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PIP kinases: a versatile family that demands further therapeutic attention

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Abstract

Phosphoinositides are membrane-localized phospholipids that regulate a plethora of essential cellular processes. These lipid signaling molecules are critical for cell homeostasis and therefore their levels are strictly regulated by the coordinated action of several families of lipid kinases and phosphatases. In this review, we provide a focused perspective on the phosphatidylinositol phosphate kinase (PIPK) family and the three subfamilies that compose it: Type I PIPKs or phosphatidylinositol-4-phosphate 5-kinases (PI4P5Ks), Type II PIPKs or phosphatidylinositol-5-phosphate 4-kinases (PI5P4Ks), and Type III PIPKs or phosphatidylinositol-3-phosphate 5-kinases (PIKfyve). Each subfamily is responsible for catalyzing a hydroxyl phosphorylation on specific phosphoinositide species to generate a double phosphorylated lipid, therefore regulating the levels of both substrate and product. Here, we summarize our current knowledge about the functions and regulation of each PIPK subfamily. Further, we highlight the roles of these kinases in various in vivo genetic models and give an overview of their involvement in multiple pathological conditions. The phosphoinositide field has been long focused on targeting PI3K signaling, but growing evidence suggests that it is time to draw attention to the other phosphoinositide kinases. The discovery of the involvement of PIPKs in the pathogenesis of multiple diseases has prompted substantial efforts to turn these enzymes into pharmacological targets. An increasingly refined knowledge of the biology of PIPKs in a variety of in vitro and in vivo models will facilitate the development of effective approaches for therapeutic intervention with the potential to translate into meaningful clinical benefits for patients suffering from cancer, immunological and infectious diseases, and neurodegenerative disorders.

Keywords

Phosphoinositides; Phosphatidylinositol phosphate kinase; PI4P5K (PIP5K); PI5P4K (PIP4K); PIKfyve; Mechanisms of disease

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Conflict of Interest

The authors declare no conflict of interest.

1 Introduction

Phosphoinositides are low-abundant lipid signaling molecules that govern key cellular processes including membrane trafficking, cytoskeletal rearrangement, and cell signaling to regulate cell growth, metabolism, survival, and proliferation [1, 2]. Phosphoinositide levels are tightly regulated by several kinases, phosphatases, and lipases, and dysregulation of these enzymes is linked to multiple diseases, including immune and developmental disorders, inflammatory diseases, and cancer [2].

All phosphoinositides derive from the addition or removal of phosphate groups to the inositol ring of phosphatidylinositol (PI) [1]. PI contains two distinct modules: a polar inositol head bound to glycerol and two non-polar fatty acid tails. The inositol ring can be phosphorylated at positions 3, 4, and 5 to generate seven phosphoinositide species: single phosphorylated PI(3)P, PI(4)P, and PI(5)P; double phosphorylated PI(3,4)P_2, PI(3,5)P_2, and PI(4,5)P_2; and triple phosphorylated PI(3,4,5)P_3 [3]. Dynamic interconversion of all these phosphoinositide species is regulated by specific lipid kinases and phosphatases to maintain the structural and functional integrity of the cell and to allow efficient adjustments to the changing cellular requirements.

The PI phosphate kinase (PIPK) family uses PI monophosphates to generate respective PI bisphosphate (PIP_2) products [2]. The PIPK family is divided into three major subfamilies: Type I PIPKs or phosphatidylinositol-4-phosphate 5-kinases (PI4P5Ks / PIP5Ks), Type II PIPKs or phosphatidylinositol-5-phosphate 4-kinases (PI5P4Ks / PIP4Ks), and Type III PIPKs or phosphatidylinositol-3-phosphate 5-kinase (PIKfyve / PIPKIII). Each subfamily phosphorylates specific hydroxyl groups of PI monophosphates but has different substrate specificity. Type I PIPKs phosphorylate PI(4)P to PI(4,5)P_2, Type II PIPKs catalyze the phosphorylation of PI(5)P to PI(4,5)P_2, and Type III PIPKs primarily generate PI(3,5)P_2 from PI(3)P but can also phosphorylate PI to produce PI(5)P [2, 4, 5] (Figure 1). Even though Type I and Type II PIP kinases generate the same lipid product, their functions are very different [6]. All members have a highly conserved catalytic kinase domain but have sequence variability in the activation loop region at the C-terminus, which is responsible for substrate specificity [7]. This review aims to give an overview of the knowledge we have today on the three PIPK subfamilies.

1.1. Type I: Phosphatidylinositol-4-phosphate 5- kinases (PI4P5Ks)

1.1.1. Substrate and product—PI4P5Ks are responsible for generating PI(4,5)P_2 by phosphorylating PI(4)P at the 5th position of the inositol ring. Phosphorylation of PI(4)P by Type I PIPKs is the major route for PI(4,5)P_2 production as opposed to the phosphorylation of PI(5)P by Type II PIPKs. Evidence for this comes from pulse-labeling studies with [^{32}P] orthophosphate, which showed a higher labeling rate at the 5th position of the inositol ring as compared to the 4th position [8, 9]. Further reasoning comes from studies demonstrating that PI(5)P levels within cells are extremely low (~2% of total PI monophosphates), about 10-fold less than PI(4)P, making Type I-dependent activity the major synthetic pathway for PI(4,5)P_2 [10]. It is the localization of Type I kinases as well as the activation loop sequence which dictates the substrate specificity, since specific amino acid changes in the activation loop of PI4P5Ks to the corresponding amino acids in PI5P4Ks alters the substrate specificity

from PI(4)P to PI(5)P [6, 11]. PI4P5Ks can additionally phosphorylate PI(3,4)P_2 at the 5th position to generate PI(3,4,5)P_3 *in vivo* [12]. This ability has also been demonstrated for Its3, the *Schizosaccharomyces pombe* homolog of PI4P5K [13]. The physiological significance of the other products possibly generated by Type I kinases is mostly unknown.

1.1.2. PI4P5Ks location and structure—There are three isoforms of Type I PIPKs (designated as PI4P5Kα, PI4P5Kβ, and PI4P5Kγ, which are encoded by the genes *PIP5K1A, PIP5K1B*, and *PIP5K1C*, respectively; however, historically the nomenclature for murine and human α and β isoforms are reversed) with multiple alternative splice variants [14, 15]. The structural details for Type I PIPKs have been discussed in detail in several reviews [16]. The isoforms are widely expressed and have been shown to have distinct localizations as well as functions to ensure the generation of optimal, localized, and functionally distinctive pools of PI(4,5)P_2 [15, 17, 18]. The isoforms are thus subjected to extensive feedback regulations. PI4P5Ks are located primarily on the plasma membrane but have also been shown to be present in the nucleus, perinuclear region on intracellular organelles such as endosomes and Golgi [17, 19] where they generate the multifunctional lipid PI(4,5)P_2 [1, 20].

1.1.3. PI4P5Ks functions—The product of PI4P5Ks, PI(4,5)P_2, is central to cellular functioning since it controls fundamental processes such as vesicular transport, membrane dynamics, actin cytoskeleton remodeling, cell cycle regulation [21–23] and therefore impaired homeostasis results in neurological diseases, metabolic disorders, ciliopathies and cancer [20, 24]. PI(4,5)P_2 also acts as a substrate for Phospholipase C (PLC) signaling and for PI3K to generate PI(3,4,5)P_3 [25]. Both of these pathways trigger major signaling alterations in cells, with critical implications in carcinogenesis. Further, pathogens are capable of hijacking PI(4,5)P_2 to allow entry as well as replication [26]. These details have been described in several reviews [15, 27, 28].

1.1.4. Regulation of PI4P5Ks—There have also been extensive studies on understanding the regulation of Type I kinases [15, 29], where it is mostly evident that these kinases are regulated by the Rho family of GTPases [30] such as Rho [31], Rac1 [32] and Cdc42 as well as ARF GTPases [33]. Arf6 and Rho have been proposed to regulate PI4P5Ks to maintain high levels of PI(4,5)P_2 at the cleavage furrow for efficient cytokinesis in mammalian cells [22]. Further, phosphatidic acid, generated by Phospholipase D has been shown to stimulate Type I PIP kinase isoforms [34, 35]. Several studies have also indicated a phosphorylation-mediated alteration in the activity of PI4P5Ks. These details are described in detail in several reviews [15, 36].

Interestingly, recent studies have highlighted a novel negative regulation of the Type I kinases through the formation of a complex with the Type II kinases [37, 38] to maintain PI(4,5)P_2 levels because even excess of the lipid can be detrimental to cellular survival.

1.1.5 PI4P5Ks and pathology—Given the role of Type I PIP kinases in cytoskeletal functions and in the generation of plasma membrane PI(4,5)P_2, which is a substrate for PI3K-based signaling, it is not surprising that PI4P5Ks have relevance in human diseases.

Cancer: Higher expression of PI4P5Kα in both prostate and triple-negative breast cancers correlates with poor patient outcome [39, 40] and knockdown of PI4P5Kα in prostate and breast cancer cells inhibited tumor growth through reduced AKT signaling. Further, a positive correlation between PI4P5Kα and AR expression was observed in prostate cancer biopsy samples and compared with primary tumors, metastatic lesions were found to express significantly higher levels of *PIP5K1A* and *AR* mRNA. In both studies, treatment of the cancer cells with a PI4P5Kα inhibitor, ISA-2011B, had the same effect on signaling and tumorigenesis [39, 40]. PI4P5Kα has recently been shown to interact directly with p53 in the nucleus [41] and with oncogenic KRAS [42], providing a rationale for a potentially stronger and personalized therapeutic benefit of PI4P5K inhibitors in cancers with *KRAS* or *TP53* mutations. Indeed, downregulation of PI4P5Kα reduced the viability of *KRAS*-mutant PDAC cell lines. PI4P5Kα downregulation by protein degradation by NEDD4, a ubiquitin ligase, resulted in impairment of proliferation of breast cancer cells through PI3K-Akt pathway [43]. Ubiquitination-mediated regulation of protein expression has also been demonstrated for PI4P5Kγ, where Smurf1 was shown to directly interact with and degrade PI4P5Kγ, with implications in lung cancer, providing a rationale for the development of PROTAC-based PI4P5K inhibitors. PI4P5Kγ knockdown decreased the proliferation of lung cancer cells and also repressed their ability to form tumors and the signaling for this was driven through the Wnt/β-catenin pathway [44]. PI4P5Kγ has been implicated in breast cancer [45] where PI4P5Kγ along with talin [46] regulates epithelial to mesenchymal transition and can promote malignancy again through the Wnt/β-catenin pathway. Further, PI4P5Kγ has also been implicated in the invasiveness of colorectal cancer due to its role in focal adhesion assembly and disassembly as well as in the facilitation of Warburg effect [47] and pharmacological inhibition with the inhibitor UNC3230 targeting PI4P5Kγ also disrupted glycolysis and tumorigenesis. Besides ubiquitination, phosphorylation modifications of PI4P5Kγ by EGFR [45, 48] and CDK5 [49] also influence tumor formation and metastasis.

Immunological diseases: $PI(4,5)P_2$ metabolism is being increasingly linked to immunological diseases due to its role in cytoskeletal dynamics including the organization of the immunological synapse, and due to it being a precursor for second messengers and a substrate for PI3K, and the downstream effects of both these pathways in the cellular immune response [50–52]. Each of the three isoforms of PI4P5K is expressed in the T lymphocytes [36, 53, 54]. PI4P5Kα has been demonstrated to be the predominant isoform to be recruited by CD28 [53] and it regulates both CD28 costimulatory as well as CD28 autonomous signals and use of PI4P5Kα inhibitor, ISA-2011B, significantly impairs both the signals, resulting in disruption of TCR-stimulated Ca^{2+} influx, NF-AT transcriptional activity, gene expression of cytokines and NF-κB activation [55]. NF-κB activation was also disrupted with PI4P5Kα knockdown in ganglioside-stimulated astrocytes indicating a potential role of PI4P5K in the immune response under pathologic neurological conditions also [56]. Metabolic disorders such as diabetes are also inflammatory conditions and ISA-2011B had promising anti-inflammatory effects in type 1 diabetes patients suggesting PI4P5Kα could be a promising anti-inflammatory target [55].

Host-pathogen Interaction: The α and γ isoforms of PI4P5K, and its product PI(4,5)P_2 are crucial for the HIV cycle, specifically the entry phase and for the targeting of $Pr55^{Gag}$ at the plasma membrane for HIV assembly [57]. The involvement of Type I PIP kinases in controlling the pathogen's cycle comes from their ability to generate plasma membrane PI(4,5)P_2 [26] and to alter cytoskeletal dynamics.

1.1.6. Genetic models of targeting PI4P5Ks and their phenotypes—

Budding yeast expresses only one type I PIP kinase, Mss4, without which cells are not viable and inactivation of Mss4 results in altered cellular morphology and phenocopies defects seen in mutants of actin-binding proteins [58, 59]. Plants also express multiple copies of a protein showing similarity to PI4P5K [60]. However, there are distinct structural differences between the yeast and plant homologs when compared to the vertebrates signifying organism-specific variations in cellular functions. Fission yeast also expresses a single homolog of PI4P5K, Its3, and similar to its budding yeast homolog Mss4, mutations result in actin-associated aberrations in cellular structural integrity [61, 62]. Functionally, Its3 has been shown to also generate phosphatidylinositol 3,4,5-trisphosphate, and considering the absence of a class-I PI3K in fission yeast, the production of PI(3,4,5)P_3 by PI4P5K signifies the early evolution for PI(3,4,5)P_3 synthesis.

Caenorhabditis elegans expresses a single homolog of the Type I PIP kinase referred to as *ppk-1*. Downregulation of *ppk-1* in *C.elegans* resulted in impairment of ovulation and sterility due to defects in cytoskeletal organization in the somatic gonad [63]. The *C. elegans* model also helped in demonstrating the involvement of *ppk-1* in the nervous system and specifically in the formation of growth cones suggesting the importance of optimal PI(4,5)P_2 levels in preventing neurodegeneration [64]. The functional significance of Type I PIP kinase in fertility, as well as brain function through effects on cytoskeletal organization, is evident also from studies in mouse models of PI4P5Ks [65, 66]. There are two genes in the *Drosophila* genome that encode PI4P5K, *sktl* [67] and *dPIP5K* [68]. *sktl* is required for cellular and organismal viability as well as germline development and has been demonstrated to be involved in cytoskeletal-associated processes such as vesicle trafficking, apical polarity [69], and wound healing [70]. *dPIP5K* is required for regulating PI(4,5)P_2 dynamics in photoreceptors cell for phototransduction in *Drosophila* [68].

Among the mouse models for PI4P5Ks, the most striking phenotype was observed in mice lacking *Pip5k1c*, which die soon after birth due to neuronal defects [71], whereas mice lacking *Pip5k1a* or *Pip5k1b* (based on the nomenclature of human PI4P5Ks) continued to adulthood, only with certain specific cell type phenotypes [65]. In fact, a single allele of *Pip5k1c*, in the absence of both *Pip4k1a* and *Pip4k2b*, resulted in survival to adulthood [65]. On the other hand, the inactivation of PI4P5Kγ by the gene-trap method resulted in embryonic lethality with neural tube closure defects consistent with results showing that PI4P5Kγ isoform is the major contributor of PI(4,5)P_2 in the brain. Inhibition of PI4P5Kγ using in utero electroporation also revealed its role in the neuronal migration [72]. The relevance in brain function is also seen in humans since disruption of the kinase activity of PI4P5Kγ by homozygous (D253N) mutation results in perinatal lethality associated with spinal defects, referred to as Lethal congenital contractural syndrome (LCCS) [73]. In a

recent study, inhibition of *Pip5k1c* in the mesenchymal stem cells was seen to induce osteopenia in adult mice [74] (Figure 2).

1.1.7. Therapeutic targeting of PI4P5Ks—There has been development of inhibitors to target PI4P5Ks and the details of the inhibitors have been described in the recent review [75]. Briefly, as discussed above ISA-2011B [39] and UNC3230 [76] are compounds that have been shown to target PI4P5Kα and PI4P5Kγ respectively, with studies showing inhibitory effects on the proliferation of multiple cancer models [39, 40, 47]. ISA-2011B demonstrated similar potency to the chemotherapeutic docetaxel at stimulating tumor regression, however, the combination therapy significantly reduced the toxic effects of docetaxel [39], indicating a potential for possible combination therapies with existing drugs.

Moreover, a combination therapy of enzalutamide with ISA-2011B has also been proposed for prostate cancer to overcome resistance difficulties faced with antiandrogen therapies [77] and another combination therapy of tamoxifen and ISA-2011B resulted in increased tumor regression in castration-resistant prostate cancer [78]. Recently, a pan-isoform PI4P5K selective inhibitor was discovered using a high throughput screen of the AstraZeneca collection and this study also provides the opportunity to continue the development of potent and selective inhibitors since there have always been issues of having off-target effects on other lipid kinases with drugs targeting PI4P5Ks [79]. Besides the need for these inhibitors to be more specific, more studies are required to determine the pharmacokinetics and toxicity profiles *in vivo* so that they could make a quick transition to the clinics. The above studies, along with the potential to effectively target KRAS-mutant cancers, highlight that PI4P5Ks could be a promising therapeutic target for multiple cancer types and potentially other disease conditions (Figure 2) and thus requires increased attention from drug discovery and development point of view.

1.2. Type II: Phosphatidylinositol 5-phosphate 4- kinases (PI5P4Ks)

1.2.1. Substrate and product—PI5P4Ks generate PI(4,5)P_2 by phosphorylating the 4-position of PI(5)P [10]. The majority of PI(4,5)P_2 is generated by type I PIPK by phosphorylation of PI(4)P, which is far more abundant than PI(5)P. Thus, it is generally assumed that the main function of PI5P4Ks is to reduce the amount of cellular PI(5)P or to generate a minor pool of PI(4,5)P_2 at specific cellular locations [80–82].

1.2.2. PI5P4Ks location and structure—This family of kinases includes three different isoforms α, β, and γ, encoded by the genes *PIP4K2A*, *PIP4K2B*, and *PIP4K2C*, respectively. PI5P4Kα is the most active of the three, being considerably more active than PI5P4Kβ and especially than PI5P4Kγ, which has little catalytic activity [82, 83]. PI5P4Ks are largely located in intracellular membranes. PI5P4Kα localizes to autophagosomes, lysosomes, and peroxisomes; PI5P4Kβ is predominantly located in the nucleus but can also be found in autophagosomes, and PI5P4Kγ is present in autophagosomes, Golgi, and endomembrane compartments [84–88]. The three PI5P4K isoforms have been reported to homodimerize or heterodimerize with each other, which can influence their localization [84, 89, 90]. PI5P4Ks consist of a dimerization domain and a lipid kinase domain with variable N- and C-terminal lobes that mediate specific functions for each isoform [16]. PI5P4Kα

and PI5P4Kβ are more homologous to each other than either are to PI5P4Kγ. Although the three PI5P4K isoforms share significant sequence similarity, there are certain structural differences that account for their distinct enzymatic activities, such as their ATP affinity [90]. Another major difference is the glycinerich loop, which is supposed to interact with the substrate [83].

1.2.3. PI5P4Ks functions—PI5P4Ks play a role in a variety of essential cellular processes by facilitating the recruitment and allosteric activation of proteins at specific intracellular locations, modulating integral membrane protein signaling, and regulating lipid transport. It is worth noting that besides PI phosphorylation, PI5P4Ks have been reported to perform catalytic-independent functions [37, 91–94].

PI5P4Ks regulate insulin signaling and autophagy and are key for surviving metabolic stress by modulating PI3K/Akt/mTORC pathways [37, 85, 91, 95–99]. It has been shown that the Mesencephalic Astrocyte-derived Neurotrophic Factor (MANF) increases the localization of PI5P4Kβ in the endoplasmic reticulum to regulate insulin signaling [100]. Additionally, evidence has demonstrated that loss of PI5P4Ks results in an increase in $PI(4,5)P_2$ levels, which serve as a substrate for insulin-stimulated production of $PI(3,4,5)P_3$ by PI3K [37, 91]. Silencing of the three PI5P4K isoforms and the subsequent changes in PI(5)P levels result in an increase in autophagosome formation [87]. Consistently, loss of PI5P4Kα and PI5P4Kβ limits the ability to metabolize lipid droplets and leads to an accumulation of autophagic vesicles. This autophagy defect results in a reduction of nutrient supply, which impairs mTORC1 activation and triggers the activation of the transcription factor EB (TFEB), responsible for the expression of lysosomal and autophagic genes [85]. Recent data has shown that PI5P4Kα can interact with the long noncoding RNA lncSAMD11–1:1 in human endometrial stromal cells, which results in the inhibition of Akt phosphorylation and stabilization of the nuclear localization of FoxO1 to promote endometrial decidualization and successful pregnancy [101]. PI5P4Ks also regulate oxidative stress. PI5P4Kα overexpression reduces the expression of NRF2 target genes by H_2O_2, decreases the rate of reactive oxygen species (ROS) accumulation, and reduces resistance to oxidative stress [102]. Interestingly, it has been shown that induction of oxidative stress mitigates PI5P4Kα and PI5P4Kβ activity [80, 103].

Type II PIP kinases can bind to both ATP and GTP. In particular, the PI5P4Kβ isoform has a strong preference for GTP over ATP and its activity changes as GTP levels change, working as an intracellular GTP sensor [104]. It has been demonstrated that the ability of PI5P4Kβ to couple changes in GTP into changes in PI(5)P levels influences tumor growth. The preference of PI5P4Kβ for GTP over ATP was evolutionarily acquired by the generation of the guanine efficient association (GEA) motif, which uses its main chain atoms for adenine recognition and the side chain atoms for guanine recognition [105].

Cellular trafficking is also regulated by these lipid kinases. The activity of these enzymes is required for the crosstalk between peroxisomes and mitochondria. Loss of PI5P4Kα and PI5P4Kβ leads to changes in the peroxisomal $PI(4,5)P_2$ pool, which impairs the trafficking of very long fatty acids to peroxisomes and results in mitochondrial dysfunction [106]. PI5P4Kα regulates intracellular cholesterol transport from lysosomes to peroxisomes by

maintaining PI(4,5)P_2 homeostasis on peroxisomes [107]. Moreover, PI5P4Kγ regulates the Notch pathway by controlling the transit of the receptor through recycling endosomes [108]. In *Drosophila*, the PI5P4Ks homolog dPIP4K regulates the trafficking of secretory granule proteins [109]. Similarly, dPIP4K regulates clathrin-mediated endocytosis by controlling the localization of rhodopsin 1 [94].

Evidence supports a role for PI5P4Ks in immune modulation. The lack of PI5P4Kγ in mice results in high levels of proinflammatory cytokines, an increase in T-helper cells, and a decrease in regulatory T-cells [110]. This enhanced immune response results from mTORC1 signaling hyperactivation upon *Pip4k2c* deletion and can be reduced after rapamycin treatment. Interestingly, various studies have described *PIP4K2C* single nucleotide polymorphisms (SNPs) associated with different autoimmune diseases [111, 112]. In addition, a recent study has shown that PI5P4Kβ and PI5P4Kγ regulate the immunosuppressive activity of regulatory T-cells by regulating PI3K, mTORC1, and MAPK signaling pathways and controlling the expression of the transcriptional regulator FOXP3 [113].

PI(5)P in the nucleus, regulated by PI5P4Ks, interacts with chromatin-associated proteins and regulates gene transcription [82]. For instance, PI(5)P can induce a conformational change in UHRF1 that mediates binding to H3K9me3, and can interact with ING2, which results in the repression of ING2 target genes [114, 115]. Consistently, overexpression of PI5P4Kβ results in the release of ING2 from chromatin [116]. Furthermore, PI5P4Kβ influences TAF3 association with H3K4me3 and gene expression during myoblast differentiation [117].

1.2.4. Regulation of PI5P4Ks—Although delineating the upstream regulation of PI5P4Ks requires further investigation, some studies suggest that these enzymes are negatively regulated by phosphorylation. Protein kinase D (PKD) phosphorylates PI5P4Kα at Thr^{376}, located within the activation loop, and reduces its enzymatic activity [118]. Under stress conditions, PI5P4Kβ activity can be inhibited through direct phosphorylation at Ser^{326} by the p38 stress-activated protein kinase [80]. mTORC1 phosphorylates PI5P4Kγ at Ser^{324} and Ser^{328} depending on nutrient availability, which results in a decrease in the kinase functions. The protein kinase CK2 phosphorylates PI5P4Kα at Ser^{304}, and mutation of this residue to aspartate to mimic phosphorylation leads to a redistribution from the cytoplasm to the plasma membrane [119]. Besides phosphorylation, the activity of PI5P4Ks might be regulated by interaction with other proteins. For instance, the interaction of PI5P4Kα and PI5P4Kβ with the proline isomerase Pin1 reduces the activity of the kinases *in vitro* [102]. Moreover, PI5P4Kβ can interact with the ubiquitin ligase complex Cul3-SPOP, which mediates its ubiquitylation [120].

1.2.5. PI5P4Ks and pathology—PIs are crucial signaling molecules involved in a variety of essential cellular processes and therefore it is imperative to maintain a tight balance between the enzymes that regulate the cellular pool of PIs. Alterations in PI5P4Ks have been linked to several pathological processes including cancer, psychiatric disorders, and infectious diseases.

Cancer: The expression of the three PI5P4Ks is altered in different cancer types such as breast cancer, leukemias, soft tissue sarcomas, and glioblastomas [121]. PI5P4Kα and PI5P4Kβ are overexpressed in breast tumors and their depletion results in impaired tumor growth, selectively in p53-deficient tumors [122]. Depletion of the kinases is linked to increased AKT phosphorylation, oxygen consumption and ROS, and reduced glucose metabolism. Therefore, under oxidative stress (i.e., p53 deficiency), PI5P4Ks are required for cancer cell survival. Along these lines, downregulation of PI5P4Kα and PI5P4Kβ has also been shown to impair cell proliferation in multiple triple-negative breast cancer models *in vitro* [106]. A different study showed that both high and low PI5P4Kβ expression compared with intermediate expression levels correlate with poor patient survival [123]. Moreover, high throughput proteomics of breast cancer interstitial fluid identified PI5P4Kβ among a panel of 10 biomarkers for stratification of breast cancer subtypes [124]. PI5P4Ks are also involved in the pathogenesis of hematological malignancies including acute myeloid leukemia (AML) and acute lymphocytic leukemia (ALL). PI5P4Kα is essential for the proliferation and survival of AML cells but not for primary normal hematopoietic stem and progenitor cells [125]. Similarly, PI5P4Kα is overexpressed in multiple types of leukemia and its expression is significantly associated with unfavorable clinical outcomes [126]. High PI5P4Kα and PI5P4Kγ expression levels are associated with unfavorable cytogenetic risk and correlate with worse survival outcomes in AML patients [127]. Additionally, genome-wide association studies (GWAS) in multiple patient cohorts have identified *PIP4K2A* SNPs associated with ALL susceptibility through inducing PI5P4Kα overexpression [126, 128–131]. Although most studies point towards oncogenic roles for PI5P4Ks, *in vivo* RNAi screening of glioblastoma patient-derived xenografts identified PI5P4Kα as a putative tumor suppressor [92]. The authors report that in *PTEN*-deficient glioblastomas, PI5P4Kα inhibits PI3K signaling by inducing the degradation of the p85 regulatory subunit of PI3K.

Neurodegenerative / Psychiatric disorders: Both PI5P4Kα and PI5P4Kβ are present in axon terminals and dendritic spines, suggesting a role in synaptic vesicle trafficking [132]. Silencing or inhibition of PI5P4Kγ reduces the levels of mutant huntingtin protein in fibroblasts, clears aggregates in neurons, and ameliorates neuronal dysfunction and degeneration in *Drosophila* [133]. Multiple GWAS studies have revealed that genetic variants of *PIP4K2A* are associated with an increased predisposition to schizophrenia [134–137] and with a poor antipsychotic response [138]. In addition, genetic variants of *PIP4K2A* have been associated with antidepressant treatment response, time to recurrence of depressive and manic/mixed episodes, and depression severity among patients with depression and bipolar disorder [139].

Infectious diseases: PI metabolism plays a key role in the life cycles of several infectious agents by favoring viral replication and assembly [140]. Furthermore, it has been shown that PI5P4Kα is imported from red blood cells into *Plasmodium falciparum, Plasmodium berghei*, and *Toxoplasma gondii*, where it associates with specific parasite RNAs [93, 141]. The RNA binding activity of PI5P4Kα is conserved across species from *Drosophila* and *C. elegans* to humans, suggesting that this kinase is also important for posttranscriptional gene regulation [93].

1.2.6. Genetic models of targeting PI5P4Ks and their phenotypes—PPK-2 (PI5P4K ortholog) deficient *C. elegans* have reduced lifespan and a decrease in lipid storage, show autophagy defects, and are more sensitive to metabolic stress [85]. Deletion of PIP4K in *Drosophila* leads to a decrease in mTORC1 signaling, lower body weight, and a shortening of larval development [96]. In mice, homozygous germline deletion of *Pip4k2a* results in normal embryonic development [122]. *Pip4k2b* knockout mice are viable but show a mild reduction in growth rates, decreased fat content, and are hypersensitive to insulin, which protects them from obesity, insulin resistance, and type 2 diabetes when exposed to a high-fat diet [142]. Mice lacking *Pip4k2c* are normal regarding growth and viability, but they show hyperactivation of the immune system. These mice have increased mTORC1 activation in multiple tissues, which results in a higher expression of proinflammatory cytokines, increased immune cell infiltrates, increased T-helper cell populations, and decreased regulatory T-cell populations [110]. Strikingly, germline deletion of *Pip4k2b* in mice lacking *Trp53* results in early embryonic lethality, with pups dying within 10–12h after birth [122]. It is important to mention that deletion of *Pip4k2a in* $Pip4k2b^{+/-}$ $Trp53^{-/-}$ mice lead to viable mice with a dramatic reduction in tumor formation compared to littermates $Trp53^{-/-}$ and wild-type for the kinases, uncovering a critical role for PI5P4Ks in maintaining glucose metabolism and ROS homeostasis in the context of p53 loss. Further, deletion of the kinases in a sarcoma mouse model ($Kras^{LSL\text{-}G12D/+}$ $Trp53^{flx/flx}$ $Pip4k2a^{flx/flx}$ $Pip4k2b^{-/-}$) also impaired tumor formation [106]. Mice with liver-specific deletion of *Pip4k2a* and germline deletion of *Pip4k2b* show a deficiency in the ability to metabolize lipid droplets in the liver upon fasting, indicating an autophagy defect [85] (Figure 2).

1.2.7. Therapeutic targeting of PI5P4Ks—Multiple studies have demonstrated the potential of PI5P4Ks to become effective therapeutic targets for the treatment of metabolic and psychiatric disorders, infectious diseases, and cancer. The isoform-specific and multi-isoform PI5P4K inhibitors developed to date have been thoroughly discussed in [75].

Briefly, isoform-specific inhibitors include the ATP-competitive inhibitor of PI5P4Kα I-OMe Tyrphostin AG-538 [143], the pyrimidine-2,4-diamine SAR088m which specifically inhibits PI5P4Kβ [144], and the specific inhibitors for PI5P4Kγ NCT-504 and NIH-12848 [133, 145]. Efforts to improve the physicochemical properties of NIH-12848 have led to the development of “compound 40” [146]. More recently, two potent and highly selective PI5P4Kα inhibitors, named BAY-091 and BAY-297 were identified using high throughput screening and subsequent structure-based optimization. However, although cellular target engagement was demonstrated, inhibition of PI5P4Kα with these compounds did not inhibit proliferation in p53-deficient cancer cells [147].

Inhibitors that target more than one PI5P4K isoform include a131, which selectively kills cancer cells but not normal cells [148]. THZ-P1–2 is also a potent and reasonably selective pan-PI5P4K covalent inhibitor that showed modest anti-proliferative activity and compromised mitochondrial homeostasis and autophagy in a panel of leukemia cell lines [149, 150]. Optimization of this molecule lead to the development of a compound with improved selectivity and potent biochemical and cellular activity [151]. CC260 is a highly potent and selective noncovalent dual inhibitor for both PI5P4Kα and PI5P4Kβ which

incorporates a hydrophobic side chain that occupies a pocket unique to the lipid kinase family [152]. Inhibition of the kinases with this compound leads to disruption of cellular energy homeostasis, AMPK activation, and mTORC1 inhibition. This induced energy stress was selectively toxic for p53-null tumor cells. However, this compound showed some off-target activity against PI3K-δ.

1.3. Type 3: phosphatidylinositol-3-phosphate 5-kinase / PIKfyve

1.3.1. Substrate and product—Type III PIPK is comprised of a single member: phosphatidylinositol-3-phosphate 5-kinase type III or PIKfyve [153]. PIKfyve primarily functions in biosynthesis of PI(3,5)P_2 from the substrate PI(3)P via phosphorylation of the 5th position of the PI(3)P inositol ring, however it has also been demonstrated to generate PI(5)P through direct and indirect processes [4]. The alteration of lipid combination from PI(3)P to PI(3,5)P_2 is thought to drive vesicle maturation, regulating cellular endocytosis (reviewed in:[154]).

1.3.2. PIKfyve location and structure—In the cell, PIKfyve is found throughout the endosomal compartments, and colocalizes with early and late endosomal markers, including EEA1, along with endo-lysosomal markers and the retromer complex [155]. PIKfyve functions as a multidomain protein complex with the scaffolding protein VAC14 and lipid phosphatase Fig4, catalyzing the reverse reaction of PI(3,5)P_2 to PI(3)P [153], for which structure and individual reactions have been thoroughly discussed [154, 156]. Interestingly, while interactions contribute to both PIKfyve autophosphorylation and lipid kinase activity, members of this complex have been shown to contribute to the vacuolization defect phenotype associated with PIKfyve inhibition [157]. The PIKfyve protein itself is a large 240kDa protein and contains three generally recognized domains including the FYVE, GroEL-like chaperonin, and lipid kinase domains [158]. Several isoforms of PIKfyve lacking the lipid kinase domain have been suggested by deep sequencing analyses but have not been experimentally verified [159]. The FYVE zinc finger domain, named for its association with the proteins Fab1, YOTB, Vac1, and EEA1, exhibits high specificity to PI(3)P lipids and is associated with endosomal trafficking [160]. The kinase domain of PIKfyve has been suggested to exhibit dual specificity as both a lipid kinase and protein kinase [161], and highlighted in a recent review by Hayakawa *et. al.*[160], the structure of which has yet to be solved despite shared homology with PI kinases. Combined, the lack of mechanistic understanding and characterization of PIKfyve and its proteincomplex highlights a need for further research in this area.

1.3.3. PIKfyve functions—At the cellular level, PIKfyve is associated with numerous cell functions including signaling, membrane homeostasis, and migration through its regulatory function in vesicle trafficking. PIKfyve is best known as a regulator of cellular membrane homeostasis and vesicular membrane trafficking, including autophagy, macropinocytosis, and receptor-mediated endocytosis [4]. This endocytic association is supported by evidence suggesting that PIKfyve regulates early endosomes in simian-derived fibroblasts [162] and phagosome maturation in macrophages [163]. Numerous studies using genetic and pharmacological inhibitors have also identified PIKfyve to be essential for lysosome function and acidification in neurons, fibroblasts, and cancer cells, further

complicating its role in vesicle compartments [164–167]. Early studies reported that PIKfyve/Fab1p possessed protein kinase and autophosphorylation activities in addition to its lipid kinase activities [161]. These observations support evidence suggesting that protein kinase activity [168] and signaling effects [169] by PIKfyve are involved in vesicle trafficking, though these aspects have not been thoroughly investigated. Despite recent advancements, full understanding of the pleiotropic functions of PIKfyve remains to be explored despite its fundamental role in cell biology and clinical relevance.

1.3.4. Regulation of PIKfyve—PIKfyve has been found to be positively regulated by the class III PI3 lipid kinase (PIKC3 or Vps34), which produces the lipid PI(3)P, but can also be activated by cell stress responses via phosphorylation, increasing intracellular PI(3,5)P_2 pools [155, 170]. These regulatory processes may in part explain the mechanism in which PIKfyve can regulate dynamic cellular processes such as macropinocytosis, autophagy, phagocytosis, and lysosomal function [165, 166].

1.3.5. PIKfyve and pathology—Recent translational interest of this lipid kinase has been spurred by the identification of PIKFyve as the target of the small molecule inhibitors apilimod and YM201636, implicating PIKFyve in several disease phenotypes. Alternatively, Loss-of-function PIKfyve mutations have been associated with development of Fleck Corneal Dystrophy, which is characterized by small white flecks, consisting of vacuolized keratocytes that are found in the corneal stroma of patients [171], and congenital cataracts [172].

Infectious diseases: To date, PIKfyve, through pharmacological inhibition, has been identified as a potential antiviral against SARS-CoV-2, Ebola, and Malburg viruses, among others [173–178]. Multiple publications have suggested that PIKfyve inhibition viral entry into endocytic vesicles and cellular cytoplasm [173, 176]. Complementary evidence has suggested that viral infection is prevented by blocking endolysosome fusion upon PIKfyve inhibition [175].

Neurodegenerative disorders: PIKfyve has also been demonstrated to be a potential therapeutic target in the tauopathies, such as Alzheimer's disease, and neurodegenerative disorders reviewed in: [178–180]. Evidence from studies in taupathies suggests that PIKfyve inhibition impairs the trafficking of proteins into lysosomes [181].

Cancer: Targeting PIKfyve exhibits therapeutic efficacy in both preclinical and clinical studies in a variety of cancer types, including non-Hodgkin's B cell lymphoma, small cell lung cancer, and liver cancer, among others (reviewed in [182]. Studies using pharmacological PIKfyve inhibitors have associated this lipid kinase with transcription factor EB (TFEB) [183] and epidermal growth factor receptor (EGFR) [184] signaling involved in tumor progression. Expectedly, PIKfyve has also been shown to be essential in endocytic pathways which contribute to tumor progression and aggressiveness including autophagy [167] and macropinocytosis [166].

The role of PIKfyve in these pathologies has been extensively highlighted and will not be discussed in depth in this review.

1.3.6. Genetic models of targeting PIKfyve and their phenotypes—Type III PIPK function was originally identified in the ortholog formation of aploid and binucleate cells protein (Fab1p) in *S. cerevisiae* where loss-of-function mutations resulted in the development of cytoplasmic vacuoles and defects in nuclear division [185]. PIKfyve/Fab1p was also found to be functionally conserved across eukaryotes and is thought to be the only enzyme to produce intracellular $PI(3,5)P_2$ [156]. In mammals, the PIKfyve protein was originally known as p235 which was shown to regulate actin fiber length in platelets [186] prior to lipid kinase identification by domain homology and verification of $PI(3,5)P_2$ synthesis [4]. Parallel studies of PIKfyve orthologs including the phosphatidylinositol phosphate kinase-3 (PPK-3) in *C. elegans* [187] and fab1 in *D. melanogaster* [188] have contributed to the mechanistic understanding of PIKfyve function and its regulation at the cellular and physiological level. Given the variety of orthologs, often referenced interchangeably, PIKfyve is referred to as multiple aliases including: 1-phosphatidylinositol 3-phosphate 5-kinase, Fab1, or type III PIPK.

Mounting evidence using *in vivo* models strongly supports the integral role of PIKfyve expression at the organismal level despite incomplete understanding of its cellular mechanisms. PIKfyve expression has also been shown to be essential for embryonic survival and development in genetic knockout studies of *D. melanogaster* and mouse models [187–189]. Interestingly, heterozygous expression was shown to be sufficient for normal physiological function in mouse models, suggesting compensatory processes [189]. In the nervous system, PIKfyve and $PI(3,5)P_2$ synthesis have been shown to be necessary for normal function through regulation of synaptic signaling [190]. Additionally, secondary systemic effects from tissue-specific knockout studies in the intestine and macrophage populations suggest that PIKfyve expression is not only important to tissues but also physiological homeostasis [191, 192] (Figure 2).

1.3.7. Therapeutic targeting of PIKfyve—Pharmacological inhibition of PIKfyve occurs through targeting of the kinase domain [169] and is generally observed to induce vesicle trafficking and endosomal defects, leading to the formation of characteristic cytoplasmic vacuoles. This phenomenon is observed in tauopathies, where PIKfyve inhibition by YM201636 reduced the aggregation of tau protein, a pathologic signature of multiple neurodegenerative diseases, where lysosomal trafficking is prevented [181]. Similarly, it is the inhibition of lysosome function by apilimod treatment that prevents viral infection of SARS-CoV-2 and ebola virus [173], and induces anti-cancer effects in Ras-driven cells and B-cell lymphoma [166] [193]. In other examples, PIKfyve targeting results in the inhibition and dysregulation of autophagy, in both prostate and liver cancers [167, 184]. Several of these preclinical studies have progressed to clinical trials of apilimod with varied effectiveness along with numerous confounding factors including low plasma concentration [173, 194]. Novel inhibitory compounds targeting PIKfyve are currently being developed and tested to overcome limited clinical effectiveness. Despite these results, PIKfyve is still considered to have significant therapeutic potential but its utility as a target will need to overcome the challenge of completely understanding the mechanism of action for the various compounds being developed.

2 Concluding remarks

Research on phosphoinositide signaling has underscored how indispensable these minor lipids are for the regulation of a myriad of fundamental cellular processes [2]. However, more cellular and physiological studies are required to further understand the phosphoinositide signaling pathways and importantly the controlling enzymes themselves. The importance of spatial and temporal organization of phosphoinositide levels proves beyond a doubt that there is a need for techniques capable of achieving a subcellular resolution to follow changes in live cells. Some tools such as the use of the widely used PH domain of PLCδ to image $PI(4,5)P_2$ are used for specifically detecting phosphoinositides, but these techniques have limitations [195]. Super-resolution imaging would allow visualization of these low-abundant lipids with great precision to better interrogate phosphoinositide metabolism. A greater understanding of the spatial and temporal regulation of phosphoinositide signaling promises to determine how alterations in phosphoinositide pools at specific cellular and organelle membranes translate to human disease.

It has become clear that PIPKs represent potential and druggable targets for the treatment of these diseases and the development of multiple molecules to inhibit PIPKs proves that substantial efforts are currently being made to turn these proteins into druggable targets [75]. These efforts are most likely to have a significant translational value, however, there is still a need to further improve the pharmacological properties of PIPK inhibitors. The current development of potent and specific small molecule inhibitors to target PIPKs gives hope for future therapeutic options for the treatment of multiple diseases. It is important to consider that the emerging proteolysis targeting chimeric (PROTAC) technology offers the possibility to therapeutically target proteins that have proved difficult to modulate using small molecules [196]. These heterobifunctional compounds induce the degradation of a protein of interest by linking it to an E3 ligase, which induces ubiquitination and subsequent proteasomal degradation [197]. Given that PROTACs could target both catalytic and non-catalytic functions of the kinases, these molecules could certainly be more advantageous than other compounds to pharmacologically target PIPKs and are definitely worth exploring.

The three different PIPK families are implicated in a variety of mostly overlapping diseases (Figure 3) and despite the prospects of each of the PIP kinase family members to be a promising therapeutic option on their own, there is also a need to study them in combination. Targeting multiple PIPKs families and isoforms may be necessary to eliminate the constant metabolic rewiring that cancer cells undergo to survive various stresses and could represent a significant step forward in cancer research and possibly help to resolve the issues of drug resistance and relapse. The PIP kinase combinatorial targeting approach could also extend to other diseases and might prove to be more effective. The existing knowledge on the PIP kinases establishes beyond doubt that this family of enzymes demands further scientific as well as therapeutic attention.

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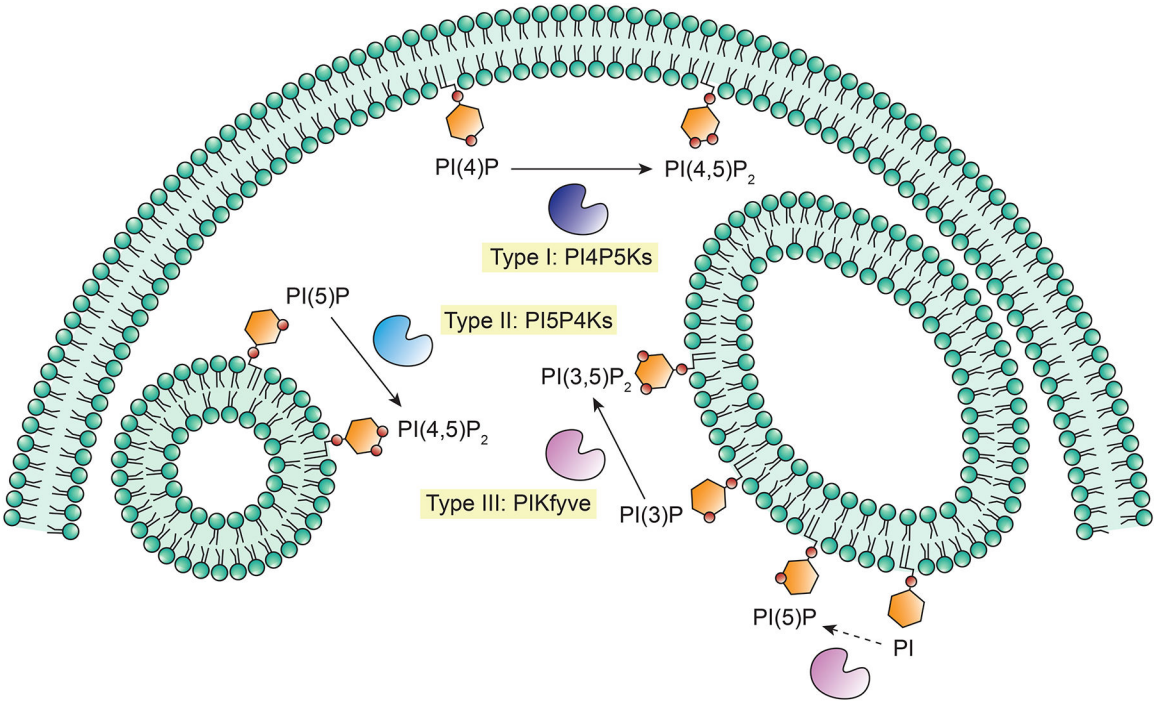

Figure 1. The Phosphatidylinositol phosphate kinase (PIPK) family.

Phosphoinositides are generated by the addition or removal of phosphate groups to the 3, 4, or 5 positions of the hydroxyl groups of phosphatidylinositol's inositol ring. Their levels are strictly regulated by several kinases and phosphatases. Among these enzymes, the PIPK family is responsible for the conversion of singly phosphorylated phosphatidylinositol (PI) to phosphatidylinositol bisphosphates (PIP_2). The PIPK family contains three subfamilies: Type I, Type II, and Type III, with significant sequence homology but distinct substrate specificity. Type I PIPKs phosphorylate PI(4)P to $PI(4,5)P_2$ mainly at the plasma membrane and are named Phosphatidylinositol-4-phosphate 5-kinases (PI4P5Ks). Type II PIPKs are called Phosphatidylinositol 5-phosphate 4-kinases (PI5P4Ks) and also generate $PI(4,5)P_2$, but they do it by phosphorylating PI(5)P at intracellular locations. There is only one Type III PIPK, named phosphatidylinositol-3-phosphate 5-kinase or PIKfyve. This kinase mainly phosphorylates PI(3)P at endosomal compartments to generate $PI(3,5)P_2$ but can also generate PI(5)P by phosphorylation of PI.

Family	Model	Ortholog	Genetic Model	Phenotype	References
Type I: PI4P5Ks	*S. cerevisiae*	*mss4*	*mss4Δ::HIS3/MSS4*	Embryonic lethality	Homma et al., 1998
		mss4	temperature-sensitive mutant *mss4-2ts* (SD102)	Actin cytoskeleton defects	Homma et al., 1998
	S. pombe	*its3*	*its3-1* mutant	Actin cytoskeleton defect	Deng et al., 2005; Zhang et al., 2000
	C. elegans	*ppk-1*	*ppk-1 (RNAi)*	Impaired ovulation and sterility; cytoskeletal defects	Xu et al., 2007
		ppk-1	*Punc-47:ppk-1* overexpression mutant	Impaired neuronal growth and integrity	Weinkove et al., 2008
	D. melanogaster	*sktl*	multiple *sktl* loss-of-function mutations	Loss of viability; impaired germline development	Hassan et al., 1998
		dPIP5K	$dPIP5K^{18}$ and $dPIP5K^{30}$ mutants	Defects in the electrical response to light in photoreceptors	Chakrabarti et al., 2015
	M. musculus	*Pip5k1a; Pip5k1b*	$Pip5k1a^{-/-}$, $Pip5k1b^{-/-}$	Live to adulthood, cell type-specific phenotypes	Volpicelli-Daley et al., 2010
		Pip5k1a; Pip5k1b	$Pip5k1a^{-/-}$ $Pip5k1b^{-/-}$	Male sterility	Hasegawa et al., 2012
		Pip5k1c	$Pip5k1c^{-/-}$	Embryonic lethality; defects in neural tube	Wang et al., 2007
Type II: PI5P4Ks	*C. elegans*	*ppk-2*	*ppk-2 (pk1343)* mutant	Reduced lifespan; increased sensitivity to energy stress due to autophagy defects; decreased lipid storage	Lundquist et al., 2018; Ravi et al., 2021
	D. melanogaster	*dPIP4K*	$dPIP4K^{29}$ mutant	Decreased mTORC1 signaling; larval growth defects; reduced body weight	Gupta et al., 2013
	M. musculus	*Pip4k2a*	$Pip4k2a^{-/-}$	Normal embryonic development	Emerling et al., 2013
		Pip4k2b	$Pip4k2b^{-/-}$	Normal life span; reduced growth rates; insulin hypersensitivity; decreased peripheral fat storage	Emerling et al., 2013; Lamia et al., 2004
		Pip4k2c	$Pip4k2c^{-/-}$	Normal embryonic development; immune system hyperactivation; increased T-helper cells and reduced regulatory T-cells	Shim et al., 2016
		Pip4k2b	$Pip4k2b^{-/-}$ $Trp53^{-/-}$	Embryonic lethality	Emerling et al., 2013
		Pip4k2a; Pip4k2b	$Pip4k2a^{-/-}$ $Pip4k2b^{+/-}$ $Trp53^{-/-}$	Dramatic reduction in tumor formation	Emerling et al., 2013
		Pip4k2a; Pip4k2b	$Pip4k2a^{flx/flx}$ $Pip4k2b^{-/-}$	Liver-specific *Pip4k2a* deletion; restricted ability to metabolize lipid droplets in the liver due to autophagy defects	Lundquist et al., 2018
		Pip4k2a; Pip4k2b	$Kras^{LSL\text{-}G12D/+}$ $Trp53^{flx/flx}$ $Pip4k2a^{flx/flx}$ $Pip4k2b^{-/-}$	Reduction in tumor formation	Ravi et al., 2021
Type III: PYKfyve	*S. cerevisiae*	*fab1*	*fab1-Δ1* mutant	Vacuolization; defects in nuclear division	Yamamoto et al., 1995
	C. elegans	*ppk-3*	*ppk-3(n2668)* mutant	Enlarged lysosomes; embryonic lethality/growth inhibition; impaired autophagy	Nicot et al., 2006
	D. melanogaster	*fab1*	$fab1^{31}/Df(2R)^{w30}$ mutant	Accumulation of endosomal receptors; swollen morphology with Fab1 inactivation	Rusten et al., 2006
	M. musculus	*pikfyve*	$pikfyve^{flx/flx}$	Embryonic lethality complete knockout	Ikonomov et al., 2011
		pikfyve	$pikfyve^{flx/flx}$ *LysM-Cre*	PIKfyve deletion in myeloid cells; systemic inflammation	Min et al., 2019
		pikfyve	$VilCrePipkII^{flx/flx}$	Intestinal inflammation; fibrosis	Takasuga et al., 2013

Figure 2. Selected genetic models used as *in vivo* tools for PIPK research.

Summary of the main phenotypes of representative Type I, Type II and Type III PIPKs *in vivo* models used to study the role of these enzymes in development and disease.

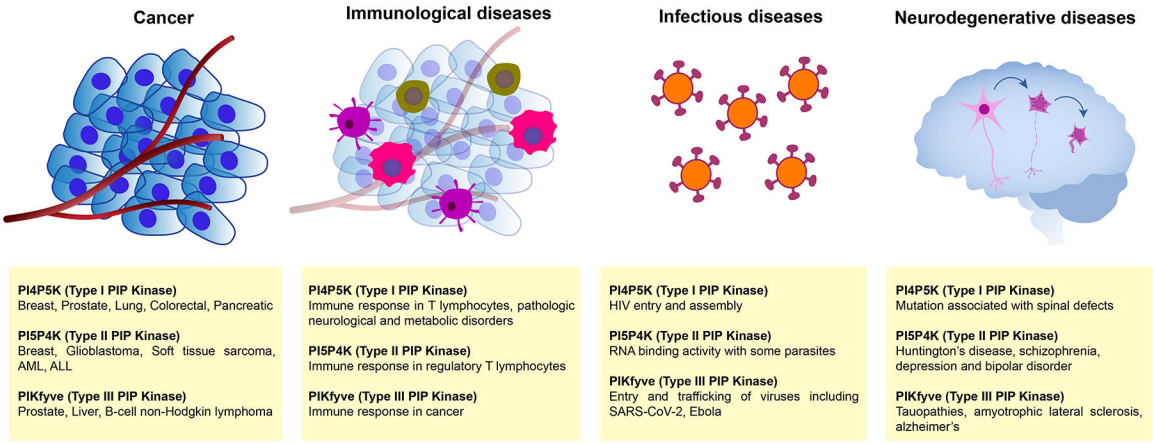

Figure 3. The PIPK family is involved in various human diseases.

Schematic overview of the human pathologies that have been associated with alterations in PIPKs and have the potential of benefiting from therapies that are targeted towards these enzymes. Members of Type I, Type II and Type III PIPKs subfamilies have been linked to multiple types of cancer, immunological, infectious and neurodegenerative disorders.