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Review

Phosphatidylinositol 4-kinases: hostages harnessed to build panviral replication platforms

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Several RNA viruses have recently been shown to hijack members of the host phosphatidylinositol (PtdIns) 4-kinase (PI4K) family of enzymes. They use PI4K to generate membranes enriched in phosphatidylinositide 4-phosphate (PtdIns4*P* or PI4P) lipids, which can be used as replication platforms. Viral replication machinery is assembled on these platforms as a supramolecular complex and PtdIns4*P* lipids regulate viral RNA synthesis. This article highlights these recent studies on the regulation of viral RNA synthesis by PtdIns4*P* lipids. It explores the potential mechanisms by which PtdIns4*P* lipids can contribute to viral replication and discusses the therapeutic potential of developing antiviral molecules that target host PI4Ks as a form of panviral therapy.

RNA viruses use host membranes for replication

Once inside the host cell, viral pathogens need to initiate replication as quickly and efficiently as possible. This is a kinetically challenging problem: the first few synthesized viral replication proteins must find each other and assemble into a replication complex that can replicate the viral genome, in a hostile cellular environment that has vast excesses of host proteins and nucleic acids. Many types of RNA viruses solve this problem by assembling their replication machinery on host intracellular organelle membrane bilayers [1]. Assembling and partitioning replication enzymes in a bilayer can provide significant kinetic advantages for replication reactions, including increasing the probability that replication components will encounter each other; increasing steady-state reaction rates; increasing the effective local concentrations of enzymes and substrates; properly orienting reaction components; and enhancing the sensitivity and speed of responses to changes in enzyme or substrate concentrations [2].

The host membranes that are hijacked by RNA viruses include the endoplasmic reticulum (ER), Golgi apparatus, trans-Golgi network (TGN), endosomes, plasma membrane and mitochondrial outer membrane [1]. The viral replication machinery is assembled in a supramolecular complex on the cytosolic leaflet of these membranes. Recent studies have revealed that the membrane lipid composition plays a crucial role in regulating viral RNA synthesis. PtdIns4*P* lipids (Figure 1) within the cytosolic leaflet of the bilayer have been shown to be required for the replication of a wide variety of RNA viruses, including members of *Picornaviridae* [poliovirus (PV), coxsackievirus, Aichi virus, and enterovirus 71] and *Flaviviridae* [hepatitis C virus (HCV)] families [3–9]. These viruses generate discrete membrane platforms highly enriched in PtdIns4*P* lipids for replication, and they achieve this by selectively recruiting host PI4Ks (Box 1) to phosphorylate PtdIns lipids within membranes (Figure 1). Remarkably different RNA viruses exploit both common and distinct mechanisms by which to hijack host PI4Ks to generate PtdIns4*P*-enriched replication platforms.

Generation of PtdIns4*P* lipid-enriched replication platforms by PV and coxsackievirus

PtdIns4*P*-enriched replication platforms were first observed within host cells infected with either PV or coxsackievirus B3 (CVB3), which are both plus-strand RNA viruses of the genus *Enterovirus* within *Picornaviridae* [3]. Plus-strand RNA viruses make up a large fraction of animal and plant pathogenic viruses, have a significant health and economic impact, and include many notable human pathogens such as PV, HCV, rhinovirus, West Nile virus (WNV), severe acute respiratory syndrome (SARS) virus and Chikungunya virus [1].

Plus-strand RNA serves as both a genetic template for replication and as mRNA from which to synthesize structural and nonstructural viral proteins; the latter comprising the viral replication machinery [1]. In cells infected with enteroviruses, a dramatic remodeling of the host secretory membrane pathway takes place over the course of infection [3] (Figure 2). Within the first ~2 h of PV or CVB3 infection, the replication proteins, translated from the original infecting viral RNA, localize to the Golgi and TGN compartments and commence viral RNA synthesis [3,10]. Later, at peak of replication kinetics (~4 h post-infection), the growing pool of viral replication proteins and viral RNA are found on membrane-bound organelles adjacent to ER exit sites [3] (Figure 2; 4 h). These organelles are 350–700 nm and become the *de facto* sites of viral RNA synthesis for the rest of the infection period, which is another ~6 h for PV or CVB3 [11,12]. Throughout infection, the viral replication membrane platforms contain high levels of the host enzyme phosphatidylinositol 4-kinase IIIβ (PI4KIIIβ) [3]. PI4KIIIβ generates PtdIns4*P* at these membranes [3] (Figure 2) and

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Keywords: virus; replication; PI4 kinase; PI4P lipids; panviral therapy.

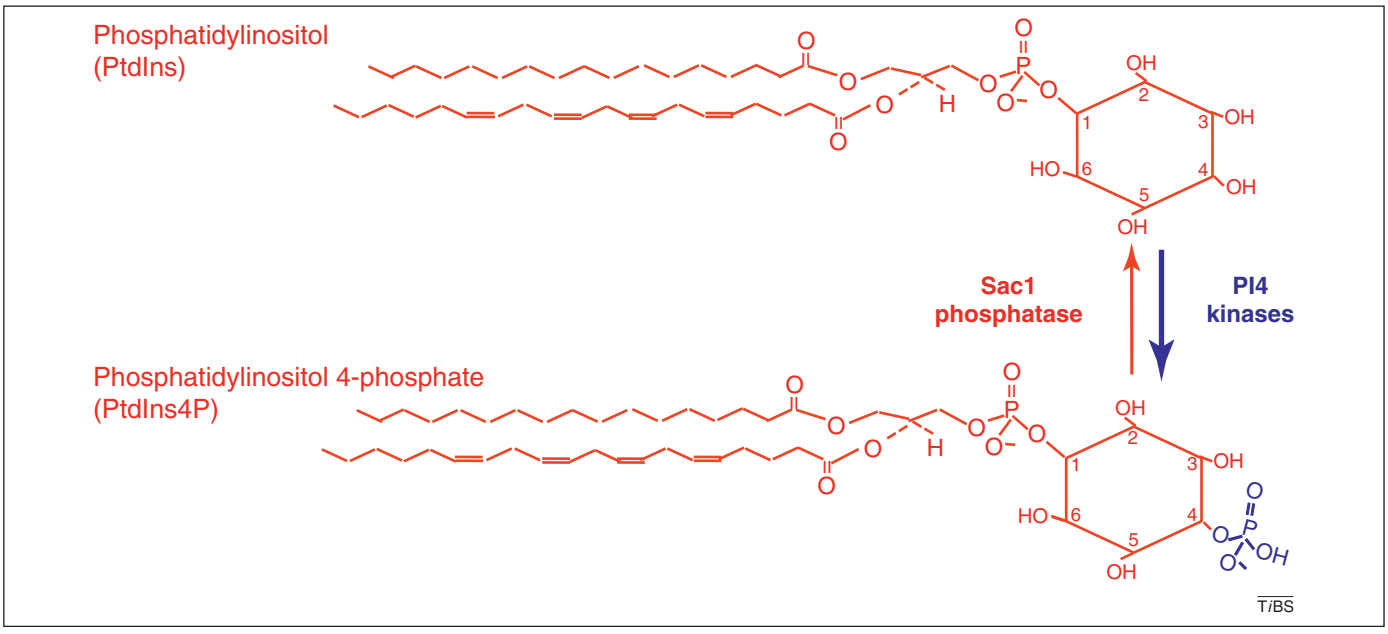

Figure 1. PtdIns4*P* lipids are produced by phosphorylation of the precursor lipid PtdIns. Yeast and mammalian cells have several different PI4Ks (Table 1), which all produce the same enzymatic product: PtdIns4*P*. PtdIns4*P* can be converted back to PtdIns by Sac1 phosphatase.

depletion of PI4KIIIβ activity from host cells, with siRNA or pharmacological kinase inhibitors such as PIK93 [13], potently block both PV and CVB3 RNA synthesis [3,4,8].

In uninfected cells, the Golgi apparatus and TGN contain PtdIns4*P*, a large fraction of which is produced by PI4KIIIβ, a cytosolic enzyme that is recruited to and activated at the cytosolic leaflet of Golgi and TGN membranes by the small GTPase Arf1 [14] (Figure 2, PI4KIIIβ, 0 h, and Table 1). The Arf1-GDP/GTP switch is controlled by guanosine exchange factors and GTPase-activating proteins such as GBF1 and ARFGAP1, respectively [15]. Membrane-bound Arf1–GTP can recruit a diverse array of effectors, including membrane coat proteins COPI and clathrin; cytosokeletal regulators; and lipid-modifying enzymes such as phospholipase D and PI4KIIIβ [15].

RNA viruses typically have small, streamlined genomes encoding only a few proteins. Both Arf1 and GBF1 not only colocalize with enteroviral replication machinery, but Arf1 can bind and hydrolyze GTP throughout the infection period, suggesting the virus can utilize Arf1 effectors to gain access to a wide range of host activities [3,16]. A systematic search for Arf1 effectors that localize to enteroviral replication platforms yielded a surprising finding: although PI4KIIIβ levels on viral replication membranes gradually rose, many other Arf1 effectors, notably the coat proteins COPI and clathrin, were progressively lost [3]. These phenomena were not due to enhanced PI4KIIIβ synthesis and/or COPI or clathrin degradation, but rather the result of a change in spatial distribution: PI4KIIIβ was recruited from the cytosol to the membranes, whereas COPI/clathrin was dislodged from the membranes back to the cytosol. Thus, remarkably, enteroviruses could modulate GBF1–Arf1 effector selection and reprogram the process to favor recruitment of one specific effector, PI4KIIIβ, over others (Figure 3, enterovirus).

The enteroviral culprit that modulates GBF1–Arf1 effector recruitment has turned out to be the ~10-kDa membrane tail-anchored 3A protein. Both 3A and its precursor, 3AB, localize to membrane platforms, are part of the viral replication complex, and are required for viral RNA synthesis [17]. When the CVB3 3A protein is expressed ectopically at low levels in mammalian cells, it localizes to the Golgi/TGN and enhances the recruitment of PI4KIIIβ to those membranes by ~300%, and decreases the coat protein levels at those membranes by ~50% compared to control cells [3]. Increasing ectopic expression of protein 3A causes complete Golgi disassembly and the *de novo* biogenesis of organelles adjacent to ER exit sites that are devoid of coats but contain 3A, PI4KIIIβ, GBF1–Arf1 and PtdIns4*P* lipids [3]. Thus, CVB3 3A protein expression alone is sufficient to modulate GBF1–Arf1 effector recruitment and mimic the secretory pathway remodeling observed during infection with whole virus.

One mechanism by which protein 3A can modulate effector recruitment is by directly interacting with PI4KIIIβ to bring it to the membrane. Protein 3A and PI4KIIIβ co-immunoprecipitate together, suggesting that they form a physical complex [3,8]. Proteomics experiments have also identified a host Golgi adaptor protein, acyl-CoA binding domain protein 3 (ACBD3), as part of the enterovirus 3A–PI4KIIIβ complex; depletion of ACBD3 significantly inhibits replication, suggesting that ACBD3 might mediate PI4KIIIβ recruitment by protein 3A [8] (Figure 3, Enterovirus). Protein 3A has also been shown to bind GBF1 [17]. GBF1 and COPI form a complex before Arf1 activation in uninfected cells [18], therefore, these data suggest that protein 3A might suppress COPI recruitment to membranes by allosterically inhibiting GBF1.

The selective recruitment of PI4KIIIβ during enteroviral infection results in a ~6-fold increase in bulk cellular PtdIns4*P* lipid levels within a time span of 4 h [3]. By using fluorescent protein tagged reporters such as the four phosphate adaptor protein 1-pleckstrin homology-GFP (FAPP1-PH-GFP), which binds membranes by recognizing

Box 1. Structural features of PI4Ks

Type III PI4Ks contain a conserved catalytic domain that shows similarity to those of PI3Ks and a group of Ser/Thr kinases called PI kinase-related kinases (Figure I). All type III PI4Ks also contain a lipid kinase unique domain (LKU) that is also found in PI3Ks and is predicted to be helical. In PI4KIIIα (Figure Ia), a putative PH domain is sandwiched between the LKU and catalytic domains [54]. The N-terminal ~1400 amino acids of PI4KIIIα has a Pro-rich sequence most proximal to the N terminus, then several Leu-rich regions, and putative nuclear localization and nuclear export signals [54]. It has been claimed that PI4KIIIα has an SH3 domain at the N terminus [55], but this has not been confirmed by sequence analysis. PI4KIIIα interacts with the nonstructural NS5A protein of HCV through PI4KIIIα residues 401–600; this activates the lipid kinase activity of PI4KIIIα [5,56].

For PI4KIIIβ and its yeast homolog Pik1p (Figure Ib), the LKU domain is followed by the frequenin (Fq)/NCS-1 binding site [57]. The solution structure of Fq1 with the N-terminal Fq1-binding region of Pik1 shows a helical conformation of the Pik1 peptide, and it is suggested that Fq binding keeps Pik1 in a closed conformation [57]. The Hom2 region is conserved between Pik1p and PI4KIIIβ, and bears some similarity to the LKU domain. It has been identified as the Rab-binding site for both mammalian and *Arabidopsis thaliana* PI4KIIIβ [58]. The Hom2 region is preceded by a conserved Ser-rich segment that contains several phosphorylation sites, including the PKD phosphorylation site [19]. A splice variant of PI4KIIIβ extends this region with an extra 15 residue Ser-rich cassette [59]. PI4KIIIβ also contains an N-terminal Pro-rich sequence, the importance of which is unknown. There are several basic-stretches and Leu-rich sequences within PI4KIIIβ that could serve as nuclear localization signals and nuclear export signals [54], respectively, but it has not been formally proven that these contribute to the nucleo-cytoplasmic shuttling of the enzyme.

Type II PI4Ks are smaller proteins with a kinase domain that shows little sequence homology with those of the type III enzymes (Figure Ic). Their kinase domain contains two stretches that are highly conserved from yeast to humans, separated by an insert that is longer in the yeast and *Drosophila melanogaster* ortholog. Both PI4KIIα and PI4KIIβ contain a conserved Cys-rich domain (CCPCC), which is palmitoylated in both proteins, although to a lesser extent in PI4KIIβ [60]. The yeast homolog Lsb6 contains only one Cys in this region, but has hydrophobic residues in place of the other Cys residues to provide the hydrophobicity needed for membrane interaction. The sequence diversity between the PI4KIIα and PI4KIIβ is larger at the N terminus, and the α enzyme is especially rich in Pro, whereas the β enzyme is highly acidic. PI4KIIα contains a conserved di-Leu motif after the Pro-rich segment that confers binding to the AP-3 clathrin adaptor [61].

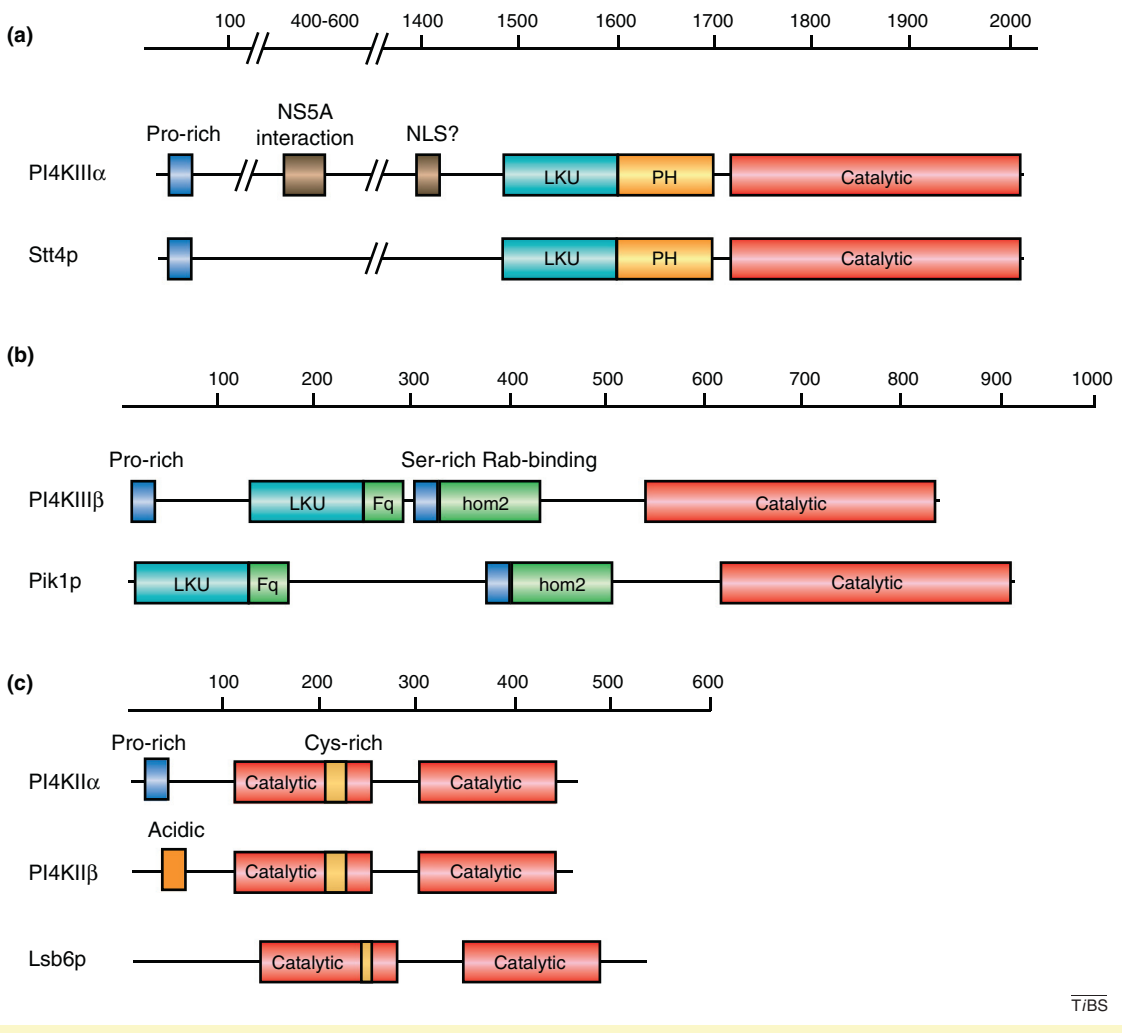

Figure I. Domain organization of PI4Ks. **(a)** Mammalian PI4KIIIα and its yeast homolog, Stt4p. LKU, lipid kinase unique domain; PH, pleckstrin homology domain. **(b)** Mammalian PI4KIIIβ and its yeast homolog, Pik1p. hom2 refers to a domain that shows similarity to the LKU and is the site of binding Rab11. **(c)** Mammalian PI4KIIα and β and its yeast homolog, Lsb6p. The scale at the top of the figure refers to amino acid numbers.

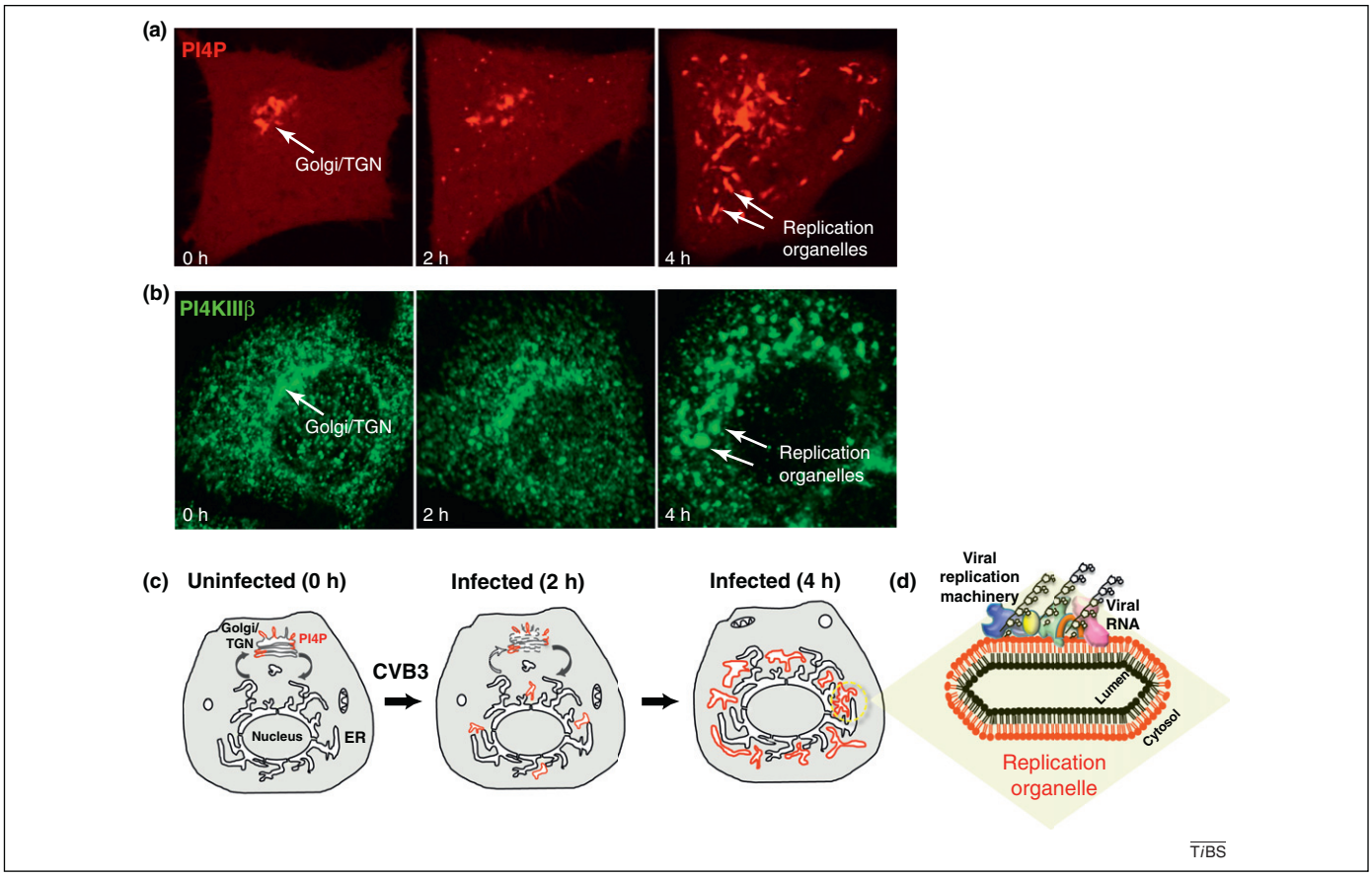

Figure 2. The host cell secretory pathway is remodeled to generate PtdIns4*P* lipid enriched replication organelles. (**a–c**, 0 h) In uninfected cells the Golgi/TGN maintains a steady-state small pool of PI4KIIIβ and PtdIns4*P* lipids. (**a–c**, 2 h) 2 h post-infection with CVB3; the Golgi apparatus begins being resorbed into the ER, while new membranes begin emerging out of ER exit sites enriched in PI4KIIIβ and PtdIns4*P* lipids. (**a–c**, 4 h) By peak replication times, the Golgi is completely disassembled, secretory trafficking is blocked and new replication organelles (white arrows) highly enriched in PI4KIIIβ and PtdIns4*P* lipids are generated near ER exit sites. **(d)** These replication organelles serve as a platform for viral replication: viral replication proteins assemble on the cytosolic PtdIns4*P* rich leaflet of these membranes and synthesize viral RNA. The top panels are a sequence of confocal time-lapse images of a human cell infected with CVB3. In (a), the cell expresses FAPP1-PH-GFP, a live-cell PtdIns4*P* lipid reporter. In **(b)**, native PI4KIIIβ is immunostained.

both PtdIns4*P* and Arf1, the levels of PtdIns4*P* have been shown to increase at the replication platforms compared to surrounding membranes [3] (Figure 2). Given their small volume, the increase in PtdIns4*P* lipid concentration is likely even greater at the replication platforms than the sixfold increase observed in whole cells. Depleting PtsIns4*P* at these sites, by either blocking PI4KIIIβ kinase activity pharmacologically or by converting PtdIns4*P* back to PtdIns through ectopic expression of Sac1 phosphatase (Figure 1), potently inhibits viral RNA synthesis, pointing to a crucial role for PtdIns4*P* lipids themselves in replication [3,4,8].

It is questionable whether recruitment of PI4KIIIβ to the membrane alone is sufficient to generate the high levels of PtdIns4*P* lipids observed. One possibility is that PI4KIIIβ activity may be stimulated; for instance, protein kinase D (PKD) is known to phosphorylate PI4KIIIβ and stimulate its kinase activity [19]. However, it is unknown whether PKD can localize to the replication platforms. Alternatively, PI4K activity could be directly stimulated by viral machinery, as is the case with HCV [5] (discussed later). Testing the effects of different enteroviral proteins on PI4KIIIβ activity using cell-free, liposome-based phosphorylation assays will shed light on this question.

Utilizing the Golgi/TGN membranes with their ready-made PtdIns4*P* platform to initiate replication in the first few hours post-infection would be kinetically advantageous for enteroviruses. The buildup in protein 3A levels through successive rounds of RNA synthesis and translation would impact GBF1–Arf1 effector selection, and lead not only to enhancement of PI4KIIIβ recruitment to the Golgi/TGN, which would increase PtdIns4*P*, but also to the progressive loss of coat proteins from the same membranes. The latter may explain why the Golgi/TGN membranes are not utilized as replication platforms throughout the infection period (Figure 2). The Golgi/TGN is generated and maintained in cells by membrane trafficking between the plasma membrane, endosomes, TGN, Golgi apparatus and ER compartments. Coat proteins are in part responsible for the trafficking, by facilitating the sorting and sequestration of cargo [20]. For example, when COPI is dispersed from membranes, trafficking from the ER to the Golgi is disrupted and the Golgi is resorbed back into the ER [20]. Similarly, in infected cells, the Golgi is disassembled by peak replication times and the membranes that emerge from ER exit sites, lacking coats, cannot sequester or sort Golgi-bound cargo to form a new Golgi apparatus. Rather, they become so-called 'replication organelles' [3] (Figure 2): a unique organelle highly enriched in PI4KIIIβ enzymes,

Table 1. PI4Ks

Gene name	Enzyme name	Isoforms	Gene location	Cellular localization[b]	Functions	Regulation
Type III PI4Ks (human and yeast)						
PI4KA, PIK4CA (human)	PI4KIIIα, PI4K230 EC 2.7.1.67	Isoform 1[a] Isoform 2	22q11	ER/Golgi [62]; nucleolus [54]	Supplies PtdIns(4,5)P_2 for the PM [63]; regulates ER exit [51]	Unknown; sensitive to Wm and some other PI3K inhibitors [59]
STT4 (yeast)	Stt4p		ChrXII	PM at PM–ER contact zones [64]	Essential gene in most yeast strains [65,66]; supplies PtdIns(4,5)P_2 for the PM [67]; regulates Pkc1 pathways [67] and sphingolipid synthesis [68]; required for cell wall integrity [68]; controls mitotic checkpoints [69]	Upstream: Sfk1 [67], Ypp1p, Efr3 [64,92]; downstream: Rom2 [67]
PI4KB, PIK4CB (human)	PI4KIIIβ, PI4K92 EC 2.7.1.67	Two splice forms [59]	1q21.3	Golgi [62]; nucleus [70]	Regulates exit of certain cargos from the Golgi [14]; supports CERT-mediated ceramide transport to the trans-Golgi [71]; required for cytokinesis in fly spermatogenesis [72]	Arf1[14], NCS-1 [49] phosphorylation by PKD [19] controlling 14-3-3 interaction [73]; Rab11 binding [58]; sensitive to Wm and some other PI3K inhibitors [59]; potently inhibited by PIK93 [13]
PIK1 (yeast)	Pik1p		ChrXIV	Golgi [74]; nucleus [40]	Essential gene [75,76]; required for late Golgi to PM secretion [73,77]; cytokinesis [78]; translation	Frq1 [79], Arf1; downstream: Ypt31 [80]
Type II PI4Ks (human and yeast)						
P4K2A, PIK42A	PI4KIIα, PI4K55		10q24.2	TGN; early and late endosomes [81–83]	Recruits clathrin adaptors AP1, AP3 and GGAs to TGN [61,83,84]; controls fate of endocytic vesicles; promotes EGF receptor degradation [82]; supports Wnt signaling [85]	Palmitoylated [86]; interacts with AP3 [61]
P4K2B, PIK42B	PI4KIIβ		4p15.2	TGN; early and late endosomes [81,87]	No specific function has been assigned; may be redundant with PI4KIIα	Palmitoylated [60]; Rac regulates its membrane recruitment [60]; associates with Hsp90 [88]
LSB6	Lsb6p		ChrX	PM; vacuolar membrane [89]	Can partially reverse Stt4p but not Pik1p defects [89]; regulates actin binding and movements of endosomes [90]	Binds Las17p (a homolog of the mammalian Wiscott–Aldrich syndrome protein, WASP [90]

[a]The existence of isoform 1 at the protein level is questionable; if it exists, it is a catalytically inactive protein [91].

[b]EGF: epidermal growth factor; GGA: GGA: Golgi-localized, gamma adaptin ear-containing, ARF-binding protein; PM: plasma membrane.

PtdIns4*P* lipids, viral replication proteins and associated host molecules [3,4,8]. One consequence of Golgi disassembly that is beneficial to the virus is that there is a block in trafficking of MHC proteins and cytokines to the cell surface, hence compromising the immune system reaction to the viral invader. Consistent with this, 3A expression in cells has been shown to slow MHC-class 1-dependent antigen presentation [21].

Generation of PtdIns4*P* lipid-enriched platforms by hepaciviruses

The hepacivirus HCV, unlike PV or CVB3, is an enveloped virus that establishes chronic infections within human liver cells and utilizes the host secretory trafficking pathways for assembly and export of its virions [22]. Worldwide, 180 million people are infected with the virus. Currently, there is no effective vaccine and there are limited antiviral treatments available, many of which are toxic to patients. HCV establishes its highly vesicular–tubular and often multi-membrane replication platforms from ER regions devoid of exit sites [23].

HCV has been shown, through human genome siRNA screening, to depend on PI4Ks for RNA synthesis [24–31]. Subsequently, the ER membrane replication platforms of cells infected with HCV have been shown to be highly enriched in PtdIns4*P* lipids, and the total cellular levels of PtdIns4*P* lipids are >3-fold higher than the levels in uninfected cells [3,5,7,32]. Within liver biopsies from HCV-infected individuals, high levels of PtdIns4*P* are observed in infected cells relative to uninfected cells [5]. Finally, depletion of PtdIns4*P* from infected liver cells significantly inhibits HCV RNA synthesis [3,5,7,9,32]. These data indicate that PtdIns4*P* lipids are required for HCV replication and are an important clinical hallmark of the disease.

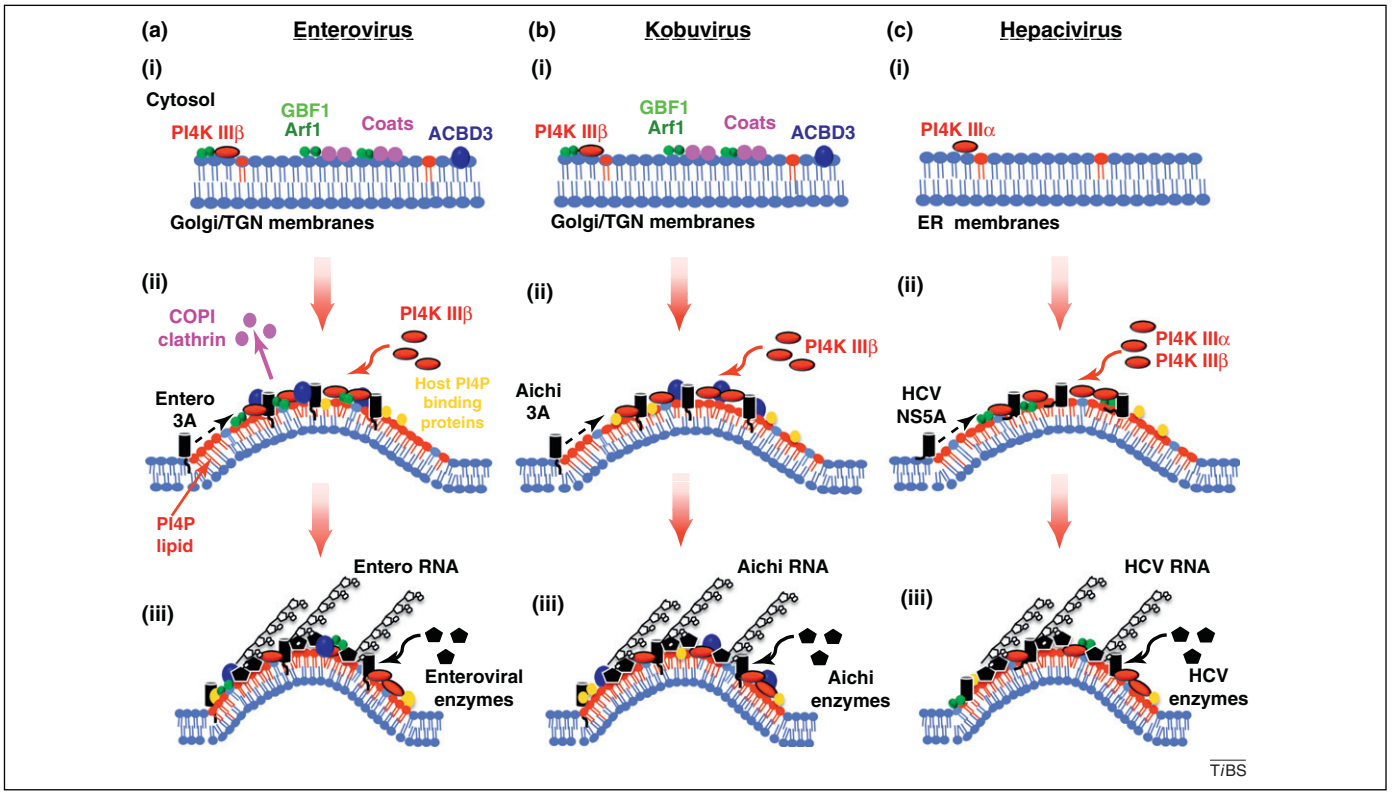

Figure 3. Biogenesis of PtdIns4*P* lipid replication platforms in enterovirus, kobuvirus and hepacivirus infections. Enterovirus replication protein 3A, kobuvirus replication protein 3A and hepacivirus (HCV) replication protein NS5A all hijack host PI4K family members. **(a–c)** Enterovirus and kobuvirus begin building replication platforms on the host Golgi/TGN, whereas HCV initiates replication platforms on the host endoplasmic reticulum (ER) **(i)**. **(a,b)** Both enterovirus and kobuvirus 3A proteins recruit PI4KIIIβ through an intermediary host Golgi protein ACBD3 **(ii)**. **(a)** In enterovirus infections, a rising level of viral 3A proteins (due to viral replication) results in enhanced PI4KIIIβ recruitment to Golgi/TGN membranes and concomitant loss of the coat proteins COPI and clathrin **(ii)**. This leads to the eventual disassembly of the Golgi apparatus and relocation of replication machinery to newly formed organelles that have emerged from ER exit sites **(iii)**. **(b)** In kobuvirus infections, although PI4KIIIβ recruitment is enhanced and the Golgi is disassembled, the fates of coat and GBF1–Arf1 proteins are yet unknown **(ii)**. **(c)** In HCV-infected cells PI4KIIIα and/or PI4KIIIβ enzymes are required for replication and the HCV NS5A protein selectively enhances the recruitment of PI4KIIIα to the ER membrane **(ii)**. **(a–c)** In entero-, kobu- and hepacivirus infections, recruited PI4K enzymes catalyze the formation of high levels of PtdIns4*P* lipids within membrane bilayers. High levels of PtdIns4*P* lipids can modify the structure and organization of the membrane platform **(iii)**. This can regulate viral replication through electrostatic interactions and/or by recruiting other host PtdIns4*P*-binding proteins, which in turn may further differentiate the membrane into specialized microdomains to which viral proteins can partition into, concentrate and assemble into a supramolecular complex for RNA synthesis. Furthermore, PtdIns4*P* lipids themselves potentially help recruit viral proteins through direct interaction (e.g., enteroviral RdRp).

HCV has been reported to enhance PtdIns4*P* lipid production through both recruitment as well as direct activation of host PI4Ks. Specifically, the HCV NS5A protein, which is a component of the replication complex and required for RNA synthesis, has been found to interact with PI4KIIIα and stimulate its kinase activity *in vitro* [5,7,9,33]. Furthermore, ectopic expression of NS5A alone can enhance host PtdIns4*P* lipid levels [5,9]. Remarkably, unlike picornaviruses, which specifically depend on PI4KIIIβ, HCV strains show more flexibility in their usage of host PI4Ks, relying on both PI4KIIIα and PI4KIIIβ for their PtdIns4*P* needs [3,5,7,9,28,32] (Figure 3, hepacivirus). Whether NS5A can also stimulate PI4KIIIβ is unknown. Lastly, GBF1 and Arf1 colocalize with HCV replication complexes, and viral RNA synthesis is sensitive to their inhibition [32,34]; however, it is yet to be determined if they regulate replication through modulation of PI4KIIIβ activity or another effector.

Other RNA viruses that depend on PtdIns4*P* lipids for replication

The picornaviruses enterovirus 71, Aichi virus, bovine kobuvirus and the SARS coronavirus have all been shown to depend on PI4KIIIβ and PtdIns4*P* for replication [4,6,8,35]. Enterovirus 71, whose infection symptoms range from mild effects to severe neurological disease, and for which no effective vaccine or antiviral exists, has been shown to be highly sensitive to the PI4K inhibitor PIK93 [4]. Aichi virus belongs to the *Kobuvirus* genus, which contains a number of emerging viruses. Aichi virus, bovine and porcine kobuvirus infections are being increasingly seen in humans, cattle and swine, respectively [36]. Aichi virus was first isolated in a gastroenteritis outbreak in 1989 in Japan and has since become a causative agent for gastroenteritis outbreaks across the globe. Like the other viruses discussed, Aichi virus generates replication organelles with membranes that are highly enriched in PI4KIIIβ and PtdIns4*P* lipids [6] (Figure 3, kobuvirus). The Golgi apparatus also appears to be disrupted in Aichi-virus-infected cells, although whether this is the result of perturbation of coat protein recruitment to membranes, as with enteroviral infection [3], is unknown [6]. The Aichi virus 3A protein can interact with PI4KIIIβ through ACBD3 [6,8]. Recently, enviroxime compounds, which block replication of rhinovirus, rubella virus and Theiler's murine encephalomyeitis virus [37,38], have been shown to target PI4KIIIβ [4]. Finally, 3A proteins from bovine kobuvirus and rhinovirus 14 have also been shown to complex with PI4KIIIβ [8]. Together, these data suggest the involvement of PI4KIIIβ in a broader spectrum of RNA viruses.

Roles for PtdIns4*P* lipids in viral RNA replication

Plus-strand viral RNA replication consists of two critical processes of viral RNA translation and viral RNA synthesis. The translated replication machinery produces more RNA, which is then either translated or packaged into virions. In cell-free PV replication assays, where translation can be decoupled from RNA polymerization, depleting PtdIns4*P* lipids specifically affects RNA synthesis [3].

The question arises: by what mechanisms do PtdIns4*P* lipids regulate picornaviral or hepaciviral RNA synthesis? In addition to protein–protein interactions, we conjecture that binding to PtdIns4*P* lipids and/or partitioning into PtdIns4*P*-rich domains may facilitate the membrane attachment and concentration of soluble and transmembrane viral proteins to form a functional replication complex (Figure 3). Supporting this idea, the PV RNA-dependent RNA polymerase (RdRp) was shown to exhibit remarkable selectivity for binding to PtdIns4*P* over other lipids [3]. RdRps lack a canonical PtdIns4*P*-binding domain, such as a PH or Epsin N-terminal homology (ENTH) domain; therefore, they probably have a novel PtdIns4*P*-binding motif. In addition to facilitating membrane assembly, PtdIns4*P* might also induce conformational changes in RdRp or other viral proteins in the replication complex, which could modulate their enzymatic activity. Indeed, this is the case for mammalian DNA polymerase α, whose activity can be stimulated upon PtdIns4*P* binding [39].

The discoveries that PtdIns4*P* regulates viral RNA synthesis and that RdRps have specific PtdIns4*P* binding sites could have significant implications for our understanding of mammalian RNA metabolism. PI4Ks shuttle in and out of the nucleus [40]; perhaps contributing to the pools of intranuclear PtdIns*P*s that localize to nuclear speckles [41]. Nuclear speckles are compartments that are enriched in pre-mRNA processing machinery. How PtdIns*P*s are produced and maintained within speckles is unknown, but blocking the production of the PtdIns*P*s within speckles has been linked to disruptions in splicing, 3′ processing and mRNA export [41]. Furthermore, PI4K trafficking to the nucleus is essential in yeast, indicating a crucial nuclear role [40]. Intriguingly, replication organelles share many similarities with mammalian nuclear speckles: both contain RNA, RNA binding proteins and PtdIns*P*s. Thus, understanding the mechanisms by which PtdIns4*P* regulates viral RNA synthesis may help elucidate roles of PtdIns*P*s in mammalian RNA metabolism. Identification of the PtdIns4*P*-binding domain sequence of RdRp may also help uncover other viral, prokaryotic and eukaryotic PtdIns4*P* binding proteins.

High-resolution electron microscopy (EM) studies of enteroviral and dengue replication organelles have revealed that replication platforms have a highly complex 3D organization that has many positive and negative curvature domains [1]. Enrichment for PtdIns4*P* lipids could allow the virus to harness host proteins to modulate lipid content and membrane shape at the platforms, resulting in complex membrane curvature that could potentially drive the sorting and sequestration of viral proteins and RNA for optimal RNA synthesis, as well as protecting them from host innate immune defenses. For example, Golgi phosphoprotein 3 (GOLPH3) binds both PtdIns4*P* lipids and the unconventional Myosin18 to regulate Golgi membrane shape through the actomyosin cytoskeleton [42]. Other examples include the ceramide transport protein (CERT), oxysterol binding protein 1 (OSBP1), and four phosphate adaptor protein 2 (FAPP2), which couple PtdIns4*P* binding to lipid transfer [43] and are crucial for the generation of sterol gradients across the secretory pathway compartments [44]. Indeed, OSBP1 has been shown to be required for HCV replication [45]. Furthermore PtdIns4*P*s can reach very high levels in the small volume of replication platforms, and their negative charges alone could significantly perturb membrane curvature [46]. Membrane curvature might also be aided by other PtdIns4*P* binding proteins such as EpsinR and FAPP2, both of which can induce membrane curvature *in vivo* and *in vitro* [43,47]. Finally, in HCV infections, the biogenesis and maintenance of the unique architecture of the ER-based replication platform can be reversed by depletion of PtdIns4*P* lipids, resulting in the destruction of the unique multi-membrane vesicular–tubular architecture and shrinkage and aggregation of double-membrane vesicles [5].

PI4KIIIβ or PI4KIIIα, independently from making PtdIns4*P* lipids, could also serve as scaffolds to recruit other host proteins to the replication platform. For example, Rab11 GTPase, which regulates cycling of cargo proteins and lipids through endocytic compartments, can bind PI4KIIIβ [48]. This could potentially redirect endocytic cargo to replication organelles. PI4KIIIβ also binds neuronal calcium sensor (NCS)-1 [49], which can regulate ion channels, phosphatases and G-protein-coupled receptors. In the case of enteroviruses and Aichi virus, hijacking PI4KIIIβ through the host ACBD3 protein may function to link viral elements to a whole network of host machinery: ACBD3 has been implicated to serve as a hub connecting many host signaling pathways and cellular lipid- and ion-homeostatic mechanisms, including cholesterol synthesis and trafficking, iron metabolism, protein kinase A signaling and nuclear traffic [50].

Finally, PtdIns4*P* lipids have been implicated in regulating trafficking of some cargo through the secretory and endocytic pathways [14,51], and are also precursors for the production of PtdIns(4,5)P_2, a crucial plasma membrane lipid involved in a multitude of host signaling pathways. It remains to be determined what impact, if any, high PtdIns4*P* lipid levels have on host signaling and trafficking pathways in persistent picornaviral or hepaciviral infections.

PI4Ks as panviral host therapeutic targets

Much of the research and development of antiviral therapeutics has focused on compounds targeting the viral machinery. However, RNA viruses mutate rapidly and frequently become resistant to therapeutics. An alternative and potentially more effective approach would be to target both viral and host components needed for replication. A possible problem with this approach is that inhibition of host components may severely affect the host. This problem could be circumvented by targeting host components, without which, host cells can survive for a period of time. This may be possible if the host target belongs to a

family of proteins that have identical activities, because it could then be redundant. Furthermore, cells might be able to maintain homeostasis with only a fraction of an endogenous enzyme activity, whereas the virus may need full or even stimulated activity of that enzyme for replication.

Remarkably, chemical inhibition of PI4KIIIβ can block enteroviral RNA synthesis without having any significant impact on cell viability, even when cells are treated for several days [3,4,8]. One potential explanation for this finding is that other PI4Ks that are found in overlapping compartments can substitute for PI4KIIIβ. For example, PI4KIIIα is present in the cis-Golgi, PI4KIIα is present in the TGN, and PI4KIIIβ is found in both [52]. Yet, all of these kinases make the same lipid product, PtdIns4*P*. Thus, it is possible that when PI4KIIIβ is inhibited, these other PI4K isoforms can provide PtdIns4*P* to the Golgi/TGN. However, the question then arises as to why picornaviruses do not also compensate through these other kinases when PI4KIIIβ is inhibited. The primary reason at least for enteroviruses is that they can recruit only one specific PI4K, namely PI4KIIIβ [3,8]. The enteroviral 3A protein specifically enhances the recruitment of PI4KIIIβ to the membranes and has no effect on the levels of PI4KIIIα, PI4KIIα or PI4KIIβ [3]. The secondary reason might be that although the steady-state levels of PtdIns4*P* produced by the other family members are sufficient to sustain basic needs for the cell trafficking and signaling machinery, it is not sufficient to sustain viral RNA synthesis. However, viruses are versatile and robust, and so they might find alternative ways to hijack the host lipid kinase machinery; indeed, HCV strains can utilize either PI4KIIIα or PI4KIIIβ to satisfy their PtdIns4*P* needs.

Concluding remarks

Despite the undeniable success of PV vaccines, poliomyelitis remains endemic in Afghanistan, India, Pakistan and Nigeria, due to inadequate vaccination. In another 15 countries around the world, where PV had once been eliminated, it has now resurfaced. Individuals can be infected with multiple different RNA viruses at any given time; recombination among these viruses can occur, leading to the generation of potent viruses that are potentially resistant to vaccines generated against either parent virus. For instance, a recent poliomyelitis outbreak in Madagascar was attributed to the recombination between circulating vaccine-derived PV and a coxsackievirus strain [53]. Simply vaccinating against each of the circulating viruses is not a feasible approach. Now, more than ever, panviral antivirals are needed to control viruses along with vaccines. PI4Ks and PtdIns4*P* lipids appear to have a panviral role in regulating viral replication, and targeting their production could be very effective in inhibiting multiple different viral infections. Designing molecules that inhibit a particular PI4K family member [3,4,13], or disrupt the interaction between the PI4K and the viral hijacking machinery or intermediary host proteins (e.g., ACBD3) [8], are promising approaches to combat multiple different viral infections. However, viruses could become resistant to these types of therapeutics through multiple mechanisms, including hijacking different PI4K family members for PtdIns4*P* production, evolving ways to replicate with less PtdIns4*P* lipids, and substituting other PtdInsP lipids in place of PtdIns4*P* at their replication platforms. Elucidating the mechanisms by which PI4Ks are hijacked and PtdIns4*P* lipids are used by viruses will help us understand potential resistance mechanisms and find ways to circumvent them. Finally, understanding the role of PtdIns4*P* lipids in viral replication may also help reveal novel roles for these lipids in eukaryotic and prokaryotic RNA metabolism.

Acknowledgments

NAB was supported by funds from the National Institutes of Health (R01AI091985) and National Science Foundation (MCB-0822058). NAB thanks members of her lab for critical reading and comments on the manuscript. TB was supported by the Intramural Research Program of the Eunice Kennedy Shriver, National Institute of Child Health and Human Development of the National Institutes of Health.

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