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Roles of PIKfyve in multiple cellular pathways
Pilar Rivero-Ríos and Lois S. Weisman

Abstract
Phosphoinositide signaling lipids are crucial for eukaryotes and regulate many aspects of cell function. These signaling molecules are difficult to study because they are extremely low abundance. Here, we focus on two of the lowest abundance phosphoinositides, PI(3,5)P2 and PI(5)P, which play critical roles in cellular homeostasis, membrane trafficking and transcription. Their levels are tightly regulated by a protein complex that includes PIKfyve, Fig4 and Vac14. Importantly, mutations in this complex that decrease PI(3,5)P2 and PI(5)P are linked to human diseases, especially those of the nervous system. Paradoxically, PIKfyve inhibitors which decrease PI(3,5)P2 and PI(5)P are currently being tested for some neurodegenerative diseases, as well as other diverse diseases including some cancers, and as a treatment for SARS-CoV2 infection. A more comprehensive picture of the pathways that are regulated by PIKfyve will be critical to understand the roles of PI(3,5)P2 and PI(5)P in normal human physiology and in disease.

Introduction
Phosphoinositide lipids (PPIs) are signaling molecules that regulate critical cell processes. They are produced from phosphatidylinositol by phosphorylation of the hydroxyl groups in any combination at positions three, four or five of the inositol ring. A series of specific lipid kinases and phosphatases are responsible for the generation and turnover of the resultant seven PPI species. These enzymes are highly regulated both spatially and temporally and are generated and turned over at specific membrane subdomains. This establishes the distribution of PPIs, which act by recruiting a set of cognate effector proteins. Although a longstanding view in the field was that PPIs define organelle identity by restricting each species to one or few compartments, a growing body of evidence indicates that the localization of each PPI is more promiscuous than initially thought (reviewed in Choy et al. [1]). Importantly, the synthesis of distinct PPIs are coordinated into cascades which control complicated multi-steps pathways. An example of such a cascade is how conversion of PPI lipids coordinates clathrin-mediated endocytosis. Plasma membrane-localized PI(4,5)P2 recruits proteins involved in the early steps of endocytosis including AP-2, dynamin and endophilin, as well as the lipid kinase PI3KC2α. Moreover, endophilin promotes the recruitment of the lipid phosphatase synaptojanin, which converts PI(4,5)P2 into PI(4)P. PI3KC2α subsequently transforms PI(4)P into PI(3,4)P2, which recruits proteins that act later in endocytosis (reviewed in the study by Posor et al. [2]). Another example is the ordered conversion of PI(3)P into PI(4)P at endosomes to promote protein recycling to the plasma membrane. This mechanism involves the formation of a complex composed of the lipid phosphatase MTM1 and the lipid kinase PI4K2A. MTM1 removes the phosphate from PI(3)P, while in parallel, a phosphate group is added to position 4 by PI4K2A. The PI(4)P is then converted into PI(3,4,5)P3, which recruits proteins involved in recycling endosomes with the plasma membrane [3].

The lipid kinase PIKfyve
Studies to date show that PIKfyve (Fab1 in yeast), which phosphorylates position 5 of PI(3)P is the sole source of PI(3,5)P2. In addition, PIKfyve is responsible for virtually all of the PI(5)P pool. PI(5)P is generated from PI(3,5)P2 via 3-phosphatase activity, likely catalyzed by the myotubularin family of phosphatases [4]. There is also evidence that PIKfyve may generate PI(5)P directly from phosphatidylinositol [5]. In either scenario, knockdown or inhibition of PIKfyve results in the loss of both PI(3,5)P2 and PI(5)P. PI(3,5)P2 is particularly challenging to study as compared to other PPIs, due to its low abundance, and lack of a highly specific bioprobe.

Keywords
PIKfyve, Fig4, Vac14, phosphoinositide, endosomes, endomembrane trafficking.

Abbreviations
Phosphatidylinositol 3-kinase C2α, PI3KC2α; Myotubularin, MTM1; Phosphatidylinositol 4,5-bisphosphate, PI(4,5)P2. The lipid kinase PI3KC2α

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Indeed, PI(3,5)P₂ accounts for approximately 0.1% and 0.04% of total PPIs in yeast and mouse embryonic fibroblasts (MEFs), respectively. This makes PI(3,5)P₂ 17-fold and 125-fold less abundant than PI(4,5)P₂ in yeast and MEFs, respectively. PI(5)P is undetectable in yeast, and is present at levels similar to PI(3)P (approximately 0.5% of total PI) in MEFs, which means that PI(5)P is 16-fold lower than PI(4,5)P₂ [6]. Note that PI(4,5)P₂ is much more extensively characterized than either PI(3,5)P₂ or PI(5)P.

PI(3,5)P₂ levels dynamically and transiently change in response to specific stimuli. The best characterized example is the transient elevation of PI(3,5)P₂ that occurs during hyperosmotic shock in yeast. Within 5 min of exposure to hyperosmotic media, there is a 20-fold increase and then a rapid return to the normal levels within 30 min (Figure 1) [7]. In mammalian cells, physiological signals such as insulin and growth factors also cause an acute elevation of PI(3,5)P₂ levels [4,8,9], which suggests that this lipid plays key roles in cellular homeostasis and in adaptation.

The dynamic and rapid changes in PI(3,5)P₂ levels following these stimuli suggest that PIKfyve is tightly regulated. Genetic studies in yeast and mammalian cells revealed that PIKfyve activity requires the formation of a complex that includes the scaffold protein Vac14 and the lipid phosphatase Fig4, which converts PI(3,5)P₂ to PI(3)P [10,11]. Paradoxically, Fig4 is also required to produce PI(3,5)P₂ and mutations in the Fig4 catalytic site result in impaired Fab1 activation in yeast. At least three different regions of Fig4 are critical for the formation of the yeast Fab1-Vac14-Fig4 complex: the Fig4 catalytic site, a conserved N-terminal surface, and the C-terminus. The presence of three contact sites in Fig4 raises the possibility that Fig4 experiences conformational changes which regulate the assembly and/or activity of the complex [12].

Key insights into the complexity of the regulation of PIKfyve came from the determination of the structure of the complex using negative-stain and cryoelectron microscopy [13]. Intriguingly, Vac14 pentamerizes into a star-shaped structure, which binds a single copy each of PIKfyve and Fig4. PIKfyve binds one leg of the Vac14 pentamer, and Fig4 binds the opposite side of that leg, as well as an adjacent leg. In this solved structure, Fig4 is oriented such that its catalytic site would contact the membrane, thereby providing Fig4 with access to its substrate, PI(3,5)P₂. However, paradoxically, the PIKfyve catalytic site is rotated away from the membrane. Moreover, mammalian Fig4 has protein phosphatase activity and acts on PIKfyve to stimulate its lipid kinase activity [13]. Yet, in the solved structure, the Fig4 catalytic site is far from PIKfyve. Together, these observations strongly suggest that during PIKfyve activation, the complex would undergo a large structural rearrangement that would place the PIKfyve catalytic

Figure 1

[Diagram of PI(3,5)P₂ levels acutely and transiently rising and falling in response to hyperosmotic stress. Yeast grown in normal media were transferred to hyperosmotic media (containing 0.9 M NaCl) at time zero (perpendicular arrow) and left in 0.9 M salt for the rest of the experiment (parallel arrow). Although there were no further perturbations to the external environment, the levels of PI(3,5)P₂ (relative to PI(4,5)P₂) rapidly increased, plateau and rapidly decreased. These findings indicate that the levels of PI(3,5)P₂ are tightly controlled. Within 5 min, PI(3,5)P₂ levels rise over 20-fold, plateau for 10 min, then rapidly return to basal levels. Multiple layers of regulations within the Fab1 complex, which includes both the lipid kinase Fab1/PIKfyve and lipid phosphatase Fig4 provide tight control of PI(3,5)P₂ synthesis and turnover. Data modified from [7].]
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It is not clear whether these proteins regulate PI(3,5)P₂. WIPI-1, WIPI-2, WIPI-3/WDR45L, and WIPI-4/Mammals have four proteins that show homology with required for autophagosome formation. In addition, is best known for its roles in autophagy, where it is press two additional regulatory proteins, Atg18 and Vac7. Furthermore, the six known sites in a relatively conserved region of Vac14. Mutation of the same leucine in yeast causes a loss of the interaction of Vac14 with Fab1, and Vac14 interaction with PIKfyve, in mammalian cells [10]. Moreover, the Charcot-Marie-Tooth Type 4J (CMT4J) mutation, Fig4-I > T also shows similar mechanistic defects as the corresponding yeast mutant, with this mutation impairing the interaction between both mammalian and yeast Vac14 and Fig4 [16]. Furthermore, the six known autophosphorylation sites are conserved among vertebrate PIKfyve, and three of these sites are also conserved in yeast.

In addition to PIKfyve/Fab1, Vac14 and Fig4, yeast express two additional regulatory proteins, Arg18 and Vac7. It remains unclear whether there are analogous proteins which perform these same functions in mammals. Arg18 is best known for its roles in autophagy, where it is required for autophagosome formation. In addition, Atg18 negatively regulates yeast Fab1 activity [17,18]. Mammals have four proteins that show homology with Atg18: WIPI-1, WIPI-2, WIPI-3/WDR45L, and WIPI-4/WDR45. These function in autophagy [19,20], however it is not clear whether these proteins regulate PI(3,5)P₂ levels in mammalian cells (reviewed in Jin et al. [21]). Vac7 is a key positive regulator of Fab1 activity. Deletion of Vac7 in yeast results in nearly undetectable levels of PI(3,5)P₂ and an enlarged vacuole [22]. TMEM106B has been proposed as a mammalian homolog for Vac7 based on bioinformatics methods [23]. It is currently unknown whether this protein regulates the PIKfyve complex in mammals.

Animal models of PIKfyve deficiency and human diseases associated with defects in the PIKfyve complex

Fab1 is essential in yeast. Fab1 deletion yeast strains were initially thought to survive (Yamamoto et al., 1995). However, acute removal of Fab1 revealed that Fab1 is essential. Suppressor mutations in Fab1 deletion mutants are rapidly acquired, which allow the cells to survive [24,25]. Yeast deletion strains show defects in cell growth, with the Fab1 deletion presenting the most severe phenotype (probably due to a complete loss of PI(3,5)P₂), followed by Vac7, Vac14 and Fig4 [22,25,26].

In mice, PIKfyve is critical during early embryonic development. Global deletion of the *pikfyve* gene results in the death of embryos before they reach the 32–64 cell stage [27] or by e8.5 due to a failure of the visceral endoderm to use maternal nutrients [28]. It is not clear whether these divergent early times of death were due to differences in mouse strain background. A hypomorphic PIKfyve mouse model expressing approximately 10% of the normal levels of PIKfyve protein is viable, but exhibits extensive neurodegeneration and dies perinatally due to defects in multiple organs, including neural tissues, heart, lung, kidney, thymus, and spleen [4]. In addition, several PIKfyve knockout mice were developed using the Cre-Lox system which demonstrate essential roles of PIKfyve in specific tissues or cell types, including the intestine, muscles, oligodendrocytes and myeloid cells [28–31]. Mouse models where either Fig4 or Vac14 are deleted exhibit extensive neurodegeneration and die perinatally [32,33]. The fact that the absence of PIKfyve results in much earlier lethality than the lack of Fig4 or Vac14 is likely due to the fact that loss of PIKfyve causes total deficiency of PI(3,5)P₂ and a loss of over 80% of PI(5)P, whereas the absence Vac14 or Fig4 results in a ~50%–70% decrease in PI(3,5)P₂ and PI(5)P [4,32,33].

Defects in the PIKfyve complex are linked to human diseases, especially those of the nervous system [32,34–60], although the underlying molecular mechanisms are not clear. For example, mutations in Fig4 predicted to have a modest effect in the regulation of PI(3,5)₂ are linked to Charcot-Marie-Tooth syndrome (CMT4J), a peripheral neuropathy, as well as some cases of amyotrophic lateral sclerosis (ALS) and primary lateral sclerosis (PLS). More extensive mutations in either Vac14 or Fig4 cause Yunis–Varon syndrome, which results in infant mortality and severe pathological effects on multiple tissues, including the brain. One case of neurodegeneration with iron brain accumulation was linked to mutation of Vac14. A list of the mutations in the PIKfyve-Vac14-Fig4 complex that have been linked to diseases is provided in Table 1. In addition, the extensive vacuolization observed in the brains of mouse models when PIKfyve activity is decreased is reminiscent of prion disorders. Notably, it was recently discovered that prion infection results in lower levels of PIKfyve [61], which suggests that the pathology associated with prion disease is due to lowered PI(3,5)P₂, and PI(5)P.
While human mutations in Fig4 or Vac14 have been linked to severe neurological syndromes, the main human disease associated with mutations in the PIKfyve gene is Fleck corneal dystrophy, characterized by the appearance of white flecks in the cornea which generally do not cause vision impairment [51,52]. These frameshift or nonsense mutations, which are predicted to result in the loss of PIKfyve function, are heterozygous, and the patients each have a wild-type copy of PIKfyve. This likely explains how these patients do not show phenotypes beyond defects in their cornea. It is plausible that the expected 50% decrease in PIKfyve levels is well tolerated. Note that while mice that are heterozygous for the hypomorphic PIKfyve allele have only 50% of the normal levels of PIKfyve, they have normal levels of PI(3,5)P2 and 70% of normal levels of PI(5)P. They do not have any observable defects in any of their tissues [4].

Interest in PIKfyve has recently increased because apilimod, a selective PIKfyve inhibitor, has been tested as a treatment for several diseases both in cell and animal disease models. These include COVID-19, some neurodegenerative diseases and some types of cancer (see references in Huang et al. [62]). In addition to apilimod, other PIKfyve inhibitors have been developed. YM201636 [63], another commonly used PIKfyve inhibitor, has also inhibited Akt phosphorylation, suggesting that it may also be a PI3K inhibitor [64]; APY0201 [65] is more potent than apilimod, although there are not yet published reports on its impact on the seven PIP species, including PI(3,5)P2. WX8 [66], a more recently published inhibitor, may have less specificity than the other inhibitors. Importantly, apilimod is the most extensively characterized inhibitor and the only one that has been approved for use in clinical trials.

Apilimod was also found to have antiproliferative effects against B-cell non-Hodgkin lymphoma [74], and is currently in phase II clinical trials. Moreover, a role for PIKfyve in tumor cell invasiveness has been described in mouse embryonic fibroblasts expressing the oncogenic fusion protein nucleophosmin–anaplastic lymphoma kinase [75] and in cancer cell lines of three different origins (lung, rhabdomyosarcoma, and osteosarcoma) [76]. In addition, PIKfyve inhibition mitigated several types of cancers in animal models (reviewed by Ikono mov et al. [77]) including metastatic liver cancer in a mouse model [78]. These findings raise the possibility of using PIKfyve inhibitors to prevent hematological cancers, as well as cancers that cause solid tumors and metastasis.

Paradoxically, PIKfyve inhibition has shown beneficial effects in several cellular and in vivo models of neurodegeneration. For example, a genome-wide CRISPR interference screen showed that PIKfyve inhibition reduced α-synuclein aggregation induced with both recombinant α-synuclein fibrils and fibrils isolated from patients [79]. PIKfyve inhibition also improved the survival of motor neurons derived from iPSC from patients with ALS [80]. In addition, apilimod treatment improved the deterioration of motor neurons with

| Disease                                      | Affected gene                                      | References                      |
|----------------------------------------------|----------------------------------------------------|---------------------------------|
| Charcot-Marie–Tooth type 4J (CMT4J)          | Homozygous or heterozygous mutations leading to reduced Fig4 expression | [32,34,45,54–56]                |
| Amyotrophic lateral sclerosis (ALS)          | Mutations in Fig4                                   | [34,57–59]                     |
| Primary lateral sclerosis (PLS)              | Mutations in Fig4                                   | [34,60]                         |
| Yunis–Varon syndrome                         | Homozygous null mutation in Fig4                   | [35,36]                         |
| Central nervous system white matter disorders| Mutations in Fig4                                   | [37]                            |
| Syndrome with severe neurological and psychiatric symptoms | Homozygous point mutation in Fig4 | [38]                            |
| Yunis–Varon syndrome                         | Biallelic Vac14 variants                            | [39]                            |
| Childhood onset striato-nigral degeneration  | Mutations in Vac14                                  | [40–44,46–49]                   |
| Francois–Mouchette Fleck corneal dystrophy   | Mutations in PIKfyve                                | [50–52]                         |
| Congenital cataract                           | Mutation in PIKfyve                                 | [53]                            |
mutations in the ALS-related protein C9Orf72 in mouse models in vivo [81]. Furthermore, PIKfyve inhibition also reduced the formation of tau aggregates in neurons [82]. The potential of PIKfyve inhibition as a treatment for neurodegeneration is surprising given that decreased PIKfyve activity in mouse models causes a spongiform phenotype in the brain and that mutations in the PIKfyve complex are associated with neurodegenerative diseases. However, the fact that individuals with just one copy of the PIKfyve gene do not develop neurodegeneration, indicate that a partial decrease of PIKfyve activity is well tolerated. However, time and dose may need to be carefully adjusted to prevent the deleterious consequences of inhibiting PIKfyve.

**Functions of PIKfyve**

To understand the processes affected in diseases associated with PIKfyve, it is critical to identify the functions of PIKfyve. Although PIKfyve was initially thought to act solely on lysosomes, a growing body of evidence indicates that it plays several additional roles that are critical for cell function. Some of these pathways are directly regulated by PI(3,5)P2, as PI(3,5)P2 protein effectors have been identified. However, in other cases, the role of PIKfyve may be due to PI(3,5)P2, PI(5)P or both lipids. Due to the lack of suitable and/or sufficiently sensitive bioprobes, the localization of endogenous levels of PI(3,5)P2 and PI(5)P is inferred from the localization of PIKfyve (Figure 2). Although a bioprobe that specifically

![Figure 2](image-url)

Schematic of the cellular localization of the two lipids products downstream of PIKfyve activity, PI(3,5)P2 and PI(5)P, as well as the precursor PI(3)P. Due to the lack of suitable and/or sufficiently sensitive bioprobes, the localization of endogenous levels of PI(3,5)P2 and PI(5)P is inferred from the localization of PIKfyve. PI(3,5)P2 localizes on early endosomes, late endosomes and lysosomes. PI(5)P may also be present at all or some of these locations. CME: Clathrin-mediated endocytosis. CIE: Clathrin-independent endocytosis. EE: Early endosome. LE: Late endosome. MVB: Multivesicular body. EA: Endoplasmic reticulum. ERC: Early recycling compartment. TGN: Trans-Golgi network.
recognizes PI(3,5)P2, ML1N-2*-GFP, has been described, it is important to note that in the presence of YM201636, ML1N-2*-GFP signal was still observed on some vacuoles, raising the possibility that in the absence of PI(3,5)P2, the probe may recognize another epitope [83].

**Roles at the lysosome**

Inhibition or knockdown of PIKfyve results in the appearance of enlarged vacuoles in mammalian cells and an enlarged vacuole/lysosome in yeast [15,84–86]. These enlarged vacuoles are likely due to the combination of two different defects: alterations in ion homeostasis and pH within the endomembrane system, and alterations in membrane dynamics, particularly membrane fission and formation of tubules.

PIKfyve is critical to the maintenance of ion homeostasis in lysosomes. It is clear that these events are driven by PI(3,5)P2, which has been shown in atomic resolution structures to bind directly to key lysosomal ion channels, including the mucolipin TRP channel (TRPML1) and the Na+ two-pore channels, TPC1 and TPC2 [87]. Changes in ion homeostasis due to channel inactivation during PIKfyve inhibition, may also indirectly lead to swelling of the lysosome due to an accompanying influx of water. Similarly in yeast, PI(3,5)P2 was shown to be required for a vacuole/lysosome monovalent cation/proton antiporter [24].

Luminal pH is also closely tied to vacular function, as the acidic environment of lysosomes and vacuoles is critical for the activity of hydrolytic enzymes and to maintain a pH gradient that drives the transport of ions and metabolites. Notably, PIKfyve/Fab1 has a role in the regulation of the pH of the yeast vacuole and mammalian lysosome. Yeast mutants deficient in PI(3,5)P2 synthesis have less acidified vacuoles. This effect is mediated in part by interaction of PI(3,5)P2 with the vacuolar ATPase (v-ATPase), which is key for organelle acidification and cellular pH control (reviewed in [88]). Indeed, hyperosmotic shock generated an almost two-fold increase in V-ATPase activity, accompanied by increased levels of V1 subunits in wild-type but not Vac14 deficient yeasts [89]. Similarly, using a stably expressed genetically encoded ratiometric probe, it was found that treatment with a low dose of the PIKfyve inhibitor, apilimod for 3 h, resulted in an elevation of lysosomal pH in a mammalian cell line [90]. It is tempting to speculate that this may be due in part to PI(3,5)P2 regulation of mammalian v-ATPase.

Membrane trafficking defects in the endolysosomal system due to impaired PIKfyve activity likely also contribute to vacuole and lysosome enlargement. Indeed, PIKfyve activity is required to drive lysosome reformation from endolysosomes [91], which suggests that PIKfyve has a role in membrane fission. Moreover, the large vacuoles in PIKfyve inhibited cells failed to undergo fission, an effect that is partly mediated by TRPML1 and is critical to redistribute degraded cargo back to the endosomal network [92]. The block in lysosome fission during PIKfyve inhibition may contribute to lysosomal enlargement via an overall steady-state increase in lysosomal fusion [93]. The full mechanism for how PI(3,5)P2 plays a role in membrane fission is not known. However, WIP1, a known PI(3,5)P2 binding protein, with an independent role in autophagy, participates in fission of endosomal tubules [94]. In yeast, PI(3,5)P2 also plays a role in vacuole fusion [95].

**Roles at endosomes**

PIKfyve is necessary for the generation of Stage I melanosomes, which are derived from early endosomes [96]. Moreover, PIKfyve is required for retrograde traffic of proteins from endosomes to the trans-Golgi network (TGN). Knockdown or inhibition of PIKfyve impairs the trafficking of several proteins to the TGN, including the cation-independent mannose-6-phosphate receptor, sortilin, furin, and Shiga Toxin B, although the mechanistic basis of these defects remains unknown [97,98].

In addition, PIKfyve plays a role in endocytic recycling to the plasma membrane. Early evidence comes from studies at synapses, where decreased PIKfyve activity results in an increase in the surface levels of AMPA-type glutamate receptors (AMPARs). This is in part due to increased recycling from endosomes to the plasma membrane, as apilimod treatment for 10 min promoted the recycling of AMPARs, although an additional effect of endocytosis cannot be excluded [15,99].

Our recent studies gained mechanistic insight into the role of PIKfyve in endocytic recycling. We found that PIKfyve is a key regulator of the recently discovered Retriever pathway. PIKfyve inhibition causes a loss of Retriever and the CCC complex from endosomes, and mutation of the lipid binding site on COMMD1, a CCC subunit, impairs its endosomal localization and delays the recycling of several SNX17 cargoes including z5-integrin, b1-integrin and LRP1. We also found that PIKfyve coordinates with VPS34, the enzyme that generates PI(3)P from PIP2, in a phosphoinositide cascade to promote the ordered assembly of SNX17, WASH, Retriever and the CCC complex [100].

Intriguingly, PIKfyve has a positive role in b1-integrin recycling but has a negative regulatory role in AMPAR localization to the plasma membrane. This could be due to the fact that AMPAR recycling is dependent on the SNX27-Retromer pathway, while b1-integrin depends on the SNX17-Retriever pathway. This would open the possibility that PIKfyve differentially regulates different recycling pathways that emerge from endosomes.
The role of PIKfyve in recycling from endosomes to the plasma membrane may also explain the reduction in steady-state levels of exogenously expressed FLAG-TGFβ-R2 at the cell surface, as well as the accumulation of the tight junction proteins Claudin-1 and Claudin-2 in intracellular compartments upon inhibition of PIKfyve activity [101,102]. Note that the recycling pathway utilized by these proteins are currently unknown.

While PIKfyve may separately regulate ion homeostasis and membrane dynamics, a recent study suggests that observed changes in membrane dynamics upon PIKfyve inhibition, might be solely accounted for by defects in ion flux [103]. This study proposes that two-pore channels, which are regulated by PI(3,5)P2, release sodium ions accompanied by water, and as the organelle loses volume, it crenelates, which recruits curvature sensing protein required for membrane tubulation and fission.

**Regulation of transcription**

Although the majority of studies on PIKfyve have focused on its roles in the endosomal membrane, PIKfyve also plays roles in regulating the transcription of some genes. PI(3,5)P2 modulates transcription in yeast by promoting the assembly of the Tup1/Cyc8/Cti6 transcription complex on the yeast vacuole via direct interaction of Tup1 and Cti6 [104]. In mammalian cells, PIKfyve also plays roles in the regulation of transcription. Apilimod inhibits IL-12 expression by transcriptional upregulation of the repressor ATF3, although the molecular mechanism is unknown [105]. Moreover, PIKfyve is a key regulator of Transcription factor EB (TFEB), a master regulator of autophagy and lysosomal biogenesis, and inhibition of PIKfyve induces the nuclear translocation of TFEB [93]. PIKfyve acts by positively regulating mTORC1-dependent phosphorylation of Ser-211 on TFEB [106]. Upon PIKfyve inhibition, protein phosphatase 2A dephosphorylates TFEB Ser-211, which results in TFEB translocation into the nucleus. While positive regulation of mTORC1 by PIKfyve is required for TFEB phosphorylation, and retention on the lysosome, mTORC1-dependent phosphorylation of other downstream targets does not require PIKfyve activity. Importantly, PIKfyve inhibition results in TFEB translocation to the nucleus, and the upregulation of the TFEB targets tested. These findings support a role for PIKfyve in the regulation of transcription. Future studies will be necessary to determine the PIKfyve-dependent transcriptome, which may provide important insights into the roles of PIKfyve in cellular physiology [106].

**Concluding remarks**

Studies on PIKfyve have increased our knowledge of PPIs. That PIKfyve is present in multiple places within the cell supports the view that PPIs do not define organelle identity, and instead, the transient appearance of PPIs in specific compartments allows for the regulation and coordination of signaling and trafficking pathways. PPI formation is tightly controlled in space and time and is triggered by specific stimuli. A clear example is the acute elevation of PI(3,5)P2 upon hyperosmotic shock in yeast. Since PIKfyve is the only source of this lipid, it is easier to observe a more dramatic effect upon genetic manipulation of PIKfyve levels. Similar acute responses may be true for other PPIs but will be more difficult to detect due to the existence of multiple sources of some PPI, which may provide a compensatory effect. An important property of PPIs is that they often participate in phosphoinositide cascades, in which the ordered appearance of PPIs at one compartment allows for the progression of a cellular pathway, as shown by the coordinated action of VPS34 and PIKfyve to regulate endocytic recycling.

PIKfyve has gained attention from different sources in recent years, when it became a promising drug target for neurodegeneration, cancer, and COVID-19. However, the difficulty of measuring the low levels of PI(3,5)P2 and the lack of specific biosensors to monitor its spatial and temporal dynamics have been major challenges to elucidate the functions of PIKfyve. Although considerable progress has been made, a more comprehensive picture of the pathways that are regulated by PIKfyve will be critical to understand the roles of PI(3,5)P2 and PI(5)P in disease. In addition, since ablation of PIKfyve activity results in very early embryonic lethality, the successful development of PIKfyve inhibitors as therapies for some diseases necessitates a deeper understanding of the diverse pathways that require PIKfyve.

**Author contributions**

PRR and LSW co-wrote the manuscript.

**Conflict of interest statement**

Nothing declared.

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