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Review Article

Phosphatidylinositol 4-kinases: Function, structure, and inhibition

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ABSTRACT

The phosphatidylinositol 4-kinases (PI4Ks) synthesize phosphatidylinositol 4-phosphate (PI4P), a key member of the phosphoinositide family. PI4P defines the membranes of Golgi and trans-Golgi network (TGN) and regulates trafficking to and from the Golgi. Humans have two type II PI4Ks (α and β) and two type III enzymes (α and β). Recently, the crystal structures were solved for both type II and type III kinase revealing atomic details of their function. Importantly, the type III PI4Ks are hijacked by +RNA viruses to create so-called membranous web, an extensively phosphorylated and modified membrane system dedicated to their replication. Therefore, selective and potent inhibitors of PI4Ks have been developed as potential antiviral agents. Here we focus on the structure and function of PI4Ks and their potential in human medicine.

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1. Phosphatidylinositol 4-phosphate (PI4P) and phosphoinositides (PIPs)

The phosphatidylinositol 4-kinases (PI4Ks) synthesize phosphatidylinositol 4-phosphate (PI4P), a member of the phosphoinositide family. Phosphoinositides (PIPs) are synthesized from phosphatidylinositol (PI), a lipid containing the *myo*-inositol head group. PI can be phosphorylated at positions 3, 4, and 5 of the inositol ring (Fig. 1), which allows for seven different PIPs. Indeed, all of them have been identified in the cell [1]. For instance, one prominent function of PIPs is to serve as membrane markers typically in concert with organelle specific proteins. PI(4,5)P_2 is the main lipid determinant of the plasma membrane and PI3P and PI(3,5)P_2 of the early and late endosomes. PI4P is the main lipid determinant of the Golgi and trans-Golgi network (TGN) [2] but, additionally, helps to define the acidic character of the plasma membrane [3].

PIPs are also instrumental in signal transduction. Specifically, hydrolysis of PI(4,5)P_2 by phospholipase C generates two second messengers – membrane-bound diacylglycerol (DAG) and cytosolic inositol 1,4,5-triphosphate – that together initiate downstream signaling cascades. Here we intend to discuss PI4P and PI4 kinases, for a thorough review of all the functions PIPs have in cellular physiology, please see [1]. Initially, PI4P was believed to be

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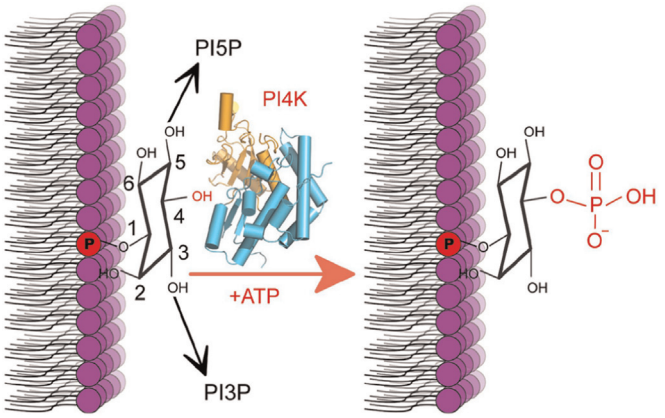

Fig. 1. Schematic layout of the phosphorylation reactions at the inositol ring. PI4 kinases (PI4K IIα depicted) attach the phospho-group to the position four of the inositol ring. Positions three and five might be also phosphorylated by the appropriate PI3 and PI5 kinases.

merely a precursor for PI[4,5]P_2 and PIP_3 [4], two lipids already well known for their roles in cells signaling. Later, essential roles of PI4P conserved from yeast to humans were described [5] and PI4P is now considered an essential molecule in signaling and cellular trafficking especially in the Golgi and TGN [4,6–8]. PI4P achieves its functions by regulating of cellular localization of its effector proteins. PI4P is recognized by proteins containing PIP binding domains such as the Pleckstrin Homology (PH), Phox homology (PX), Epsin N-Terminal Homology (ENTH), Kinase Associated-1 (KA1), MVB12-associated beta-prism (MABP) and others [9–11]. Most PIP binding domains show little preference for a specific PIP, however domains preferentially binding the PI4P were described [12]. Surprisingly, the most specific high affinity binders of PI4P yet identified come from SidC and SidM proteins of the intracellular bacterial pathogen *Legionella pneumonia* [13–15]. These domain fused to GFP are high-affinity, high-specificity PI4P biosensors that uncovered additional PI4P pools beyond the Golgi such as Rab7 positive late endosomes/lysosomes [16]. Recent review on PIPs binding domains is available [17]. Importantly, five years ago the role of PI4P in the replication of positive strand RNA (+RNA) viruses was described for the first time [18–22] putting the PI4P lipid in the center of scientific scrutiny.

2. Phosphatidylinositol 4-phosphate kinases

As stated above, PI4P *in vivo* is primarily produced by the action of PI4Ks, although it can be also produced by dephosphorylating a higher PIP. All eukaryotes have two families of PI4Ks conserved from yeast to man called type II and type III PI4Ks [23] that have different domain organization (Fig. 2). The missing type I PI4Ks is a historical artifact as it was later discovered that the type I PI4Ks were actually PI3Ks [24,25]. Type III kinases are known as typical PI4Ks due to their similarity to PI3Ks and type II are known as atypical due to their dissimilarity to any other lipid kinases.

2.1 Type II PI4Ks

The type II kinase of the yeast *Saccharomyces cerevisiae* is named Lsb6p and has been implied in the regulation of actin polymerization and in endosome mobility as it binds Las17p (Wiskott–Aldrich syndrome protein homolog) but is not an essential gene for yeast survival [26,27]. Its deletion has only a mild phenotype in endosome mobility that can be rescued by a catalytically inactive construct suggesting that Lsb6p might have a scaffolding role and, thus, its catalytic activity is not required for proper function [28]. Humans, on the other hand, have two type II kinases – PI4K IIα and IIβ. Both contain a CCPCC motif within the kinase domain that can be palmitoylated (Fig. 2). Palmitoylation is also the main method for their regulation as upon palmitoylation they stably associate with the membrane and become active. Little is known about PI4K IIβ membrane targeting and activation [29]. It seems to be largely localized in an inactive cytoplasmic pool stabilized by Hsp90 (Heat Shock Protein 90) [30] and was identified to be activated by platelet-derived growth factor (PDGF) [31]. PI4K IIβ was also identified as a component in early T cell activation mechanisms [32]. PI4K IIα is the most active PI4K in humans and is responsible for the synthesis of ~50% of PI4P. It is localized mostly in the Golgi and endosomes, plays a role in vesicle and endosomal trafficking [33–36], as well as in Wnt signaling [37,38]. Together with the PI4K IIIβ it controls the delivery of Gaucher's disease enzyme *β*-glucocerebrosidase (GBA) to the lysosomes [34]. In *Arabidopsis thaliana* several type II PI4K isoforms contain ubiquitin within their coding sequence; correspondingly the human PI4K IIα has a binding site and is regulated by the E3 ubiquitin ligase Itch [39]. It also contains a binding motif for AP-3 [36] and the association with PI4K IIα as well as its kinase activity are important for

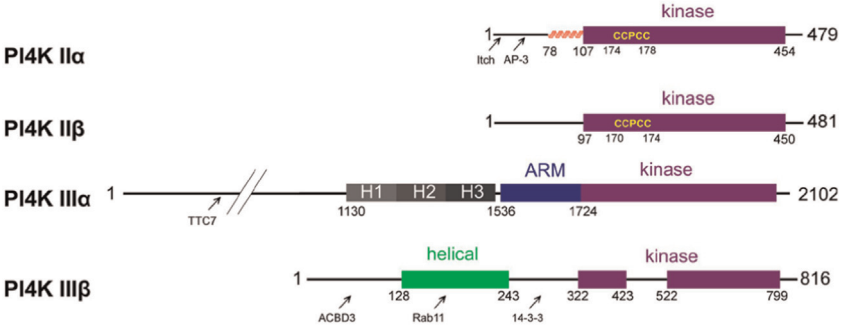

Fig. 2. The domain organization of all four PI4 kinases. (i) PI4K IIα has a binding site for ubiquitin ligase Itch and the AP-3 adaptor complex within the disordered N-terminus. The kinase domain is superseded by a short flexible helix. The domain boundaries are based on recent crystal structures [46,48]. (ii) PI4K IIβ also has an unstructured N-terminus but without the flexible helix. The hallmark of type II kinase domain is the CCPCC motif that is palmitoylated *in vivo* when the kinase is active. (iii) PI4K IIIα has a binding site for the TTC7 (Ypp1 in yeast) which anchors PI4K IIIα in the helical scaffold of the TTC7:ERF3 protein complex and ensures its proper membrane localization. The kinase domain is located at the very C-terminus and is superseded by an ARM (Armadillo repeat domain) domain and three predicted helical subdomains (here named H1, H2, H3). The domain boundaries are based on experimental and modeling work of Harak and colleagues [80]. (iv) PI4K IIIβ has a helical domain in front of the kinase domain. How helical domain recruits the Rab11 protein was shown at the atomic level [64]. PI4K IIIβ contains three disordered regions; the very N-terminus that binds the ACBD3 protein, a disordered loop between the helical and kinase domain that has a binding site for the 14–3–3 proteins, and a disordered loop within the N-lobe of the kinase domain that has an unknown function. The last 15 residues are flexible and thus are not depicted as a part of the kinase domain but it is worth noting that a deletion of these 15 terminal residues creates a kinase dead mutant. The domain boundaries are based on recent crystal structure [64] but are annotated according to the isoform 1 (some authors [64] use the 15 amino acid shorter isoform 2 for numbering).

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the function of AP-3 [40]. Surprisingly, given the well described and important functions, mice lacking PI4K IIα kinase activity initially appear normal and only later manifest neurodegeneration [41]. It is well documented that proteins can regulate the physical properties and/or shape of the biological membranes [42–45]. However, the structural and functional analysis of PI4K IIα suggests that in fact the membrane regulates the activity of PI4K IIα enzyme [46–48]. Zhou and colleagues [46,47] suggest that the fluidity of the membrane (i.e. the cholesterol content) regulate its activity. Our own structural analysis revealed a lateral hydrophobic pocket within the C-lobe of the kinase domain that is important for proper alignment of the kinase with respect to the membrane and might be involved in binding a suitable hydrophobic ligand at the surface of the cellular membrane [48].

2.2 Type III PI4Ks

Early studies in yeast identified the 2 isoforms of type III PI4 kinases [49–51] Stt4p and Pik1p. Both Stt4 and Pik1 are essential genes in *Saccharomyces cerevisiae*. The elegant functional studies conducted in yeast revealed that Stt4p functions mainly at the cytoplasm and provides the PI4P substrate for the Mss4p, a PI4P-5 kinase that synthesizes the cytoplasmic pool of $PI(4,5)P_2$ [52]. Later it was found that Stt4p is localized in distinct patches at the cytoplasmic membrane where it forms a complex responsible for PI4P synthesis with Efr3p and Ypp1p [53,54]. Structural analysis revealed that Efr3p and Ypp1p are α-helical scaffolds that recruit and hold the PI4 kinase at the membrane patches [55].

Stt4p corresponds to the human PI4K IIIα enzyme. PI4K IIIα also localizes to the plasma membrane where it forms a complex with ERF3 and TTC7 (analogs of the yeast Efr3p and Ypp1 proteins). A very recent study identified that the transmembrane protein TMEM150 is another regulatory component of the PI4K IIIα complex. TMEM150A reduces the association of TTC7 with the EFR3-PI4KIIIα complex but without impairing the localization of PI4KIIIα at the plasma membrane [56]. Given that the deletion of Stt4 is lethal for *S. cerevisiae* it is not surprising that mice lacking PI4K IIIα exhibit embryonic lethality [57]. Importantly, PI4K IIIα was identified in screens for cellular host factors of hepatitis C virus [18,19,21,22]. These discoveries fueled intense research into PI4K in both academia and pharmaceutical companies, as they implied the PI4K IIIα as a potential drug target.

Pik1p was identified to function mainly at the Golgi and nucleus [58] but the function of Pik1p in the nucleus is not understood [58–60]. Perhaps it only synthesizes the precursor for $PI(4,5)P_2$ – a known regulator of nuclear proteins including RNA polymerase [61,62]. However, this has been suggested for PI4P in the cytoplasmic and other membranes and it turned out to be incorrect. In the Golgi Pik1 regulates trafficking in the secretory pathway. New functions are discovered in the regulation of autophagy, where Pik1p was shown to regulate Atg9p trafficking from the trans-Golgi to the Pre-Autophagosomal Structure (PAS) in selective and nonselective autophagy [63]. Most importantly it synthesizes PI4P – the lipid hallmark of Golgi and TGN.

Pik1p corresponds to the human PI4K IIIβ enzyme. Recent structural analysis clearly showed that it has additional functions besides the catalysis of PI4P formation. Through its helical domain (Fig. 3), PI4K IIIβ can simultaneously recruit Rab11 and its effector FIB3 to the membrane independent of enzymatic activity [64]. As PI4K IIIβ has been identified in screens for +RNA viruses, making the enzyme a potential target for broad spectrum virostatics, it is critical to understand its non-enzymatic function. It is accepted that +RNA viruses must hijack a PI4 kinase to generate membranes that are suitable for replication, providing shelter against the innate immune system of the cell. However, genetic inactivation (deletion) of type III PI4 kinases is detrimental for the animals suggesting that this target is not feasible for commercial drug design [65]. Since we now know that the enzyme has more functions beyond the catalytic domain, these experiments might need to be reexamined.

PI4K IIIβ can recruit proteins to the membrane but must itself also be recruited to endosomal and Golgi membranes. Arf1 has been hypothesized to play a role in PI4K IIIβ recruitment [66], but its function may not be direct. Another known interactor is Frq1 (NCS-1 in mammals) [67] but its role in recruitment is not clear as NCS-1 is not localized to the Golgi in mammals [68]. The multifunctional scaffolding protein 14–3–3 that binds phosphorylated binding partners including enzymes (AANAT), regulators of G-protein signaling and transcription factors such as FOXO [69–71] has been also identified to bind PI4K IIIβ upon its phosphorylation by PKD at residue Ser^{294} residue [72,73]. This binding was proposed to increase enzymatic activity of PI4K IIIβ but later structural analysis revealed that the Ser^{294} residue is located in a disordered loop distant from the kinase domain [64]. Nevertheless, 14–3–3 proteins as cytosolic proteins cannot be the recruiters of PI4K IIIβ to the Golgi. Possibly, the main Golgi recruiter of PI4K IIIβ is ACBD3 (acyl-CoA binding domain containing 3 also known as PAP7, GCP60, GOCAP1 or GOLPH1). ACBD3 was shown to bind PI4K IIIβ and its depletion due to siRNA leads to disperse cytoplasmic localization of PI4 KIIIβ [74]. Future studies are needed to elucidate the molecular mechanisms of PI4 KIIIβ membrane recruitment.

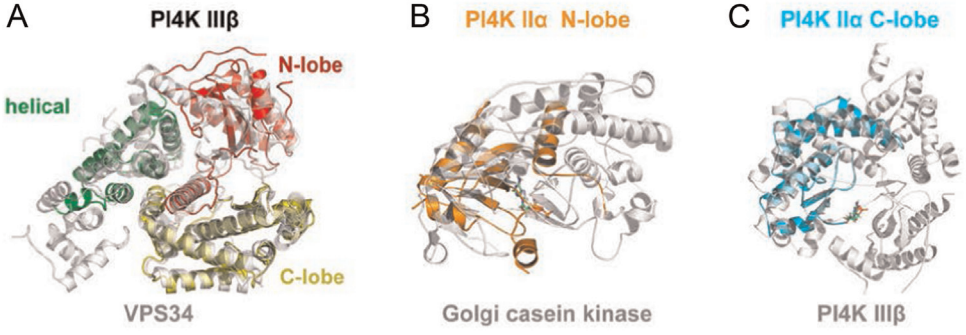

Fig. 3. Structural superposition of PI4Ks on structurally most similar template. (A) The superposition of PI4K IIIβ on the human PI3 kinase VPS34 (PDB ID: 3IHY) reveals that all the subdomains of PI4K IIIβ (helical in green, N-lobe in red, C-lobe in yellow) align very well on the respective parts of VPS34 (in grey). (B) A superposition of the N-lobe from PI4K IIα (in orange) with its structurally closest homolog-the Golgi casein kinase (in grey). (C) A superposition of the C-lobe from PI4K IIα (in cyan) with its structurally and functionally closest homolog-the PI4K IIIβ (in grey).

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3. Structural biology of PI4Ks

The year 2014 was a breakthrough for the structural biology of PI4 kinases as the first structures for PI4K IIα and PI4K IIIβ became available [46,48,64] for the academic community (although it was already available in industry) followed by the structure of PI4K IIβ in 2015 [75]. From analysis of the primary sequence it was quickly thought that type III PI4Ks are analogous to the well-studied PI3Ks [76,77] which was confirmed by structural analysis by Burke and colleagues [64]. Indeed, the human VPS34 PI3 kinase turnout to be close enough to the PI4K IIIβ that the structure could be solved by molecular replacement using the structure of VPS34 (see Fig. 3A for the structural alignment of PI4K IIIβ with VPS34). In addition, PI4K IIIβ was shown to recruit the small GTPase Rab11 through its helical domain that precedes the kinase domain. Importantly, PI4K IIIβ is capable of forming a ternary complex (PI4K IIIβ/Rab11/FIB3) with Rab11 and its effector protein FIB3 suggesting a possible scaffolding role in signaling for the IIIβ enzyme. Unlike Rab11, the 14–3–3 proteins and ACBD3 bind to unstructured parts of the IIIβ enzyme making the traditional crystallographic analysis difficult but the recently developed hybrid methods that rely on small angle X-ray scattering and molecular simulations could provide the structural information [78,79].

Primary sequence analysis and a careful experiment-guided modeling study of PI4K IIIα by Harak and colleagues [80] confirms that the IIIα kinase bears significant similarity to the PI4K IIIβ kinase. The IIIα enzyme contains ARM (Armadillo repeat) domain that is functionally and structurally similar to the helical domain of PI4K IIIβ. PI4K IIIα also has three predicted helical domains and large unstructured N-terminus (Fig. 2). Its N-terminus is responsible for the interaction with the TTC7:ERF3 helical scaffolding complex and thus for the localization in patches at the plasma membrane [53,54]. However, no direct structural information is available for the PI4K IIIα enzyme despite its importance in human health as an essential host factor for HCV. The structure of the kinase domain would be valuable as it would provide information for structure assisted inhibitor design and the structure of the PI4K IIIα N-terminus in complex with TTC7:ERF3 would provide the structural basis for membrane recruitment and patch formation. Moreover, PI4K IIIα was also reported to interact with the NS5A nonstructural protein of HCV [81]; the structural basis for this interaction is also unknown. Thus PI4K IIIα is a promising target for structural analysis.

Type II PI4Ks were predicted to have a novel lipid kinase fold which was confirmed by Zhou and colleagues and our own structural analysis [46,48,75]. Interestingly, the atypical type II kinases are structurally more similar to protein Ser/Thr kinases than to other lipid kinases. Analysis by the DALI server [82] reveals that their overall structure most closely resembles the cell translocating kinase A (ctkA) from the bacterium *Helicobacter pylori* [83]. The structural similarity becomes more profound when the comparison is done separately for the N- and C-lobe of the kinase domain. The N-lobe bears almost no similarity to lipid kinases (no lipid kinase hit in the first 100 hits of the DALI search) whereas it is highly similar to protein kinases. The best DALI hit for the separate N-lobe is the Golgi casein kinase (for structural superposition see Fig. 3B). On the other hand, the C-lobe resembles C-lobes of other lipid kinases. It superposes well with the C-lobe of PI4K IIIβ and but also with the protein kinase mTOR (Fig. 3C). These findings may be important for the evolution of type II PI4 kinases as they imply that the type II PI4K gene could be a fusion of a lipid kinase C-lobe and a Ser/Thr kinase N-lobe.

In the structures of PI4K IIα the ATP or ADP molecules are localized between the N- and C-lobes in a way to align the terminal γ-phosphate in the vicinity of the putative inositol binding pocket. Interestingly, the structural analysis also identified an unusual hydrophobic pocket within the C-lobe that was occupied by the adenine ring of the ATP in our crystals. Using molecular dynamic simulations and mutagenesis analysis it was shown that this pocket is localized in close proximity of the membrane and the key tryptophan residues W^{359} and W^{368} are able to insert their side chains into the lipid bilayer [46,48]. It was reported previously that the activity of PI4K IIα is regulated by cholesterol [84] and that cholesterol also affects its palmitoylation [85]. It is tempting to speculate that the hydrophobic pocket of the C-lobe controls the activity of the PI4K IIα either by directly sensing the fluidity of the membrane (i.e. the cholesterol content) or by directly binding a suitable biological ligand present on the surface or within the lipid bilayer. That could be another link between the cholesterol and PIP metabolism besides the oxysterol-binding proteins [86].

4. PI4Ks in health and disease

The last decade has witnessed a tremendous advance in our understanding of connections between various PI4K enzymes and pathological processes. Several excellent reviews describing these advances in detail are available [87–92], therefore we will only briefly summarize and update the most important findings paving the way toward new treatments based on these discoveries.

As previously mentioned, PI4K IIα is implicated in Wnt signaling, a critically important signaling pathways involved in the tissue development and in the formation of human malignancies. Particularly, the activation of Frizzled by Wnt3a leads to direct interaction of Disheveled with PI4K IIα resulting in an increase of PI4P and $PI(4,5)P_2$ levels. The later phosphoinositide is required for phosphorylation of lipoprotein receptor-related protein Lrp6 and related activation of downstream processes. Notably, silencing of the PI4K IIα gene resulted in suppression of a Wnt3-mediated effect on β-catenin stabilization, a fundamental feature of this signaling pathway [37–39]. While there is no direct evidence connecting PI4K IIα and cancerogenesis via the Wnt pathway, this enzyme is significantly overexpressed in a number of human cancers, malignant melanoma, fibroblastoma, breast cancer, bladder transition cell carcinoma and thyroid papillarycarcinoma [88]. Over expression leads to elevated angiogenesis and is directly correlated with increased hypoxia-induced factor (HIF-1α) expression connected with an alteration of HER/PI3K/ERK signaling. Based on this observation Li and colleagues demonstrated that suppression of PI4K IIα significantly decreased tumor growth in mice and identified this enzyme as novel tumor growth regulator [93]. They also showed that PI4K IIα knockdown can significantly enhance the antitumor effect of EGFR inhibitors, such as tyrphostin (AG 1478) [94]. However, more conclusive and convincing studies are needed to assess the role of PI4K IIα in tumorigenesis.

Class III PI4Ks are hijacked by numerous +RNA viruses and are essential in early stages of their replication. Particularly, viruses from *Flaviviridae*, *Picornaviridae* and *Coronaviridae* families have been shown to exploit PI4Ks to phosphorylate and remodel cellular membranes in order to set up a platform for the replication machinery. Although pharmaceutical companies possessed this information much earlier [95], in 2009, several studies suggested that PI4K IIIα is an essential host factor for the replication of hepatitis C virus (HCV, *Flaviviridae*) [18–20,96,97], a virus that presents a global health issue and infects approximately 80 million individuals worldwide [98]. PI4K IIIα was shown to be a necessary component in the reorganization of cellular membranes indispensable for the formation of a so-called membranous web, an extensively phosphorylated and modified membrane system derived from endoplasmatic reticulum and dedicated to the replication of HCV [99–101]. The role of PI4K IIIβ in the replication of HCV is somewhat less explored. Initially, two siRNA screens

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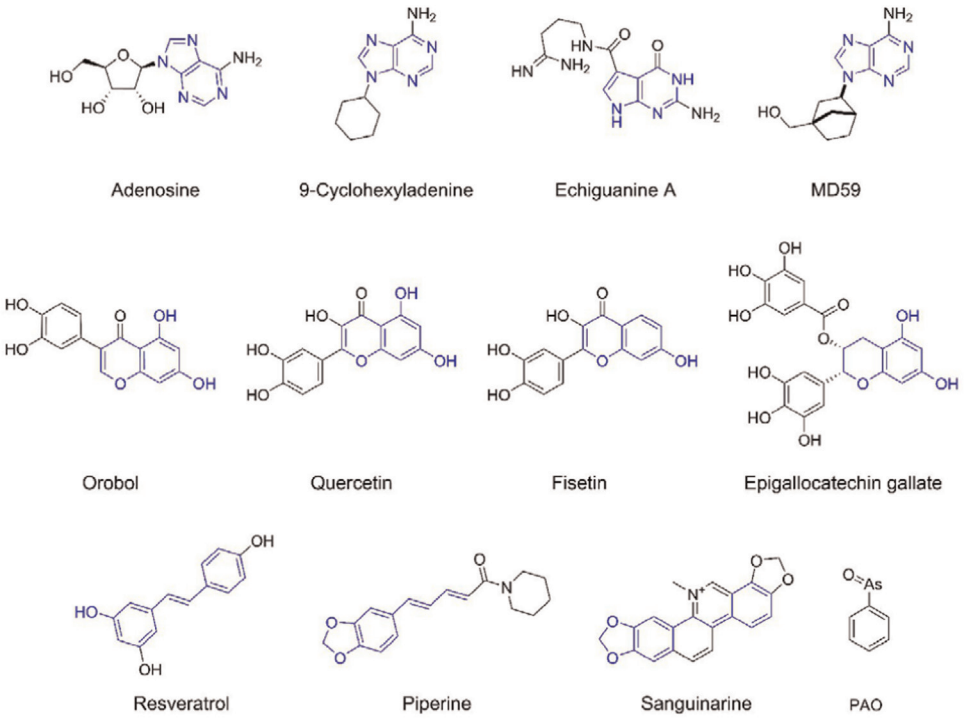

Fig. 4. Selected inhibitors of type II PI4Ks.

demonstrated that depletion of PI4K IIIβ led to the arrest of HCV replication, although this phenomenon was strongly dependent on the virus genotype [19,97]. Whereas the replication of genotypes 1a and 1b was significantly affected, genotype 2a appeared to be resistant to PI4K IIIβ knockdown [97]. Taken together these results suggest that the HCV virus can hijack either PI4K IIIα or IIIβ in order to secure PI4P rich membranes that are required for its replication.

PI4K IIIβ appears to be indispensable for the multiplication of viruses from the *Picornaviridae* and *Coronaviridae* families. The anti-Poliovirus and anti-Coxsackievirus B3 (CVB3) activity of several compounds was linked with their PI4K IIIβ inhibitory activity [102,103]. Sasaki and colleagues showed that this enzyme is also essential in the life cycle of another picornavirus, the Aichi virus (Kobuvirus) and that the virus hijacks PI4K IIIβ through the Golgi residing ACBD3 protein [74,104]. Greninger and colleagues showed that PI4K IIIβ recruitment through the interaction with ACBD3 protein is common to many picornaviruses and suggested that the IIIβ enzyme might be target for a broad anti-picornavirus therapy [105,106]. The molecular mechanism of PI4K IIIβ recruitment probably varies among different members of *Picornaviridae* family but it is generally accepted that the nonstructural protein 3A plays the major role [103]. Although, Arf1 and GBF1 seem to be involved in the membrane recruitment and activation of PI4K IIIβ in uninfected cells they are presumed to participate on this process also during the enteroviral infections [90]. Work on Aichi virus led to conclusion that Golgi adaptor protein ACBD3 is the cellular mediator of interaction between 3A protein and PI4K IIIβ [74,104] but details regarding the recruitment of the IIIβ enzyme by enteroviruses are conflicting. siRNA against ACBD3 was reported to both lower and increase the replication of the poliovirus [105,107] but not of the CVB3 or rhinovirus [108,109], which is surprising as the 3A proteins of these enteroviruses are rather similar and their 3A proteins interact with ACBD3 [105]. The level of knock-down, siRNA off-target effects or the viral strand may play an important role but perhaps an important piece of the puzzle is still missing.

PI4P is important in infection by several intracellular bacteria thought its role is not as well understood as in the case of viruses. *Listeria monocytogenes* requites PI4K IIα for internalization and it has been shown that knockdown of PI4K IIα results in significant decrease in the number of bacteria that invade cells (80% reduction) [110,111]. This reduction in invasiveness suggests that the bacterium requires a PI4K IIα generated pool of PI4P. *Chlamydia trachomatis* exploits PI4K IIα for functional establishment of a nonacidified vacuole (termed an inclusion) necessary for the bacterial replication and depletion of PI4K IIα by siRNA decreases the formation of these replication vacuoles and production of bacterial progeny [112]. *Legionella pneumophila* bacteria also exploit PI4P to anchor its secreted proteins to the *Legionella*-containing vacuoles (LCVs) [113]. The recruitment of *L. pneumophila* effector DrrA to the LCV via PI4P binding is one of the few cases where the PI4P recognition is understood at the atomic level [114]. These findings have practical implications, as PI4K IIα inhibitors could function as a novel class of antibiotics targeting intracellular bacteria.

5. Inhibitors of PI4Ks and their potential in human medicine

Only a handful of compounds that inhibit class II PI4Ks have been described so far and they are neither selective nor potent. In early 90's, several purine and 7-deazapurine derivatives from both natural and synthetic sources (e.g., 9-cyclohexyladenine or echiguanine A) were identified as inhibitors of PI4K from human erythrocytes [115–119] which was subsequently identified as PI4K IIα [120]. An ATP analog was also found to inhibit PI4K class II enzyme from sheep brain with potency comparable to that of adenosine [121]. Recently, we prepared the compound MD59 (Fig. 4), a locked carbocyclic nucleoside, which exerts inhibitory activity against PI4K IIα without affecting class III PI4Ks [122] suggesting that nucleoside scaffold is suitable for design of inhibitors specific against the atypical type II PI4Ks [75]. Furthermore, simple benzoic acid and benzaldehyde derivatives, common isoflavonoids such as orobol, and flavonoids including quercetin

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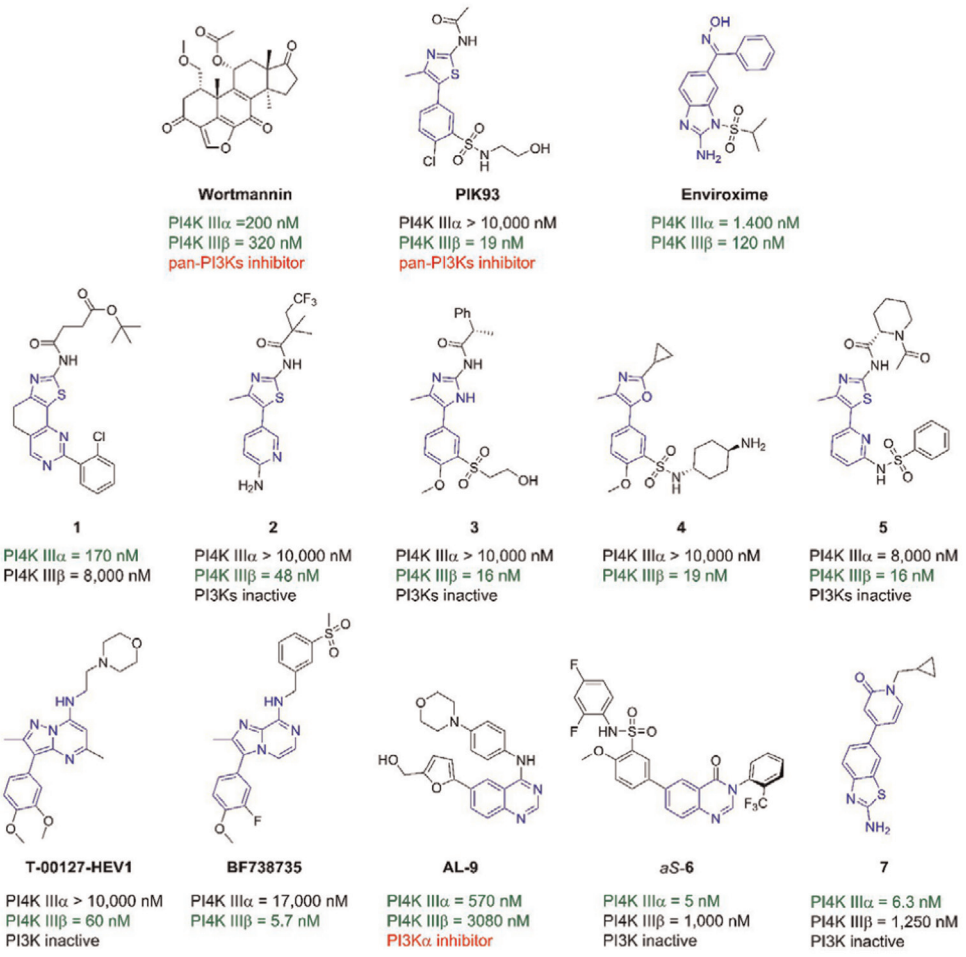

Fig. 5. Nonselective and selective inhibitors of type III PI4Ks.

and fisetin inhibited PI4Ks in early studies [123–125]. Several other natural products including epigallocatechin gallate [126], resveratrol [127], piperine [128], and sanguinarine [129] were recently shown to have potency against both class II isoforms, apart from a number of different cellular effects [130–132]. In addition, type II enzymes are also inhibited by phenylarsine oxide (PAO) and calcium ions. Selected PI4K class II inhibitors are summarized in Fig. 4. Unfortunately, most of the PI4K class II inhibition data were acquired under incomparable conditions and differ significantly; therefore, we elected to omit the nominal IC_{50} values.

The class III enzymes are not sensitive to adenosine [23] but they are inhibited by wortmannin [133–136] and LY-294002 [135], PI3K inhibitors that are structurally different. Numerous compounds with distinct selectivity towards PI4Ks and PI3Ks have been reported [137]. Among them, PIK-93 has received attention due to selectivity toward the PI4K IIIβ isoform [138,139] although this derivative significantly inhibits also PI3K isoforms at similar level [137]. The discovery of connections between phosphatidylinositol 4-kinases and viral, especially HCV, infection started a frantic struggle for novel inhibitors of both class III members. Since PIK-93 was identified as a potent inhibitor of HCV, enterovirus (PV and CVB3), and rhinovirus replication [19,103,140], it was subsequently utilized as a starting point for design of novel selective PI4K class III inhibitors. Interestingly, compounds structurally related to PIK93 are able to selectively inhibit both PI4K IIIα and PI4K IIIβ. A team from Boehringer Ingelheim Ltd. focused on the developing highly potent inhibitors of the IIIα isoform with a chemotype resembling PIK93. Their most selective compound (Fig. 5 (1)) had IC_{50} = 450 nM and exerted significant suppression of HCV replication in cellular replicon assay (EC_{50} = 170 nM). However, the selectivity was rather low with PI4K IIIβ (IC_{50} = 8000 nM) [65]. Another study performed at Boehringer Ingelheim Ltd. identified PIK93 related analogue (Fig. 5(2)) with significant anti-PI4K IIIβ activity (IC_{50} = 48 nM vs. PI4K IIIα IC_{50} > 10,000 nM) exerting only a weak inhibition of two PI3Ks [141]. LaMrache and colleagues identified derivatives similar to PIK93 by high-throughput screening of the Novartis compound library (~1.5 million compounds) as selective PI4K IIIβ inhibitors. The most potent derivative identified (Fig. 5(3)) inhibited the IIIβ enzyme with an IC_{50} = 16 nM while being inactive to other PI4Ks and PI3Ks up to 9.1 μM. The PI4K IIIβ correlated with anti HCV activities in all tested HCV genotypes [142]. The same Novartis group has recently published a structure–activity relationship (SAR) study focused on oxazole derivatives that also exert exclusive selectivity toward PI4K IIIβ (Fig. 5(4)) [143]. Waring and colleagues identified PIK93-like scaffold by focused screening of 100,000 compounds from the AstraZeneca library and explored potential enhancement of selectivity of PI4K IIIβ inhibitors resulting in a selective compound with an IC_{50} = 16 nM (Fig. 5(5)) [144].

Enviroxime [145] and related compounds are long known for their anti-picornavirus activity. Arita and colleagues were the first to unveil their mechanism of action related to the inhibition of PI4K IIIβ [102]. They identified inhibitor T-00127-HEV1 via high throughput screening, and showed that it selectively inhibits PI4K IIIβ (IC_{50} = 60 nM). Although the compound exerted profound anti-enterovirus activity against poliovirus (PV) (EC_{50} = 770 nM), it proved inactive against the hepatitis C virus. BF738735, introduced by van der Schaar and colleagues, possesses a notably similar

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structure to T-00127-HEV1. This compound inhibits PI4K IIIβ with IC_{50} =5.7 nM and reasonable selectivity (PI4K IIIα IC_{50}=17 μM). This derivative excels in broad spectrum inhibition of picornavirus replication (with EC_{50} lower than 100 nM for all 25 tested picornaviruses including PV, EV71, CVB3 and HRV) [146]. BF738735 was also active against HCV 1b with EC_{50}=56 nM. MacLeod and colleagues reported that this compound was also based on a hit from high throughput screening [147]. Enviroxime itself was also proven to inhibit PI4K class III enzymes although with rather low selectivity [89]. Recently, we have prepared compounds based on T-00127-HEV1 with excellent selectivity for PI4K IIIβ (three orders of magnitude difference in IC_{50} values comparing PI4K IIIβ and PI4K IIIα) [148] and our structural analysis revealed that the inhibitors occupy the binding site for the ATP adenine ring [148].

One of the first inhibitors with higher activity against PI4K IIIα than PI4K IIIβ was AL-9 (Fig. 5), originally a presumed direct inhibitor of the NS5A [149,150]. The compound inhibits replication of HCV with EC_{50}=290 nM (genotype 1b) and EC_{50}=750 nM (genotype 2 a) [149]. Inhibitors of NS5A seem to interfere with the function of the NS5A-PI4K IIIα complex but not with catalytic function of PI4K IIIα [151]. Another compound with the quinazoline scaffold was identified by focused screening in GlaxoSmithKline. It was optimized into compound *aS*-6 (Fig. 5) with high inhibitory activity against PI4K IIIα (IC_{50}=5 nM) and diminished potency against PI4K IIIβ and PI3Ks. It is noteworthy that the holder of the activity is only the *aS* atropoisomer whereas the *aR* isomer possesses both lower selectivity and activity against PI4K IIIα. This compound exerts low nanomolar activity in the HCV 1a, 1b and 2a replicon assays [152]. A similar activity and selectivity profile as *aS*-6 was reported for AstraZeneca compound **7** (Fig. 5 (7)) with significantly different structural pattern (one could find some resemblance with enviroxime), which inhibited PI4K IIIα with IC_{50}=6.3 nM and PI4K IIIβ with IC_{50}=1.25 μM. The activity against PI3K was largely suppressed [57,144]. Using this compound and PI4K IIIβ selective inhibitor (Fig. 5(5)), Waring and colleagues showed that PI4K IIIα unlike PI4 IIIβ notably influence the levels of PI4P and products of its phosphorylation in cells and affects cell proliferation in numerous cancer cell lines *in vitro* [144] and that specific inhibitors are useful tool in deciphering the roles of each PI4K in complex cellular environment [153].

Since a bicylic motif is one of the most frequent among kinase inhibitors, several compounds strongly inhibiting PI4K IIIβ can be found among various inhibitors primarily targeting other kinases, e.g., compound NCGC00229610-01, developed as Cdc2-like kinase inhibitor [154], and Torin2, mTOR/PI3K inhibitor [155]. The Raf-1 inhibitor GW5074 possesses a rather unique structure and also significantly inhibits PI4K IIIβ [156]. Weak PI4K IIIβ inhibitory activity was observed on several 6-chlorpurine-based derivatives that exerted activity against CVB3 virus [157].

A specific type of irreversible inhibition against PI4K IIIα was reported by Pelyvás and colleagues. Unlike all the mentioned compounds, which most likely bind to the ATP-binding site, these inhibitors are derived from cyclitol and could, therefore, bind to a different region of the active site [158]. The most important inhibitors of PI4K IIIα and PI4K IIIβ are summarized in Fig. 5.

6. Concluding remarks

+RNA viruses and several intracellular bacteria exploit PI4K to produce PI4P rich membranes. Therefore, PI4Ks are considered potential targets for broad spectrum antiviral agents but their essential role in cellular physiology dictates that the therapeutic window, if it exists, will be small. However, viruses up regulate the enzymatic function of PI4Ks and require every bit of their kinase activity. It is probable that a partial inhibition of these enzymes would cripple the viral replication while the cellular physiology would be fine. This could be true especially if the inhibitors would be applied only for a limited time needed by the immune system to combat the viral infection. As potent and selective inhibitors are now available, studies to assess the use of PI4Ks inhibitors in medicine are finally possible in animal models.

Imidazopyrazines, a new antimalarial compounds that inhibit the intracellular development of multiple *Plasmodium* species at each life stage were shown to be inhibitors of PI4K IIIβ from the parasite [159]. To prepare PI4K IIIβ inhibitors that would be highly selective for a parasite and not for the human protein might be challenging but their use in human medicine would be straightforward as compared to the inhibition of the human enzyme.

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