et al.,* 2009; Chakraborty *et al.,* 2010; Prasad *et al.,* 2011; Wundenberg *et al.,* 2014; Zhang *et al.,* 2014; Sun *et al.,* 2015). Moreover,TNP inhibits both kinase and phosphatase activities of IP6K on IP6 (Wundenberg *et al.,* 2014). Accordingly, TNP regulates IP6K-mediated cellular processes. For example, TNP hampers the tyrosine phosphorylation and membrane localization of α-actinin, which reduces migration of HeLa cells (Fu *et al.,* 2017). TNP substantially increases Cul4A–CSN binding and attenuates the UV-elicited dissociation of the Cul4A–CSN complex, which conforms with the role of 5-IP7 in this process (Rao *et al.,* 2014). Under various conditions, TNP increases Akt activity in HEK293 cells, MEFs, neutrophils, aged MSCs and diabetic cardiomyocytes (Chakraborty *et al.,* 2010; Prasad *et al.,* 2011; Xu *et al.,* 2013; Zhang *et al.,* 2014; Sun *et al.,* 2015), whereas it enhances AMPK activity in adipocytes and diabetic cardiomyocytes (Sun *et al.,* 2015; Zhu *et al.,* 2017). TNP enhances EE, whereas it inhibits fatty acid biosynthesis in 3T3L1 cells (Zhu *et al.,* 2017). TNP is also bioavailable. For example, 6 h post-administration of a single intraperitoneal dose, TNP accumulated at ~10–25 μM in liver, skeletal muscle, and adipose tissue, although to a much lesser extent (0.63 μM) in the brain. Plasma protein binding (PPB) of TNP is 90–93% which is similar to or better than a number of FDA-approved drugs (Ghoshal *et al.,* 2016).

(a)   TNP ameliorates DIO and insulin resistance—Similar to *IP6K1* deletion (Sections VI.3i,j) (Chakraborty *et al.,* 2010; Zhu *et al.,* 2016, 2017), TNP protects mice from DIO and associated metabolic abnormalities (Ghoshal *et al.,* 2016). Both short-term (2 weeks) and long-term (10 weeks) TNP treatments (10–20 mg/kg daily, intraperitoneal) reduce fat mass, reduce fatty liver, enhance insulin sensitivity and restore metabolic homeostasis in DIO mice without altering food intake. TNP decelerates both initiation and progression of DIO and insulin resistance in mice (Zhu *et al.,* 2016; Ghoshal *et al.,* 2016). These effects are abolished in *IP6K1*-KO mice, indicating that at least part of the beneficial effects of TNP on metabolism act *via* inhibition of IP6K1. TNP enhances insulin sensitivity in DIO mice *via* Akt activation (Ghoshal *et al.,* 2016). Moreover, TNP reduces weight gain by enhancing AMPK-mediated browning and EE in the adipose tissue and adipocytes (Zhu *et al.,* 2016). Thus, TNP has strong anti-obesity and anti-diabetic effects.

(b)   TNP attenuates HFD-induced loss of trabecular bone volume—*IP6K1*-KO mice display increased MSC fitness and osteogenesis (Section VI.3k). Accordingly, long-term TNP treatment (Ghoshal *et al.,* 2016) increases yield and colony-formation capacity of MSCs in DIO mice (Boregowda *et al.,* 2017). TNP conserved trabecular bone, but decreased marrow adiposity. Therefore, in addition to its beneficial effects on obesity and T2D, TNP also protects mice from HFD-induced MSC dysfunctions in the marrow (Boregowda *et al.,* 2017). Insulin sensitizers like peroxisome proliferator-activated receptor gamma (PPARγ) antagonists increase obesity and fracture risk. Thus, IP6K1 inhibitors may have greater acceptability as anti-obesity and anti-diabetic drugs due to their beneficial effects on bone properties.

(c)   TNP protects against cardiac I/R injury in leptin receptor mutant db/db mice—The cytokine OSM, which works *via* activation of the OSM receptor, alleviates cardiac ischaemic/reperfusion (I/R) injury by inhibiting cardiomyocyte apoptosis in leptin receptor-deficient obese and diabetic db/db mice (Sun *et al.,* 2015). OSM enhances mitochondrial biogenesis and function, whereas it reduces IP7 levels in db/db mice subjected to cardiac I/R injury (Section III). Conversely, OSM receptor knockout increases IP7, impairs mitochondrial biogenesis, glucose homeostasis and insulin sensitivity and augments cardiomyocyte apoptosis, together aggravating cardiac I/R injury. Short-term TNP treatment (10 mg/kg daily, tail vein injection) mimics the effects of OSM in db/db mice (Sun *et al.,* 2015). OSM exerts pleiotropic signalling effects, which may regulate 5-IP7 production, although the precise mechanism is not clear.

(d)   TNP improves aging effects of BM-MSCs and their therapeutic efficacy for myocardial infarction (MI)—Decreased viability and impaired function of aged BM-MSCs hamper their therapeutic efficacy. Aged (18-month old) BM-MSCs exhibit higher 5-IP7 levels compared to young cells (Section III). In addition, Akt activity is decreased, whereas apoptosis is increased in aged BM-MSCs. TNP treatment (10 μM, 2 h) reduces 5-IP7 level and apoptosis, whereas it enhances Akt activity in aged BM-MSCs. Levels of angiogenic factors such as vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), IGF-1 and hepatocyte growth factor (HGF), which are decreased in aged BM-MSCs, are restored by TNP treatment (Zhang *et al.,* 2014). Moreover, TNP-treated

BM-MSCs, when transplanted into infarcted hearts, display enhanced survival, which promotes their anti-apoptotic and pro-angiogenic efficacy *in vivo*. Thus, TNP-treated BM-MSC therapy decreases fibrosis and preserves heart function (Zhang *et al.,* 2014).

(2) Potential risks of IP6K inhibition

From the above discussion, it is clear that the IPP metabolic pathway regulates major cellular processes (Fig. 7), *via* diverse mechanisms (Fig. 6), modulating various phenotypes in mice (Fig. 8). Although targeting this pathway is beneficial in certain disease models, it may also have potential risks (Fig. 8). For example, *IP6K1*-KO mice exhibit premature death, impaired social interaction and male sterility (Bhandari *et al.,* 2008; Chakraborty *et al.,* 2014; Fu *et al.,* 2017; Malla & Bhandari, 2017), whereas *IP6K3*-KO mice display reduced motor learning and coordination (Fu *et al.,* 2015). *IP6K1* deletion also aggravates lung inflammation in nicotine-exposed mice (Xu *et al.,* 2013). Moreover, platelet aggregation is delayed in these knockouts, which may cause excess bleeding. *IP6K2*-KO mice are sensitive to chemical carcinogenesis (Morrison *et al.,* 2009). Therefore, it is critical to determine whether pharmacological inhibition of IP6Ks can cause these aberrations. Chronic TNP treatment (15 weeks, 10 mg/kg daily) does not lead to neuronal or reproductive anomalies (Ghoshal *et al.,* 2016). TNP does not cross the blood–brain barrier (BBB) efficiently. Functioning in a similar way to the BBB, the blood–testes barrier (BTB) blocks entry of drugs in the apical compartment (Cheng & Mruk, 2012), which may explain the ineffectiveness of TNP in these organs. Thus, developmental defects caused by germ-line deletion of *IP6K1* are not observed following its pharmacological inhibition in adult mice. Studies involving very long-term treatment should be conducted to rule out such concerns.

Does Akt activation represent a potential cancer risk in patients treated with IP6K inhibitors? Gene amplification or constitutive hyperactivation of Akt kinase (~10- to 50-fold) (Cheng *et al.,* 1996; Vincent *et al.,* 2011) causes tumorigenesis (Mundi *et al.,* 2016). However, tumours are not formed if Akt hyperactivation is not achieved (Mundi *et al.,* 2016). *IP6K1* deletion only causes a ~2- to 3-fold increase in phosphorylation stimulated by Akt in insulin-treated mice (Chakraborty *et al.,* 2010). DIO mice with *IP6K1* deletion exhibit normal Akt activity, unlike DIO mice with intact IP6K1 in which Akt activity is reduced (Zhu *et al.,* 2016). Therefore, it is unlikely that IP6K inhibition will result in Akt-mediated tumorigenesis. IP6Ks regulate various other pathways and thus have differential effects on cell proliferation and survival (Chakraborty *et al.,* 2011; Wilson *et al.,* 2013; Thota & Bhandari, 2015; Zhu *et al.,* 2016). *IP6K1* or *IP6K3* knockout mice do not display tumorigenesis (Chakraborty *et al.,* 2010; Jadav *et al.,* 2016; Moritoh *et al.,* 2016). In fact, *IP6K1*-KO mice are protected from chemical carcinogenesis and colon cancer metastasis (Jadav *et al.,* 2016). *IP6K2* deletion, on the other hand, sensitizes mice to chemical carcinogenesis (Morrison *et al.,* 2009), although it protects them from cancer metastasis (Rao *et al.,* 2015). However, TNP does not cause tumorigenesis. To summarize, the therapeutic significance of IP6K inhibition in cancer is yet to be determined.

(3) Development of safe and isoform-selective IP6K inhibitors is necessary

TNP inhibits all three IP6K isoforms. It also inhibits human cytochrome P450s (Ghoshal *et al.,* 2016), which may interfere with the metabolism of other drugs. Moreover, TNP slightly

elevates Ca^{2+} levels in human promyelocytic leukemia (HL60) cells (Chang *et al.,* 2002) and cytosolic Zn^{2+} levels in cortical neurons (Stork & Li, 2010) and thus, may cause signalling alterations. At higher concentrations, TNP inhibits IP3–3K which enhances neurite outgrowth in PC12 cells (Eva *et al.,* 2012). TNP also enhances the stimulatory phosphorylation of ERK in PC12 cells (Eva *et al.,* 2012), but inhibits the same process in endothelial cells (Sekar *et al.,* 2014). TNP has a half-life ($T_{1/2}$) of 32 and 9 min in human and mouse hepatic microsomal stability assays (Ghoshal *et al.,* 2016). The development of an IP6K1 inhibitor with greater metabolic stability is required to achieve oral efficacy in patients. Thus, although TNP is an exciting proposition, further improvements are required before it advances to the next level. To minimize undesirable effects, isoform-selective and safer inhibitors are needed. BBB- and BTB-impermeable IP6K1 (or IP6K1 + IP6K3) selective inhibitors are desirable for the treatment of diet- or age-induced metabolic diseases. Furthermore, the therapeutic window for each disease should be determined.

VIII. THE IPP PATHWAY IN HUMAN HEALTH

Pathways regulated by IPP enzymes are highly conserved in mice and humans. Thus, pharmacological targeting of IPP enzymes is expected to work similarly in human subjects. Yet, murine and human IP6Ks do exhibit certain differences. For example, although *IP6K* isoforms are expressed in human tissues, isoform-specific expression may vary between mice and humans (Moritoh *et al.,* 2016) (Section II). In addition, regulatory domains of IPP enzymes display minor variations in amino acid sequences, which may alter certain *in vivo* functions.

Limited information is available on gene expression and polymorphism profiles of IPP enzymes in diseases. An epigenome-wide association study identified higher CpG methylation upstream of *IP6K1* in the saliva of heavy (BMI = 24.1) adolescent Finnish girls compared to lean (BMI = 14.4) girls (Rounge *et al.,* 2016), suggesting diminished expression of the gene in obesity. It is conceivable that *IP6K1* expression is reduced in a natural negative-feedback mechanism. Disruption of *IP6K1* at intron 1 was observed in a single Japanese family with type-2 diabetes (Kamimura *et al.,* 2004). However, this anomaly was not found in 405 unrelated patients, indicating the disrupted *IP6K1* is either family-specific or a chance association. An intron-specific *NUDT3* single nucleotide polymorphism (SNP) (rs206936) is associated with increased BMI and obesity but not metabolic disorders in Japanese women. The rs206936 polymorphism may enhance gene expression, although confirmation is required (Kitamoto *et al.,* 2013).

IP6K2 is located in chromosome *3p21*, with an array of other tumour-suppressor genes including *NPRL2, TUSC2, RASSF1A, SEMA3B,* and *SEMA3F* (Shames & Minna, 2008). A missense mutation in *IP6K2* (R325C; arginine to cysteine) was reported in patients with low-grade serous carcinoma (LGSC) (Jones *et al.,* 2012). The arginine residue is adjacent to the FYSSSLL motif of the enzyme, which is critical for its catalytic activity. Loss of the *IP6K2* gene, among others, was reported in B-cell chronic lymphocytic leukaemia (CLL) patients (Kay *et al.,* 2010).

A genetic variability study identified *IP6K3* polymorphism in Alzheimer's disease. The SNPs rs28607030 and rs10947435, located in the 5j-flanking region of *IP6K3*, are associated with late-onset alzheimer's disease (LOAD) risk (Crocco *et al.,* 2016). The 'G' allele of SNP rs28607030 in the promoter region of *IP6K3* forms an intact binding site for the cellular myelocytomatosis viral oncogene homolog (Myc) transcription factor and thus shows higher transcriptional activity than the corresponding 'A' allele in a luciferase assay (Crocco *et al.,* 2016). The impact of this SNP on endogenous *IP6K3* is not known. The above studies are interesting, albeit inadequate, as in many cases mRNA expression does not correlate with protein or activity level (Tian *et al.,* 2004). Further studies are required to determine activity, post-translational modifications, localization and protein interactions of these enzymes in human tissues.

IX. CURRENT AND FUTURE DIRECTIONS OF IPP RESEARCH IN MAMMALS

(1) Complete characterization of *IP6K*-KO mice and generation of other models

Although *IP6K*-KO mice are fairly well characterized, further details are necessary. Transgenic and inducible mouse models of IP6K, PPIP5K and DIPP are required to determine the IPP-dependent and independent functions of these enzymes. Knockout cell lines of PPIP5K reveal that these enzymes are also critical in modulating cell proliferation and metabolism (Gu *et al.,* 2017). Therefore, genetic-knockout mouse models of *PPIP5K* and *DIPP* are needed to study their roles *in vivo*. Appropriate tissue-specific mouse models are also required. Most importantly, impacts of the pathway in human health and diseases should be assessed.

(2) Design high-throughput assays and obtain structural information for IPP-metabolizing enzymes

The only available IP6K inhibitor TNP is not an ideal lead compound (Section VIII.3). Although PAO and U73122 inhibit IP6K1 activity *in vitro* (Rajasekaran *et al.,* 2017) (Section III), they are not exciting lead compounds. The toxic arsenic atom in PAO (Ralph, 2008) and off-target effects of U73122 (Huang *et al.,* 2013) limit their use. Thus, chemical libraries should be screened to discover new inhibitors. A rate-limiting step in this approach is lack of a high-throughput assay. Although radiolabelled-HPLC or non-radiolabelled sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and mass-spectrometry-based methods (Azevedo & Saiardi, 2006; Draskovic *et al.,* 2008; Losito *et al.,* 2009) are useful for estimating IP6K activity and cellular IPP levels, they are not suitable for high-throughput screening of thousands of compounds. A recent paper addressed this issue by designing a high-throughput assay for IP6Ks (Wormald *et al.,* 2017). In addition, crystallization of human enzymes will provide an insight into active sites and regulatory regions, which is critical for structure-based drug development. To date, crystal structures of IP6K from the parasite *Entamoeba histolytica* and PPIP5Ks from humans (Wang *et al.,* 2012, 2014) are known. Similarly, structures of human IP6Ks should be resolved.

(3) Develop new chemical tools for IPP research

Lack of appropriate chemical tools hampers IPP research. Encouragingly, various laboratories have initiated efforts to design novel tools such as methods for the cellular delivery of IPPs (Pavlovic *et al.,* 2016), non-hydrolysable IPP analogs (Wu *et al.,* 2013, 2014), isolation and detection of IPPs from tissue samples (Wilson *et al.,* 2015), modification of current isolation protocols to differentiate 5-IP7 and 1-IP7 (Gu *et al.,* 2016), synthesis of IP- and IPP-based affinity reagents (Wu *et al.,* 2016), 5-IP7 analogs for traceable phosphoryl transfer (Brown, Marmelstein & Fiedler, 2016), synthetic peptides to establish pyrophosphate ester stability (Yates & Fiedler, 2015), an affinity reagent for the recognition of pyrophosphorylated peptides (Conway & Fiedler, 2015), in-gel detection of pyrophosphorylated proteins (Williams & Fiedler, 2015) and stabilized mimics of IPPs (Williams & Fiedler, 2015). A recently reported mass-spectrometry approach detected peptide pyrophosphorylation (Penkert *et al.,* 2017), which hopefully can be utilized *in vivo.* A reliable method for quantitative estimation of IPPs from biological samples is required to measure their levels in healthy and diseased subjects.

X.   CONCLUSIONS

(1)   IPPs are soluble IP derivatives with diphosphate moieties, which are metabolized by distinct classes of enzymes.

(2)   Post-translational modifications or protein interactions regulate IP6Ks.

(3)   IPP-metabolizing enzymes regulate proteins *via* diverse mechanisms.

(4)   The IPP pathway regulates various cellular functions.

(5)   Genetic deletion of *IP6K* s has detrimental effects on development, but beneficial impacts on several diseases.

(6)   Pharmacological inhibition of IP6Ks in mice reveals that this pathway has great potential to become a target in the treatment of various diseases.

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XII.   REFERENCES

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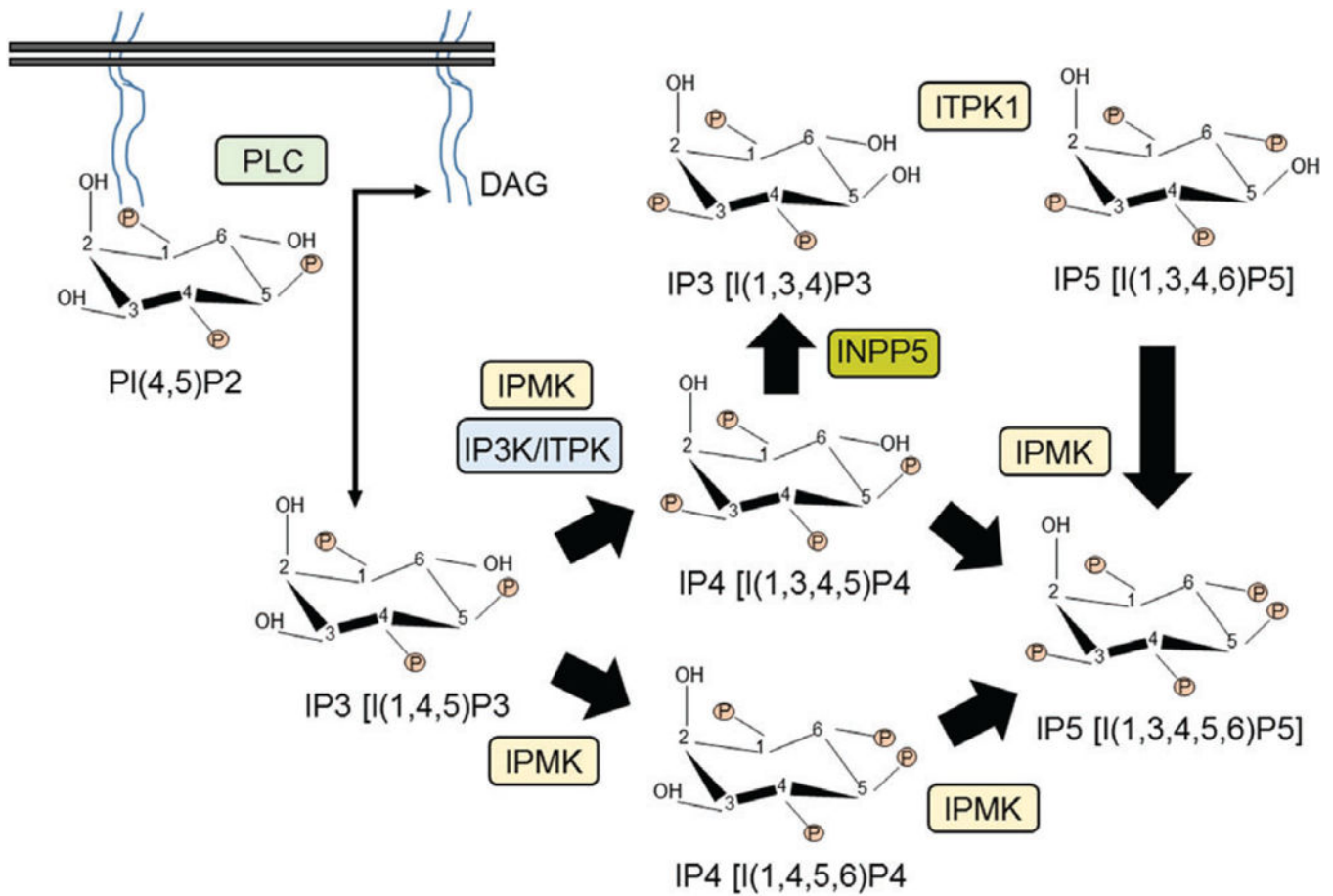
Fig. 1.
Generation of inositol phosphate IP5 from the lipid phosphoinositide phosphatidylinositol (4,5)-bisphosphate (PIP2) in mammals. Only major enzymes are denoted. DAG, diacylglycerol; INPP5, inositol polyphosphate 5-phosphatase; IP3K, IP3 3-kinase; IPMK, inositol polyphosphate multikinase; ITPK1, inositol tetrakisphosphate 1-kinase; PLC, phospholipase C.
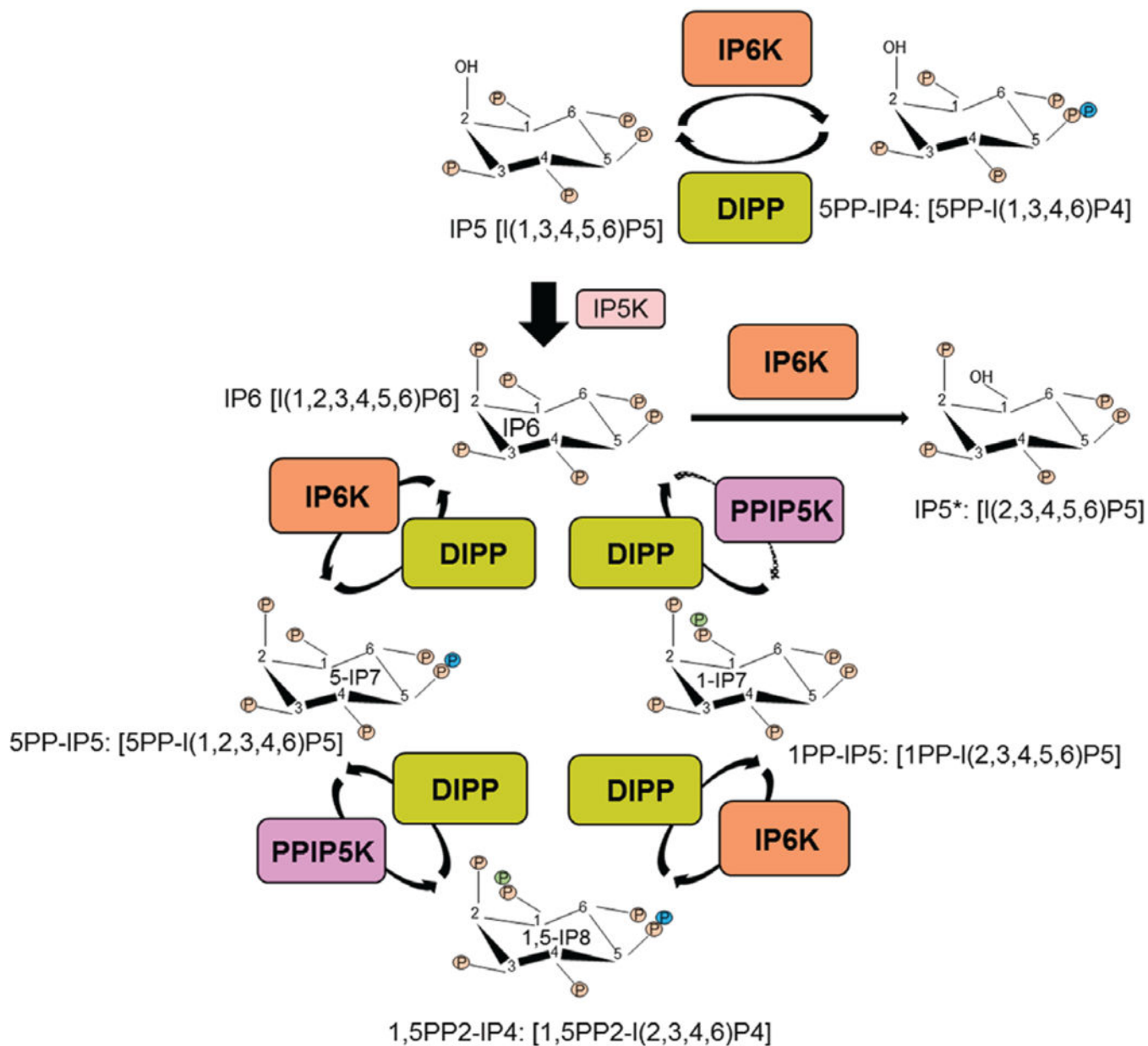
Fig. 2.
Synthesis of inositol phosphate IP6 and inositol pyrophosphates. Inositol hexakisphosphate kinases (IP6Ks) convert IP5, IP6 and 1-IP7 to generate 5PP-IP4, 5-IP7 and 1,5-IP8 respectively. Diphosphoinositol pentakisphosphate kinases (PPIP5Ks) primarily convert 5-IP7 to 1,5-IP8. PPIP5Ks also generate 1-IP7 from IP6, albeit to a lesser extent (dotted arrow). Diphosphoinositol polyphosphate phosphohydrolases (DIPPs) hydrolyse inositol pyrophosphates to regenerate the respective inositol phosphates. At a lower ATP/ADP ratio, IP6Ks dephosphorylate IP6 to IP5*. Only well-characterized enzymes are depicted.
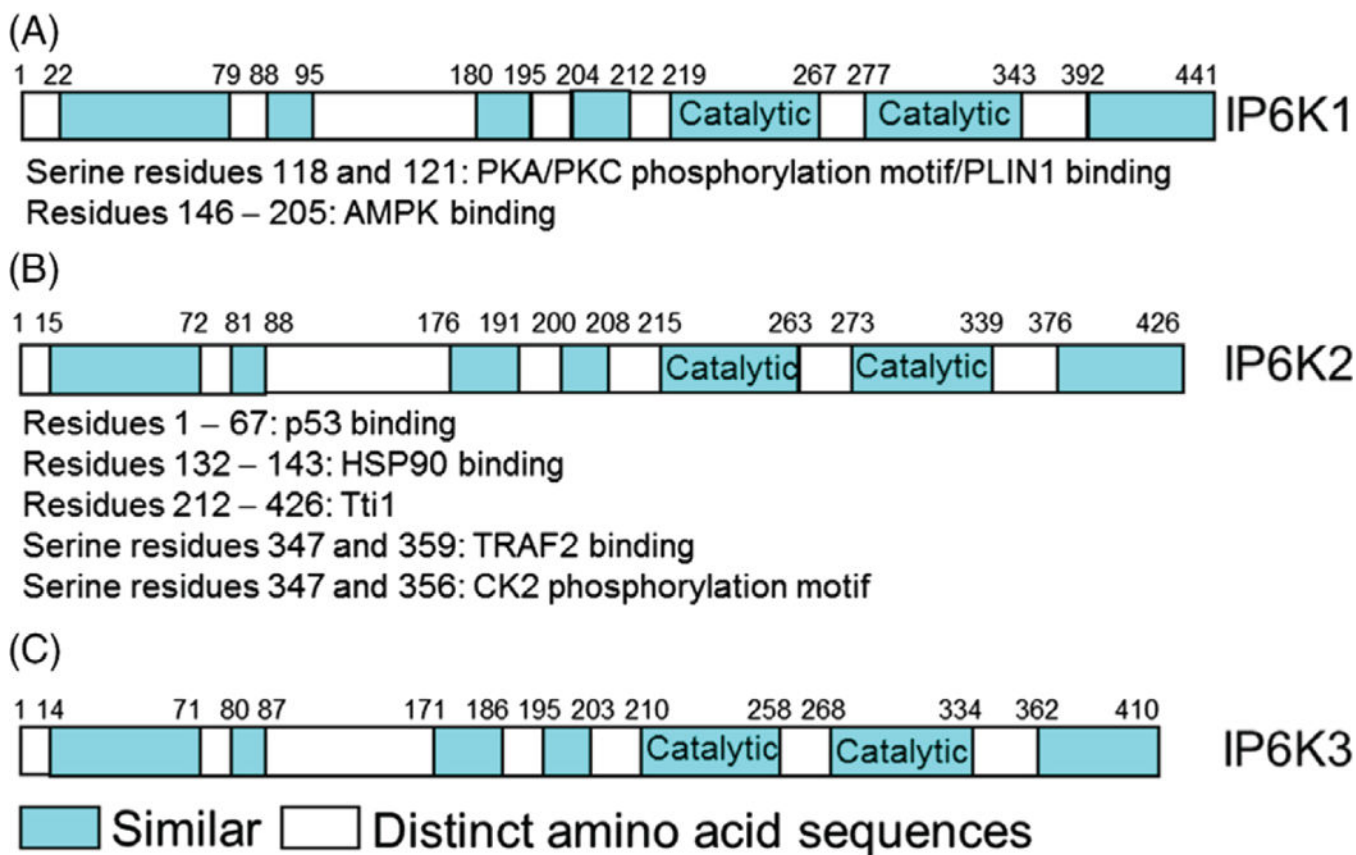
Fig. 3.

A–C. Schematic presentation of the human inositol hexakisphosphate kinase (IP6K) isoforms. In addition to the highly conserved catalytic domain (cyan: catalytic), various other regions display substantial similarities (cyan). Conversely, certain (white) regions are isoform-specific. IP6Ks interact with various protein targets in an isoform-selective manner. A few representative protein-interactors are shown for IP6K1 and IP6K2, for which the target binding sites were mapped. Some isoform-specific interactions are partly explained by substantial dissimilarity in the binding regions. IP6Ks also bind to certain targets [perilipin-1 (PLIN1) for IP6K1 and TNF receptor-associated factor-2 (TRAF2) for IP6K2] *via* phosphorylation of isoform-specific residues. Details of these and other interactions are presented in Sections IV–VI. AMPK, AMP-activated protein kinase; CK2, casein kinase 2; HSP90, heat shock protein 90; p53, tumour suppressor protein 53; PKA/C, protein kinase A/C; Tti1, telomere length regulation protein 2 (Tel2)-interacting proteins 1.
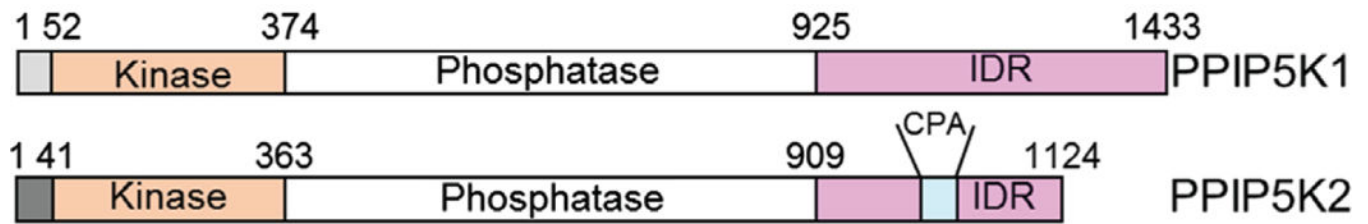
Serine residue 1006: Phosphorylation mediated regulation of nuclear localization of PPIP5K2

Fig. 4.
Domain analyses of human diphosphoinositol pentaphosphate kinases PPIP5K1 and PPIP5K2 (redrawn from Shears *et al.,* 2016). PPIP5Ks contain a kinase and a phosphatase domain, which share 86 and 77% homology between the isoforms. This group also contains an intrinsically disordered domain (IDR), essential for protein– protein interaction, which shares 3% identity. PPIP5K2 contains a penta-arginine (CPA) domain that serves as a nuclear-localization signal (NLS). Phosphorylation of the serine 1006 residue, adjacent to the NLS regulates the nuclear localization of PPIP5K2.
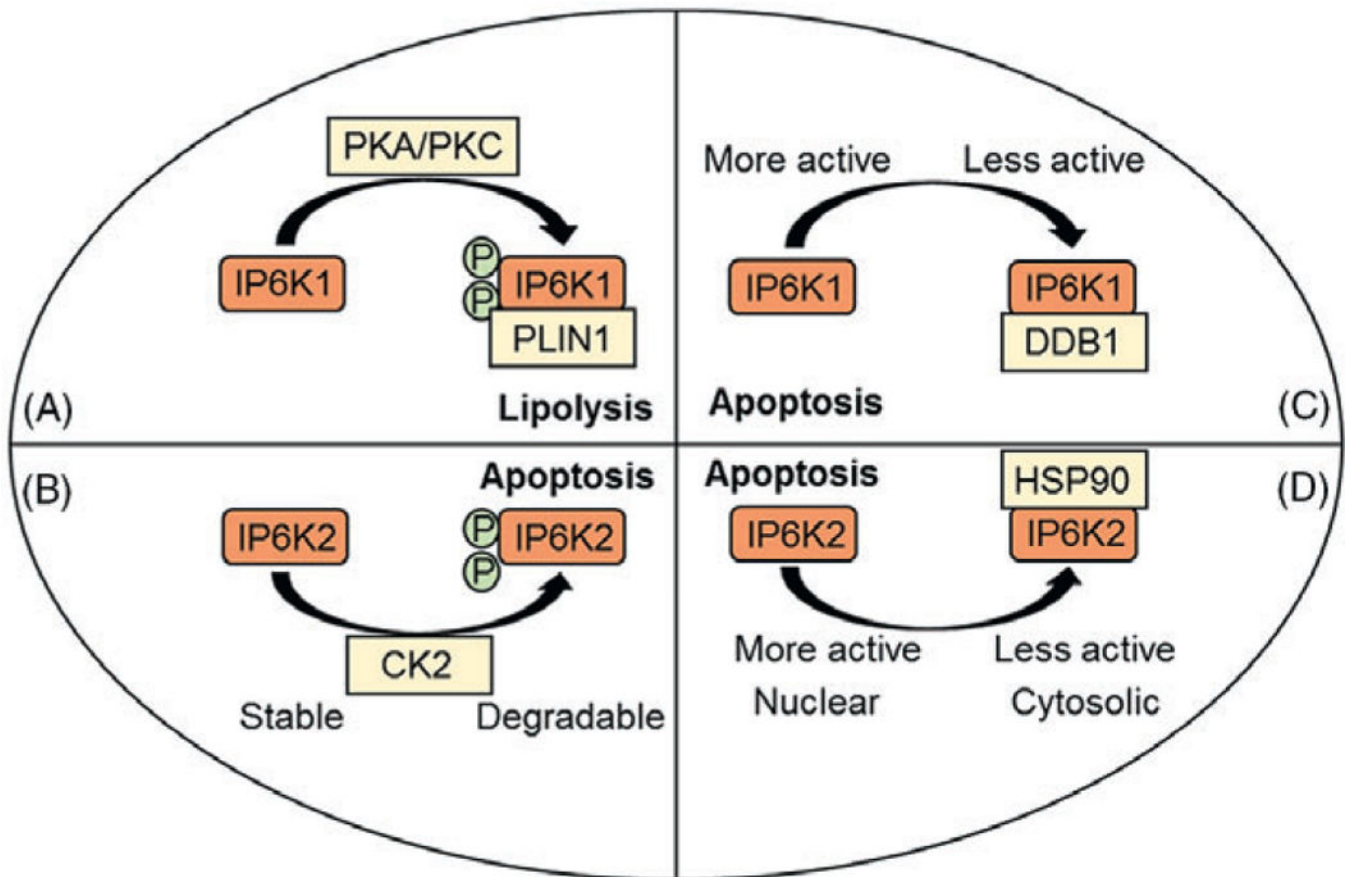
Fig. 5.
Isoform-specific post-translational modifications regulate inositol hexakisphosphate kinase (IP6K) stability or interaction with other proteins. Moreover, isoform-selective interactions with certain proteins regulate the catalytic activity of IP6K. (A) Protein kinase A/C (PKA/PKC)-mediated phosphorylation of IP6K1 mediates its interaction with the lipolytic modulator perilipin 1 (PLIN1). (B) Casein kinase-2 (CK2) phosphorylation facilitates degradation of IP6K2. (C) DNA damage binding protein-1 (DDB1) inhibits IP6K1 catalytic activity. (D) Heat shock protein 90 (HSP90) binds to inhibit the catalytic activity of IP6K2.
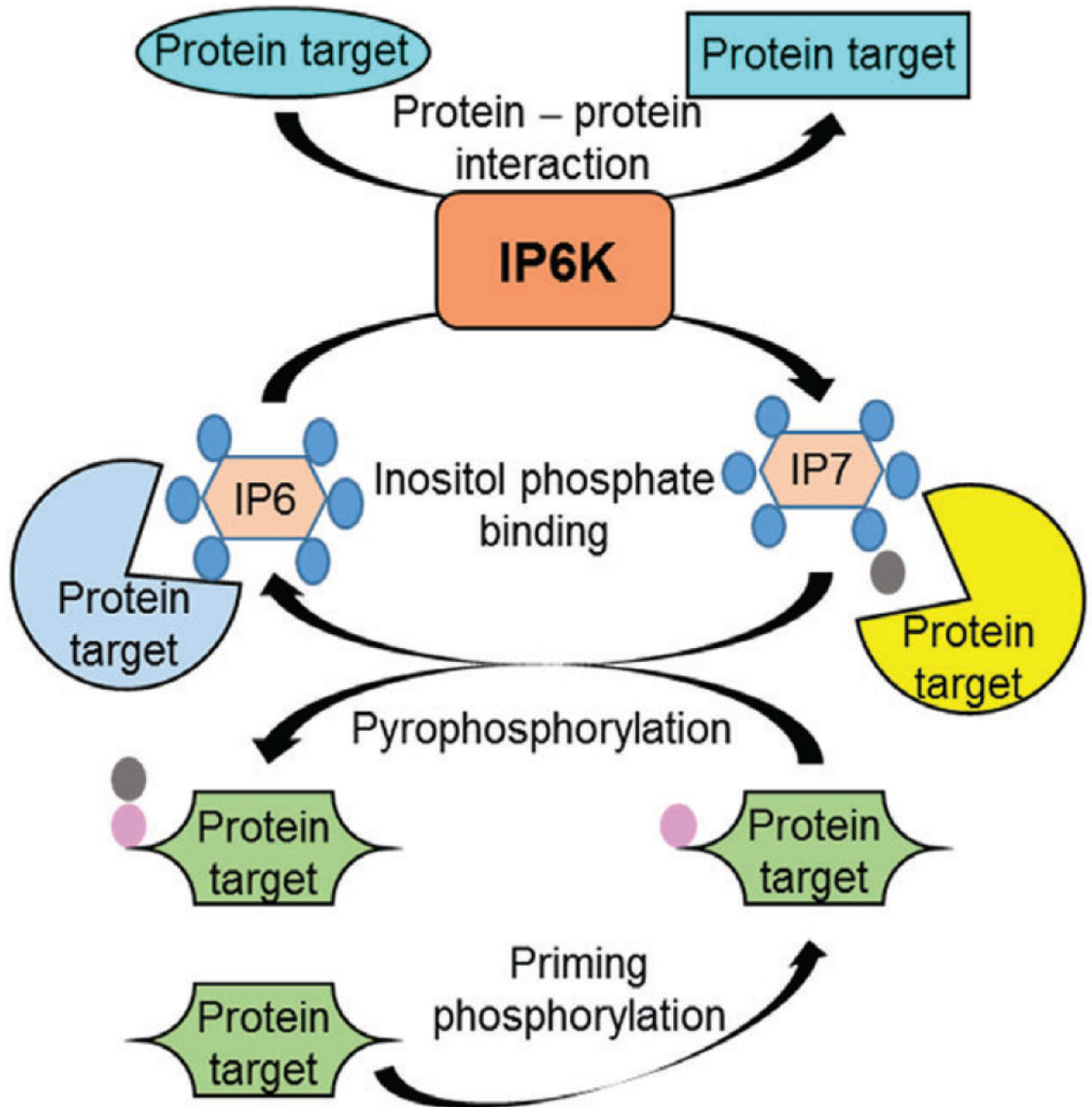
Fig. 6.

Mechanisms by which inositol pyrophosphate biosynthetic enzymes regulate protein targets. The model is based primarily on inositol hexakisphosphate kinases (IP6Ks). IP6Ks generate inositol phosphate IP7 from IP6. IP7 modulates protein (yellow) targets by direct binding. This activity also diminishes the effects of IP6 on its protein (blue) targets. Moreover, IP7 donates its β-phosphate (grey) to the serine residue of a protein (green), which is already phosphorylated (pink) by a priming protein kinase. This event is called pyrophosphorylation. IP6 is released by the process, which may enhance its interaction with protein targets. In

addition, IP6Ks bind to certain protein targets (cyan), to alter their conformations and functions. This does not require catalytic activity of IP6Ks. The catalytic activity-mediated functions of IP6K may also require protein interaction. Diphosphoinositol pentakisphosphate kinase (PPIP5K) may display similar activities. See Section V for further details.

Inositol pyrophosphate metabolic pathway

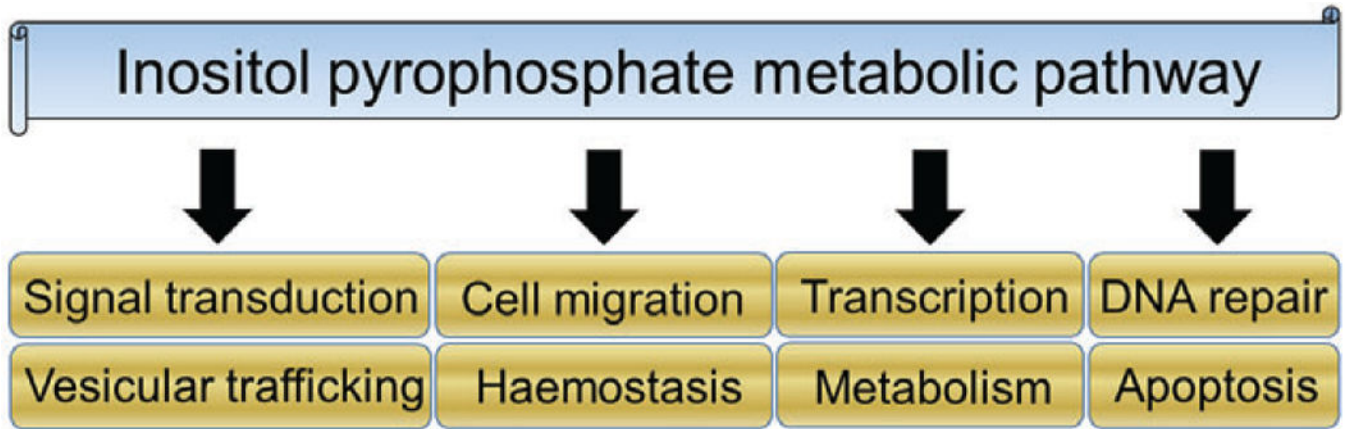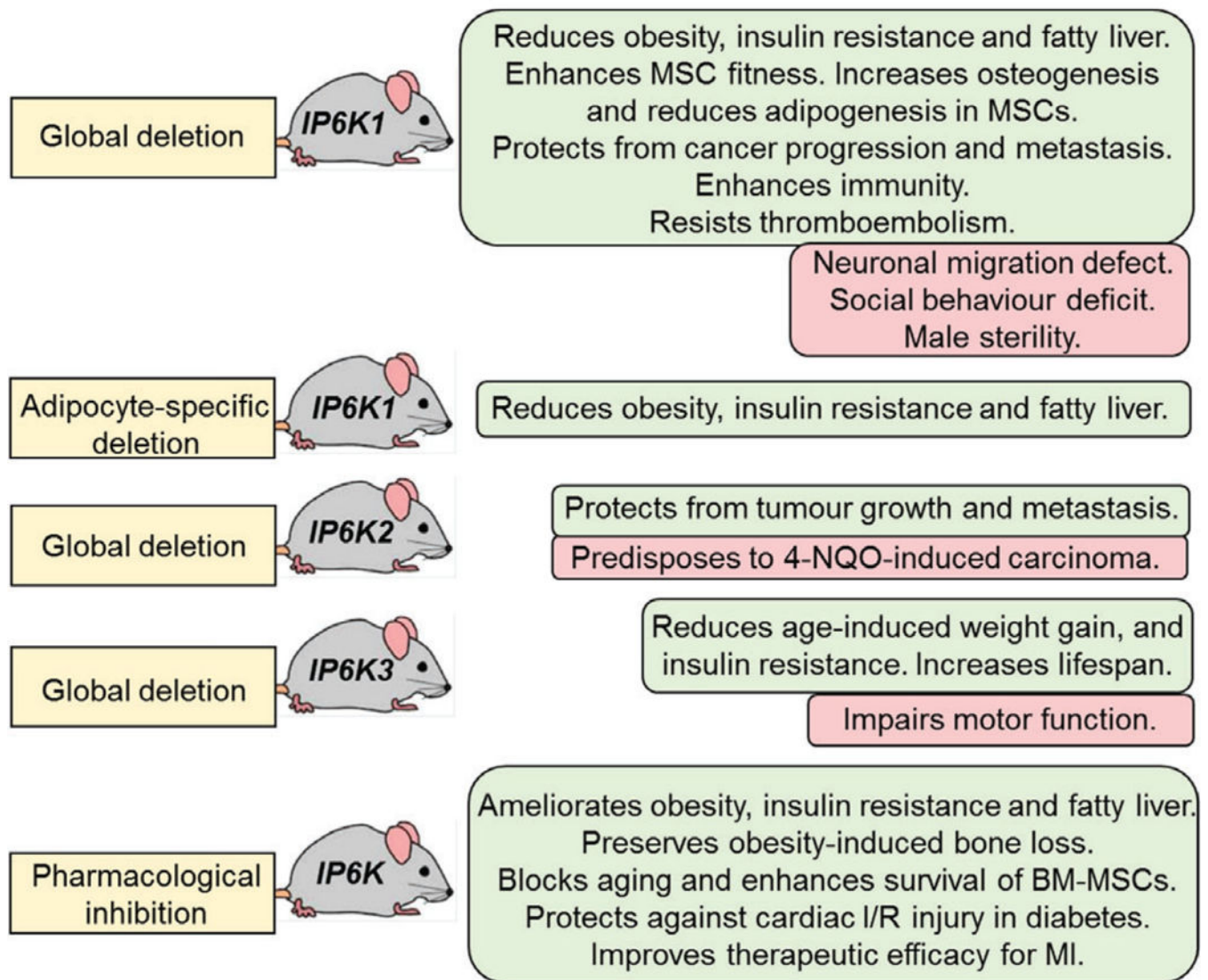| Signal transduction | Cell migration | Transcription | DNA repair |
| Vesicular trafficking | Haemostasis | Metabolism | Apoptosis |

Fig. 7.
The inositol pyrophosphate (IPP) metabolic pathway regulates various cellular processes in mammals.

Fig. 8.
Summary of benefits and potential risks of perturbation of the inositol pyrophosphate (IPP) pathway. Studies using *IP6K1-*, *IP6K2-* and *IP6K3-*knockout mouse models and pan-pharmacological inhibition reveal that disruption of the pathway is beneficial in several diseases (highlighted in green). However, the knockout mice also display isoform-specific aberrations (highlighted in pink). Thus, although the IPP pathway is a novel and encouraging therapeutic target, precautions must be taken during drug development to minimize potential risks. Development of isoform-selective inhibitors would be beneficial. 4-NQO, 4-nitroquinoline-1-oxide; BM-MSC, bone marrow-derived mesenchymal stem cell; I/R, ischaemia/reperfusion; MI, myocardial infarction; MSC, mesenchymal stem cell.