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Review

PI3K-C2α: One enzyme for two products coupling vesicle trafficking and signal transduction

Carlo C. Campa, Irene Franco, Emilio Hirsch *

Department of Molecular Biotechnology and Health Sciences, Molecular Biotechnology Center, University of Torino, Via Nizza 52, 10126 Torino, Italy

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ABSTRACT

The spatial restriction of phosphorylated phosphoinositides generated downstream activated membrane receptors is critical for proper cell response to environmental cues. The α isoform of class II PI3Ks, PI3K-C2α, has emerged as a modulator of receptor localization, acting both in the control of receptor endocytosis and resensitization. This unexpectedly versatile enzyme was found to differentially produce two distinct 3-phosphorylated phosphoinositides and to selectively control distinct steps of vesicular traffic such as endocytosis and recycling. This review focuses on the latest discoveries regarding PI3K-C2α function in vesicle trafficking and its impact on cell biology and mammalian embryonic development.
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1. Class II PI3Ks: PI3K family members emerging from neglect

Phosphoinositide-3-kinases (PI3Ks) are lipid kinases involved in a large set of biological processes, including membrane receptor signaling, cytoskeletal organization and vesicle trafficking [1]. PI3Ks catalyze the phosphorylation of phosphoinositides (PtdIns), lipid moieties derived from the modification of phosphatidylinositol, a basic constituent of plasma and internal membranes that can be reversibly phosphorylated at positions 3, 4 and 5 of its inositol ring. The combination of these modifications results in the generation of seven phosphoinositide species, each specifically enriched in a different cellular compartment and selectively involved in determination of membrane identity and propagation of intracellular signals [2].

The conserved family of PI3Ks counts 8 isoforms, each one able to phosphorylate the 3rd position of the inositol ring. Within the PI3K family, 3 different classes (named class I, II and III) can be recognized. Grouping is based on protein homology and enzyme affinity for specific phosphoinositide substrates. PI3Ks catalyze 3 reactions: PtdIns → PtdIns(3)*P*; PtdIns(4)*P* → PtdIns(3,4)P_2 and PtdIns(4,5)P_2 → PtdIns(3,4,5)P_3, but each class has preferential substrates due to structural differences in the lipid binding domain of the catalytic pocket (Fig. 1) [3,4]. Class I PI3Ks (PI3Kα, PI3Kβ, PI3Kγ and PI3Kδ) catalyze the production of PtdIns(3,4)P_2 and PtdIns(3,4,5)P_3 in vivo. These lipids are known as crucial second messengers activating signal transduction of extracellular stimuli upon engagement of both tyrosine kinase and G-protein coupled receptors at the cell membrane. Class II PI3Ks (namely, PI3K-C2α, PI3K-C2β, PI3K-C2γ) are able to generate PtdIns(3)*P* and PtdIns(3,4)P_2 in vivo and to take part in both signal transduction and vesicle trafficking [4]. Finally, the unique member of class III, PI3KC3, has been recognized as the major source of PtdIns(3)*P* in the cell, controlling the endosomal sorting of internalized receptors and autophagy [5]. In general, class I and III PI3K play an essential role in regulating tissue homeostasis and are implicated different pathologies including cancer, inflammation, diabetes, Alzheimer's disease [5–8]. However, recent work highlights the important function of class II PI3K in mouse development and angiogenesis [9,10] identifying a non-redundant role for these proteins during embryogenesis.

The three class II PI3Ks paralogues found in mammals evolved from a primordial ancestor appearing in *Caenorhabditis elegans* and *Drosophila* [11–13]. PI3K-C2α and PI3K-C2β show ubiquitous

* Corresponding author at: Department of Molecular Biotechnology and Health Sciences, Molecular Biotechnology Center (MBC), University of Torino, Via Nizza 52, 10126 Torino, Italy.

E-mail address: emilio.hirsch@unito.it (E. Hirsch).

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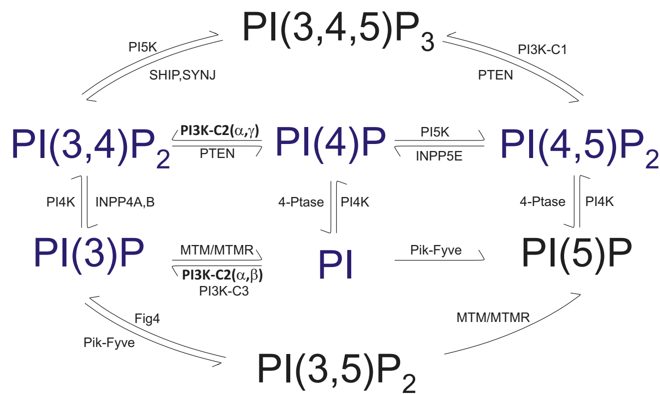

Fig. 1. Pathways of phosphoinositide metabolism. The major routes of phosphoinositide metabolism are depicted. Phosphoinositides directly involved in the control of endocytosis and exocytosis are indicated (blue), like the main enzymes thought to catalyze these conversions. MTM, myotubularin; MTMR, MTM-related protein; Ptase, phosphatase.

expression in mammalian tissues, while PI3K-C2γ is restricted to liver, pancreas and kidney [14,15]. Class II PI3Ks are involved in a multitude of different, and apparently unrelated, processes and their specific function in the cell is still incompletely understood. However, recent genetics and biochemical studies have pointed out non-redundant roles of the α isoform of class II PI3Ks [9,10,16]. These studies show that PI3K-C2α is involved in different signaling pathways and is required for the production of PtdIns3*P* and PtdIns(3,4)P_2 in specific subcellular compartments. These products are critical regulators of membrane dynamics and such findings start to shed light on how PI3K-C2α influences vesicle dynamics and consequent compartmentalization of signaling complexes [9,10,17,18]. This review thus provides an overview of the different functions of PI3K-C2α, particularly focusing on the involvement of this enzyme in membrane trafficking events (Fig. 1).

2. Localization and activation of PI3K-C2α

Modulation of PI3K-C2α localization and activation has been reported to depend on a plethora of different receptors including tyrosine kinase receptors, such as Insulin Receptor (IR) [19,20], EGFR [21], TGFβR [17], VEGFR [10], as well as G-protein coupled receptors such as, S1-P [22], and CXCR2 [23]. PI3K-C2α subcellular distribution is accordingly widespread and with the enzyme being found not only on the plasma membrane but also on the Golgi and on different endosomes ranging from early endosomes to vesicles of the recycling compartment. Such broad distribution pattern likely arises from a modular protein structure, containing multiple domains involved in protein–protein and protein–lipid interactions, such as the N-terminal clathrin-binding, the Ras-binding, two C2, one helical, one kinase and one C-terminal PX domain (Fig. 2) [9,10].

Different studies highlight the function of specific domains in PI3K-C2α recruitment to the plasma membrane. For example, the N-terminal unstructured region shows sequence similarities with the clathrin interactor protein AP-3 β3A [24]. Accordingly, the first 144 aminoacids of the protein are able to directly interact with clathrin [24]. However, deletion of the same region does not completely suppress PI3K-C2α binding to clathrin, indicating the presence of other motifs contributing to this interaction [25]. Notably, PI3K-C2α/clathrin binding modifies PI3K-C2α substrate preference, increasing the ability of this enzyme to generate PtdIns(3,4)P_2 and PtdIns(3,4,5)P_3 [24]. However, the same phenomenon cannot be observed in vivo [26] and, at the moment, the unique products identified for PI3K-C2α are PtdIns(3)*P* and PtdIns(3,4)P_2 [9,10,16].

At the C-terminal region, PI3K-C2α presents a PX-domain. This is a phosphoinositide-binding domain involved in targeting of proteins to cell membranes [27]. The crystal structure of the PI3K-C2α PX domain shows that membrane binding is initiated by non-specific electrostatic interactions with the lipid moiety followed by membrane penetration of hydrophobic residues [28]. This insertion is enhanced in presence of PtdIns(4,5)P_2, the most abundant phosphoinositide of the plasma membrane [2]. Whether PI3K-C2α binding to PtdIns(4,5)P_2 has an impact on the regulation of protein localization to plasma membrane or enzymatic activity in vivo remains to be addressed.

Although the function of the Ras binding, C2 and helical domains of PI3K-C2α have not been elucidated yet, some aspects of the biological functions of these regions can be inferred by the analyses of other PI3K family members. The Ras binding domain is a common feature of class I and class II PI3Ks [1]. In class I PI3Ks the Ras binding domain recognizes the activated form of Ras and allows the plasma membrane recruitment and activation of PI3Ks after stimulation [29,30]. While class I are considered Ras effectors, the Ras binding domain of PI3K-C2β was observed to bind the nucleotide-free form of Ras. This interaction results in the inhibition of PI3K-C2β lipid kinase activity, indicating that class II PI3Ks cannot be considered downstream effectors of Ras [31]. Nonetheless, binding of PI3Ks to small GTPases is observed in multiple contexts [32]. For example, PI3K-Cβ and PI3K-C3 are Rab5 effectors, which are recruited and activated in endosomes [33,34]. In addition, the Drosophila homologue of PI3K-C2α interacts with Rab21 [35]. These studies, together with the finding that PI3K-C2α localizes to internal membranes like Golgi or recycling compartments, suggests that members of the Rab protein family are critical determinants of PI3K-C2α localization.

C2 domains of PI3K-C2α present low affinity for phosphoinositides [28], thus suggesting that these lipids are not their preferential interactors. Identification of protein-binding partners of PI3K-C2α C2 domains can be useful to understand PI3K-C2α function. In agreement, The C2 domain of class I PI3K was shown to interact with the iSH2 of p85α subunit to negative control the lipid kinase activity [36]. The same approach can also be used to study the regulation of PI3K-C2α by the helical domain. For example the helical domain of class I PI3K was observed to interact with beta adrenergic receptor kinase (βARK) and to mediate beta adrenergic receptor (βAR) sequestration, as well as subsequent receptor desensitization [37].

PI3K-C2α share sequence similarities in the activation loop of the kinase domain with class I and III PI3K members, conferring the ability to generate three different phosphoinositides. Accordingly PI3K-C2α recognizes Ptdins, Ptdins(4)*P* and Ptdins(4,5)P_2 as a substrates to generate Ptdins(3)*P*, Ptdins(3,4)P_2 and Ptdins(3,4,5)P_3, in vitro [38,39]. Point mutations in the activation loop of the kinase domain confer substrate selectivity in vitro, which can be exploited to analyze the in vivo role of the PI3K-C2α generated lipids [16,39].

Fig. 2. PI3K-C2α domains composition. Schematic representation of PI3K-C2α and its functional domains. CBR Clathrin binding region, RBD Ras Binding domain.

3. PI3K-C2α in endocytic traffic

PtdIns(3,4)P_2 represents a minor constituent of cell membranes that is produced through the dephosphorylation of PtdIns(3,4,5)P_3 by the lipid phosphatases SHIP, SYNJ and INPP5 or by the phosphorylation of PtdIns(4)P by class I and II PI3Ks (Fig. 1). PtdIns(3,4)P_2 is enriched in the plasma membrane but its levels reaches only 2–5% of those of PtdIns(4,5)P_2 [40].

Reduction of PI3K-C2α abundance induces a decrease in PtdIns(3,4)P_2 that causes mislocalization of endocytic route markers, such as the transferrin receptor [24]. This broad alteration of endocytic markers underlines a critical role of PI3K-C2α in endocytosis. Accordingly, changes in phosphoinositide membrane composition are known to represent key events required for regulated vesicle internalization. In particular, abundant PtdIns(4,5)P_2 on the plasma membrane allows the recruitment of the AP2 complex, which in turn initiates clathrin basket assembly. However, a complex maturation process is required to condition the membrane of clathrin-coated vesicles in order to allow homotypic fusion between early endosomes. During this maturation the majority of PtdIns(4,5)P_2 present on endocytic vesicles is converted into PtdIns(3)P [41]. This process requires the intervention of several kinases and phosphatases. A known player is INPP5E, which is able to convert PtdIns(4,5)P_2 into PtdIns(4)P (Fig. 1). Consistently, depletion of this phosphatase decreases TfR internalization rate [42]. Similarly, down-modulation of INPP4A (type I α PtdIns(3,4)P_2 4-phosphatase), a Rab5 GTPase effector, controls the local enrichment of PtdIns(3)P in the nascent endosome [43]. On the base of these findings, a PI3K activity is required to convert INPP5E-produced PtdIns(4)P into a transient pool of PtdIns(3,4)P_2, which is finally degraded into PtdIns(3)P by INPP4A (Fig. 1). Time-lapse microscopy shows that PI3K-C2α is recruited to clathrin-coated pits (CCP) immediately after their formation. Here, this enzyme produces a PtdIns(3,4)P_2 pool necessary to allow SNX9 binding to the neck of the forming vesicle. This event promotes the formation of a complex containing dynamin, a GTPase involved in the fission of newly formed vesicles, thus promoting maturation of CCP into vesicles (Fig. 3). Interestingly, a PI3K-C2α mutant that has lost the ability to produce PtdIns(3,4)P_2, but retains the capacity to produce PtdIns(3)P, is unable to restore CCP maturation in cells where the PI3K-C2α gene (*Pik3c2a*) is down-regulated by RNA interference. This conclusively demonstrates that PI3K-C2α-mediated production of PtdIns(3,4)P_2 is needed to recruit the machinery necessary to clathrin dissociation and maturation of vesicles into early endosomes [16].

PI3K-C2α activity is also involved in dynamin-independent endocytosis [44]. However, the underlying mechanism is currently unknown. Dynamin-independent endocytic processes involve small GTPases, such as RhoA, CDC42 and Arf6, to regulate membrane invagination, elongation and vesicle scission [45,46]. These small GTPases exert their function by controlling actin cytoskeleton dynamics [45]. Interestingly, the activity of RhoA has been found to be regulated by PI3K-C2α [10,47]. It is thus tempting to speculate an involvement of PI3K-C2α in dynamin-independent endocytosis via small GTPase regulation.

Defects in the endocytic traffic cause reduced internalization of activated membrane receptors [48]. Consequently, proper receptor resensitization and signal transduction result impaired [49,50]. In agreement, PI3K-C2α depletion is associated with multiple dysfunctions in different signaling routes [51]. For some of these, the link between endocytosis and signaling defects has been revealed. Several examples have been found in endothelial cells, where PI3K-C2α appears as a converging point mediating different signaling routes controlling cell viability, differentiation and migration. PI3K-C2α is required for internalization of vascular endothelial growth factor (VEGFR2) [10] and sphingosine-1-phosphate ($S1P_1$) receptors [22]. As a consequence, formation of VEGFR2- and $S1P_1$-positive signaling endosomes is reduced in PI3K-C2α-deficient cells with subsequent impairment in endothelial cell response to these stimuli [10,22]. *Pik3c2a*-silenced cells are also less sensitive to TGFβ stimulation as a consequence of reduced receptor internalization and association with its downstream effector Smad anchor for receptor activation (SARA) on early endosomes [17]. In this situation, PtdIns(3)P normally accumulates in early endosomes of *Pik3c2a*-silenced cells after TGFβ stimulation. On the contrary, TGFβ signal transduction is specifically dependent on PI3K-C2α-mediated production of PtdIns(3,4)P_2 at the plasma membrane (Fig. 3) [17].

A similar involvement of PI3K-C2α-derived PtdIns(3,4)P_2 has been registered in insulin signalling [19]. PI3K-C2α is specifically engaged downstream of the IRβ receptor in pancreatic β cells. Clustering of PI3K-C2α around insulin-bound IRβ results in local production of PtdIns(3,4)P_2 and activation of a specific pool of Akt (Akt1), which in turn propagates the insulin transduction cascade [19]. Although this work does not address the effect of PI3K-C2α activity on IRβ internalization, it provides evidence for membrane compartmentalization as a mechanism ensuing isoform-specific roles of different PI3Ks in insulin signaling. The involvement of PI3K-C2α in many other signaling pathways has been still elusive, but is possible that PI3K-C2α-dependent mechanisms of receptor internalization may allow the control of a broad range of signaling pathways involved in cellular responses.

4. PI3K-C2α in exocytic traffic

Despite the mentioned evidence for PI3K-C2α-mediated PtdIns(3,4)P_2 production, depletion of *Pik3c2a* also induces reduction of PtdIns(3)P level in cells [10,52]. PtdIns(3)P is the main determinant of endosomal compartment identity [2]. This lipid specifically labels endosomal membranes and works as a platform to recruit membrane effectors that mediate endosome maturation, sorting and motility [53].

In addition PtdIns(3)P is a positive and indispensable modulator of autophagy [54]. To allow efficient degradation and recycling, damaged or useless cellular components are enclosed into special membrane compartments called auto-phagosomes before being processed at the lysosome. Class III PI3K Vps34 is recognized as a master regulator of autophagosome formation. Nonetheless, class II PI3Ks α and β play an additive affect in generation of these membrane compartments through PtdIns(3)P production. In particular, they are responsible of at least half of the residual autophagosome formation in Vps34-null mouse embryonic fibroblasts (MEFs) [55]. Involvement of class II PI3Ks in this process is supported also by evidence in *C. elegans*. Here the class II ortholog, PIK-1, produces an initial pool of PtdIns(3)P on nascent phagosomes. Vps34 then intervenes on the same vesicles by producing an additional amount of PtdIns(3)P which is necessary for further organelle maturation [56].

Class II PI3K-dependent PtdIns(3)P has specific functions also in endosomal sorting towards plasma membrane. The Drosophila class II member and PI3K-C2α homologue, Pi3k68D, is involved in protrusion formation in hemocytes, by the endosomal control of cell remodeling [35,57]. Pi3k68D produces PtdIns(3)P in the endosomal compartment. This product is dephosphorylated to PtdIns by the myotubularin phosphatase MTMR13 that is found in a complex with Sbf, a pseudophosphatase with regulatory functions (Fig. 1). This sequence of modifications is entirely required for exit of endosomes from the endocytic compartment and sorting towards the plasma membrane, where they contribute to protrusion formation. Among the effectors of this trafficking are Rab21

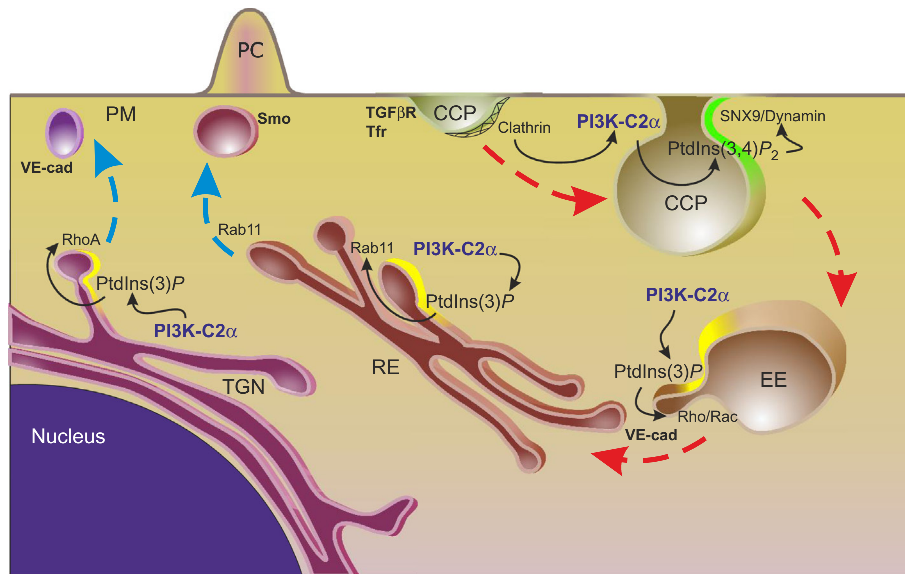

Fig. 3. PI3K-C2α function in different membrane compartments. PI3K-C2α is found in multiple subcellular compartments and its activity influences both endocytic (red arrow) and exocytic pathways (blue arrow). After stimulation of receptors for example for TGFβ (TGFβR) or transferrin (Tfr), PI3K-C2α is recruited to the plasma membrane (PM) at newly formed clathrin-coated pits (CCP) to produce PtdIns(3,4)P_2 (green). This lipid, in turn, is necessary to recruit SNX9 and allow dynamin-mediated fission of CCPs. PI3K-C2α-mediated conversion of membrane lipids is thus necessary to initiate the maturation process that allows fusion of endocytic vesicles with the early endosomal (EE) compartment. Conversely, PI3K-C2α produces PtdIns(3)P (yellow) in the endosomal compartment and this allows activation of GTPase involved in actin remodeling and vesicular trafficking. At the endosome, PI3K-C2α activity controls RhoA and Rac activation. RhoA activation, in turn, is required for trafficking of VE-cadherin (VE-cad) across the endocytic-recycling compartment and from the trans-Golgi network (TGN) to the plasma membrane. PI3K-C2α is also involved in PtdIns(3)P production in the recycling endosomes (RE) in proximity of the primary cilium (PC) base. This process activates the Rab11 pool responsible for vesicle targeting to the primary cilium. The Sonic Hedgehog effector Smoothened (Smo) is among the proteins trafficked to the cilium along this route.

and Rab11. Rab21, in particular, is thought to interact not only with PtdIns(3)P but also with a Pi3k68D/Sbf/MTMR13 complex, which favors its activation [35].

Intriguingly, PI3K-C2α exerts a similar function in mammals. In MEFs, PI3K-C2α has been found to regulate Rab11 activation and polarized trafficking of vesicles directed to the base of the primary cilium [9]. This is a microtubule-based protrusion of the plasma membrane that can be found on the apical side of almost all mammalian cells, but is absent in invertebrates. The primary cilium is a compartmentalized organelle specialized in sensory function. Structural components and signal transduction effectors are trafficked in vesicles from the cytosol and exocyted in proximity of the ciliary base [58]. Only selected proteins are allowed to enter the ciliary compartment. Hence, the primary cilium is particularly enriched for receptors and other components of the signaling machinery. Since multiple signaling pathways important during embryonic development rely on cilia for transduction, ciliary dysfunction dramatically affects embryogenesis [59]. Consistent with a role of PI3K-C2α in this organelle, *Pik3c2a*-null embryos die at midgestation and show signs of impaired Hedgehog (Hh) signaling. This is due to defective accumulation of the Hh signaling effector Smoothened (Smo) on the membrane of *Pik3c2a*-null cells. This process requires the activation of Rab11, because only constitutively active Rab11 is able to restore Hh signaling in *Pik3c2a*-silenced cells (Fig. 3) [9].

Smo is a membrane receptor that traffics to the cilium following different routes [60]. A pool of Smo is internalized from the plasma membrane and crosses the Rab11 positive recycling compartment before reaching the cilium [60,61]. Since PI3K-C2α has been shown to affect internalization of membrane receptors such as TGFβ and VEGFR2 [10,17], it is possible to speculate a similar involvement in Smo internalization. However two lines of evidence have been provided against this hypothesis. First, transfection of the mutant form of PI3K-C2α, able to produce only PtdIns(3)P but not PtdIns(3,4)P_2, in *Pik3c2a*-silenced cells is able to restore Smo trafficking and Hh signaling [9]. This shows that PI3K-C2α-mediated internalization via PtdIns(3,4)P_2 is not required in Hh pathway activation. A second evidence comes from the use of the synthetic Smo agonist, SAG. SAG stimulation, different from the endogenous ligand Sonic Hedgehog, is able to activate Hh downstream targets without the need of Smo translocation to cilia [62]. It is thought that Smo-mediated activation of downstream effectors takes place in an extra-ciliary location that still awaits identification, and may involve the signaling endosome. When *Pik3c2a*-depleted cells are stimulated with SAG they are able to activate the Hh pathway even in the absence of Smo ciliary accumulation [62]. The fact that PI3K-C2α exclusively interferes with cilium-dependent Hh pathway reinforces the hypothesis that the defect seen in *Pik3c2a*-deficient cells is due to impaired trafficking of Smo to cilia. Thus, the role of PI3K-C2α in Hh signal transduction appears to specifically rely on PtdIns(3)P production and subsequent Rab11 activation in the endocytic recycling compartment. Nonetheless, given the function of the Rab11-positive recycling compartment in vesicular trafficking of a broad number of cargo proteins to the cilium, it is envisaged that PI3K-C2α-depletion affects the ciliary targeting of a large number of ciliary proteins besides Smo. For example, low levels of PI3K-C2α correlate with impaired transport of Polycystin-2 to cilia (Franco et al., manuscript in revision). Defective Rab11-mediated trafficking of structural ciliary proteins is thus consistent with morphological defects of cilia observed in *Pik3c2a*-null cells.

Besides Rab11, PI3K-C2α has been shown to regulate the activity of several other GTPases involved in endosomal sorting. In human endothelial cells (HUVEC), PI3K-C2α promotes RhoA, Rac and Rap activation upon VEGF and S1P stimulation (Fig. 3) [10,22]. FRET experiments localized RhoA activation in PtdIns(3)P positive endosomes. In *Pik3c2a*-silenced cells, this process was abolished, together with RhoA-dependent targeting of VE-cadherin to tight junctions. Baso-lateral trafficking of VE-cadherin is indispensable for endothelial cell maturation,

tubule formation and vessel integrity. Consistently, loss of the *Pik3c2a* gene in mouse has been reported to cause defective vasculogenesis that contributes to early embryonic lethality in the mutant mice [10].

Another molecule that is defectively transported to the plasma membrane after PI3K-C2α depletion is the glucose receptor GLUT4 [52]. In muscle cells, membrane delivery of GLUT4 occurs in response to insulin stimulation. PI3K-C2α has been found to be activated by insulin and to promote GLUT4 transport via PtdIns(3)*P* production. Also in this case a small GTPase, TC10, has been found to participate to the process. However, the small GTPase TC10 works upstream and not downstream PI3K-C2α and to promote this enzyme activation upon insulin stimulation [52]. Insulin-evoked pools of PtdIns(3)*P* on the endocytic compartment are subsequently phosphorylated to PtdIns(3,5)P_2 by the 5-kinase PIK5 (Fig. 1) [18]. This lipid is necessary to define a subdomain of the endocytic membrane that favors mTOR activation and, once again, translocation of the protein complex to the plasma membrane [18]. This is another example of a PI3K-C2α-initiated phosphoinositide cascade that finally culminates in compartmentalized activation of signal transduction.

Finally, PI3K-C2α activity has been described in the late phases of exocytosis of neurotransmitters [63] and insulin granules [64]. In particular PI3K-C2α mediates fusion of exocytic vesicles with the membrane. In neuroendocrine cells, the presence of PI3K-C2α has been shown on secretory granules. Here, lack of PI3K-C2α-mediated PtdIns(3)*P* production prevents the ATP-dependent priming phase of exocytosis [63]. The effect on exocytosis is due to PI3K-C2α-dependent regulation of Synaptosomal-associated protein 25 (SNAP25) mediated vesicle fusion. PI3K-C2α is suggested to promote correct SNAP25 proteolysis and function. However, if PI3K-C2α directly participates in vesicle fusion needs further clarification [64]. Similarly, whether these effects are a consequence of reduced endocytosis and recycling is currently unknown.

A final route that is worth mentioning in respect to PI3K-C2α is the biosynthetic pathway. Newly synthetized proteins cross the Golgi apparatus and are finally enclosed in vesicles that depart from the trans-Golgi network (TGN) directed to the plasma membrane. At least two functional interactors of PI3K-C2α have important functions in TGN-plasma membrane trafficking route, namely clathrin and Rab11. Clathrin coated vesicles constantly detach from the TGN membrane [65]. Interestingly, PI3K-C2α has been reported at the TGN in a localization that overlaps with that of the clathrin adaptor complex AP-1 [24,66]. Given the crucial role of PI3K-C2α in clathrin-coated vesicles biogenesis at the plasma membrane, it is easy to hypothesize a similar role at the TGN. However, direct evidence is still lacking, and the presence of PtdIns(3,4)P_2 or PtdIns(3)*P* has never been reported at the Golgi [67]. On the other hand, Rab11 localizes at the TGN and specifically drives vesicle sorting from this location to the plasma membrane [68]. Notably, this small GTPase has also been reported to orchestrate the trafficking of Golgi proteins to the ciliary base in mammalian cells [69–71]. Another player in polarized transport to cilia is the Rab11 downstream effector Rab8 [72], which has also been found mislocalized in *Pik3c2a*-null cells [9]. Given the presence of PI3K-C2α at the Golgi [24,66], it is difficult to imagine that such a high number of PI3K-C2α-connected processes take place without the involvement of this enzyme. Further analyses are likely to shed light on the underlying molecular mechanisms.

5. Conclusion

PI3K-C2α was first discovered in 1997 [38], but its function is only now starting to emerge in all its complexity. PI3K-C2α is involved in multiple roles encompassing primary cilium assembly, Sonic Hedgehog signaling, angiogenesis and receptor-mediated endocytosis. Differently from other PI3Ks that usually generate a single lipid product in vivo, this functional versatility is apparently achieved through the production, in selected cellular compartments, of at least two distinct 3-phosphorylated phosphoinositide moieties: PtdIns(3)*P* at the endosomes and PtdIns(3,4)P_2 at the plasma membrane. Whether the enzyme is indeed able to directly generate these products is as yet debated. Classical experiments with HPLC-based analytical methods identify PtdIns(3)*P* as the most abundant, if not exclusive, PI3K-C2α product [9,10,16]. In agreement, cells lacking PI3K-C2α do not evidence general changes in PtdIns(3,4)P_2 that can be detected by HLPC analysis. However, re-expression in the PI3K-C2α-null background of the PI3K-C2α mutant mimicking the Class III catalytic pocket, and producing PtdIns(3)*P* only, cannot rescue the endocytic defect, indicating PtdIns(3,4)P_2 as a direct but highly localized product. This indicates that production of PtdIns(3,4)P_2 could be rather weak and perhaps subjected to an intense turnover. However, the rescue experiment demonstrates that PI3K-C2α is directly producing PtdIns(3,4)P_2 at least at the plasma membrane and that kinases that can produce PtdIns(3,4)P_2 from PtdIns(3)*P* like PIPKI or III are not involved [73]. At this point a question remains as to whether PI3K-C2α directly produces PtdIns(3)*P* in vivo. The combined action of lipid kinases and phosphatases such as PI3K-C2α and INNP4, a inositol polyphosphate 4-phosphatase, can control the generation and hydrolysis of PtdIns(3,4)P_2, and potentially promote PtdIns(3)*P* production [74]. While the finding of complexes with a class II PI3K coupled to PtdIns phosphatases supports this model [35], an alternative possibility is that products of PI3K-C2α enzymatic activity are determined by the relative abundance of different substrates in the diverse locations where this protein accumulates. For example, PtdIns(4)*P* is highly abundant at the plasma membrane but scarcely represented in recycling endosomes [75]. Conversely, PtdIns is clearly not present at the plasma membrane but it is easily detectable in different types of endosomal membranes [75]. If the relative abundance of substrates dictates the specificity of PI3K-C2α enzymatic activity, targeting of the enzyme to discrete subcellular locations might result the key in controlling the versatile functions of PI3K-C2α. The presence in the protein of multiple and diverse interaction domains supports this view and suggests that protein–protein and protein–lipid interactions through the discrete involvement of selected regions of the protein drive functional diversification. This implies that either membrane composition or membrane-protein complexes can modulate PI3K-C2α localization and perhaps its substrate selectivity. In line with this view, the interaction of PI3K-C2α with clathrin is able to modify not only the subcellular localization but also enzymatic activity at least in vitro [24]. Whether this could occur in vivo is as yet unclear and further cellular and biochemical studies, including the long sought 3D structure of PI3K-C2α, are likely to solve these issues and conclusively elucidate the function of this essential enzyme.

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