Phosphoinositide conversion in endocytosis and the endolysosomal system

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Phosphoinositides (PIs) are phospholipids that perform crucial cell functions, ranging from cell migration and signaling to membrane trafficking, by serving as signposts of compartmental membrane identity. Although phosphatidylinositol 4,5-bisphosphate, 3-phosphate, and 3,5-bisphosphate are commonly considered as hallmarks of the plasma membrane, endosomes, and lysosomes, these compartments contain other functionally important PIs. Here, we review the roles of PIs in different compartments of the endolysosomal system in mammalian cells and discuss the mechanisms that spatiotemporally control PI conversion in endocytosis and endolysosomal membrane dynamics during endosome maturation and sorting. As defective PI conversion underlies human genetic diseases, including inherited myopathies, neurological disorders, and cancer, PI-converting enzymes represent potential targets for drug-based therapies.

PIs[2] are a minor class of comparably short-lived membrane phospholipids that mediate crucial cellular and organismal functions, including signaling, gating of ion channels, cytoskeleton regulation and motility, development, as well as the regulation of intracellular membrane traffic (1, 2). The seven PI species found in mammalian cells differ with respect to the phosphorylation status of the 3-, 4-, and 5-OH group in the inositol ring of phosphatidylinositol and can be interconverted by specific PI kinases and phosphatases (Fig. 1) (1, 2). Different PI species display distinct subcellular distributions with PI 4-phosphates such as phosphatidylinositol 4-phosphate (PI(4)P) being concentrated in the exocytic pathway, in particular in the Golgi complex, the trans-Golgi network (TGN), and at the plasma membrane. In contrast, PI 3-phosphates such as PI(3)P and $PI(3,5)P_2$ are found predominantly within early and late endosomes or lysosomes. As PIs are essential for key functions of the organelles or membrane domains where they reside, they have been postulated to serve as spatiotemporally controlled signposts of membrane identity (1–3). While organelles or membrane transport intermediates (*e.g.* vesicles and tubules) mature, change their functional status, or fuse with each other, their PI content changes. We are only now beginning to understand how PI conversion is spatiotemporally controlled at the molecular level. In this minireview, we highlight recent advances in our understanding of PI conversion with a focus on endocytosis and the endolysosomal system.

$PI(4,5)P_2$ to $PI(3,4)P_2$ conversion controls maturation of endocytic coated pits

Plasma membrane function, including endocytosis, is intimately linked to the presence of $PI(4,5)P_2$, which is generated by consecutive phosphorylation of PI to PI(4)P and $PI(4,5)P_2$ by PI 4-kinase IIIα (4) and type I PI 4-phosphate 5-kinases (PIPKIs) (1). Among other clathrin adaptors, including FCHo, CALM/AP180, epsins, etc., $PI(4,5)P_2$ recruits the heterotetrameric AP-2 complex, which associates with and activates PIPKIs (5). This results in a feed-forward loop of PIPKI activation creating a local $PI(4,5)P_2$ pool required for the initial stages of clathrin-coated pit (CCP) formation. Assembly of clathrin then displaces PIPKIs from AP-2 (6), consistent with the absence of PIP5KIs from clathrin-coated vesicles (7) and from maturing CCPs (5, 8). Feedback inhibition by clathrin thus restricts $PI(4,5)P_2$ synthesis to the early stages of clathrin-mediated endocytosis (CME). Maturation of CCPs is accompanied by the recruitment of the $PI(3,4,5)P_3$ and $PI(4,5)P_2$ 5-phosphatases synaptojanin, oculocerebrorenal syndrome of Lowe protein (OCRL) and Src-homology 2 containing 5-phosphatase 2 (SHIP2). Loss of these enzymes impairs CME due to defective $PI(4,5)P_2$ hydrolysis, resulting in the accumulation of clathrin-coated vesicles in various biological systems (9–14). The exact timing of $PI(4,5)P_2$ turnover by synaptojanin, OCRL, and possibly SHIP2 remains to be determined but may depend on the cell type and tissue.

Recent data show that loss of $PI(4,5)P_2$ late in CME is accompanied by synthesis of phosphatidylinositol 3,4-bisphosphate ($PI(3,4)P_2$) mediated by phosphatidylinositol 3-kinase C2α (PI3KC2α) (15), suggesting a local conversion from $PI(4,5)P_2$ to $PI(3,4)P_2$ as CCPs mature. In support of this model, depletion of plasma membrane $PI(3,4)P_2$ by expression of a membrane-targeted variant of the $PI(3,4)P_2$ 4-phosphatase INPP4B stalls CCP

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[2] The abbreviations used are: PI, phosphoinositide; PI(4)P, phosphatidylinositol 4-phosphate; TGN, trans-Golgi network; PI(3)P, phosphatidylinositol 3-phosphate; $PI(3,5)P_2$, phosphatidylinositol 3,5-bisphosphate; $PI(3,4)P_2$, phosphatidylinositol 3,4-bisphosphate; $PI(4,5)P_2$, phosphatidylinositol 4,5-bisphosphate; $PI(3,4,5)P_3$, phosphatidylinositol 3,4,5-trisphosphate; CME, clathrin-mediated endocytosis; CCP, clathrin-coated pit; PIPKI, PI 4-phosphate 5-kinase; MVB, multivesicular body; GEF, guanine nucleotide exchange factor; ER, endoplasmic reticulum; MTMR, myotubularin-related protein; TRPML, transient receptor potential mucolipin; TFEB, transcription factor EB; SNX, sorting nexin; AMPK, AMP kinase; OCRL, oculocerebrorenal syndrome of Lowe; PI3K, phosphoinositide 3-kinase.

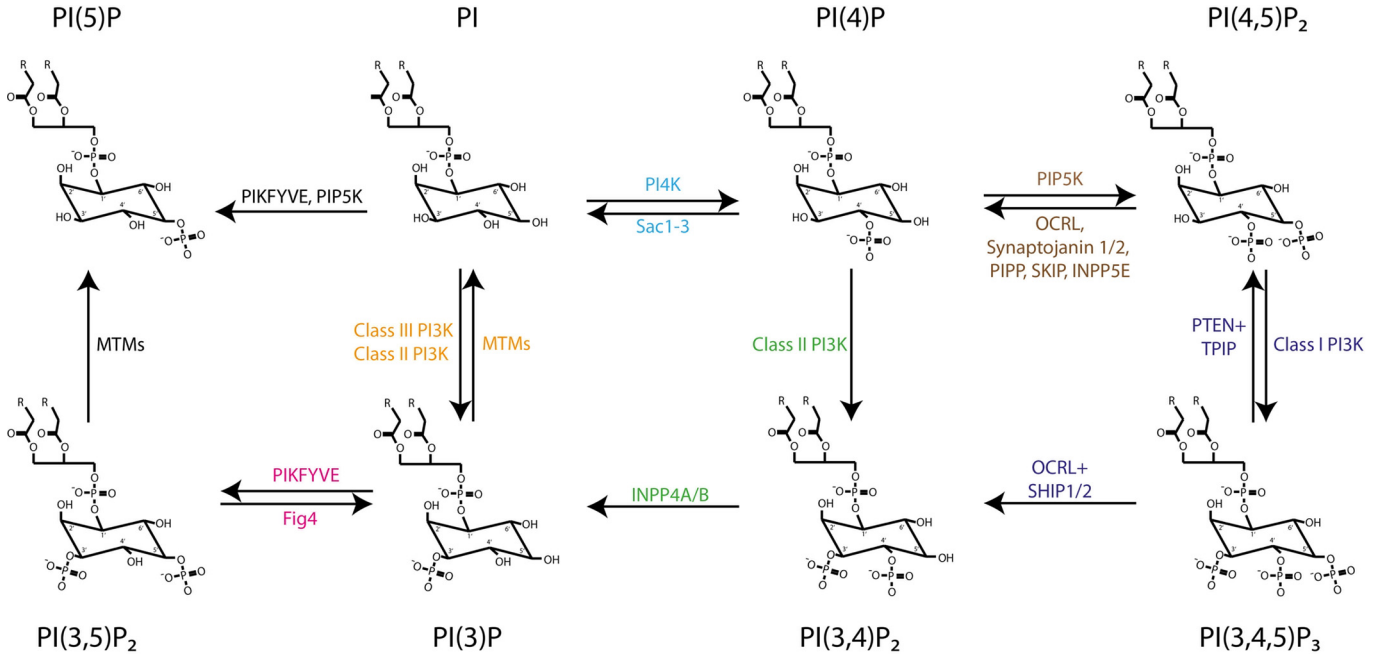

Figure 1. Interconversion of PIs by kinases and phosphatases. Phosphatidylinositol can be phosphorylated by PI4K to yield PI(4)P), which can be further phosphorylated by PIP5K to PI(4,5)P_2, which serves as a substrate for class I PI 3-kinases (*Class I PI3K*) to produce PI(3,4,5)P_3. Phosphorylation of PI at the 3-OH position by class I PI 3-kinase (termed Vps34) (*Class III PI3K*) yields PI(3)P that can be further phosphorylated by PIKFYVE to produce PI(3,5)P_2. PIKFYVE may also synthesize PI(5)P from PI. Class II PI 3-kinases (*Class II PI3K*) synthesize PI(3,4)P_2 from PI(4)P and PI(3)P from the PI. Myotubularins (*MTMs*) are 3-phosphatases that hydrolyze PI(3)P and PI(3,5)P_2. OCRL, synaptojanin 1/2, PIPP, SKIP, and INPP5E are PI(4,5)P_2 5-phosphatases, Fig4 is a 5-phosphatase for PI(3,5)P_2. PI(3,4,5)P_3 can be dephosphorylated by the 3-phosphatases PTEN and TPIP to PI(4,5)P_2 or by the 5-phosphatases OCRL and SHIP1/2 to produce PI(3,4)P_2. The 4-phosphatases Sac1–3 and INPP4A/B dephosphorylate PI(4)P and PI(3,4)P_2, respectively.

dynamics similar to depletion of PI3KC2α. In contrast, plasma membrane CCPs are lost upon PI(4,5)P_2 depletion (16). Thus, PI(4,5)P_2 and PI(3,4)P_2 exhibit distinct regulatory roles during the early and late stages of CME (Fig. 2). How the formation and turnover of these PIs is controlled in time and space remains incompletely understood. Clathrin appears to play a dual role in this process as it not only restricts PI(4,5)P_2 production to early stages but also is essential for the recruitment and activation of PI3KC2α (15, 17). It is likely that additional factors, such as plasma membrane PIs and/or small GTPases, control the nanoscale localization and activity of PI3KC2α. Moreover, it is possible that the activity of PI3KC2α is physically and functionally linked to hydrolysis of PI(4,5)P_2 by PI 5-phosphatases, which generate the PI3KC2α substrate PI(4)P. Dissecting these mechanisms in detail remains a fruitful area for future studies.

Recent computational modeling and super-resolution imaging data show that local PI(3,4)P_2 synthesis by PI3KC2α at CCPs triggers the selective recruitment of the PX-BAR domain protein sorting nexin 9 (SNX9; and its close homolog SNX18) (18) to the invagination neck where its self-assembly regulates membrane constriction (19). Interestingly, the membrane-deforming activity of SNX9 is controlled by an allosteric structural switch involving coincident detection of the clathrin adaptor AP-2 and PI(3,4)P_2 at endocytic sites (20). This mechanism thus allows the spatiotemporal coupling of SNX9/18-mediated membrane constriction to the progression of the endocytic pathway (21).

As SNX9 not only interacts with PIs but also with actin-regulatory factors (18), it is conceivable that its action in CME may couple membrane deformation to changes in actin dynamics that–given the non-essential role of actin in CME in mammals–likely are regulatory in nature (22, 23). Consistent with this idea, it has been recently proposed based on *in vitro* studies using liposomes that the concomitant presence of PI(3)P and PI(4,5)P_2 can facilitate F-actin assembly by SNX9 (24). According to this model, PI(3,4)P_2 produced by PI3KC2α is rapidly converted to PI(3)P by the 4-phosphatase INPP4A. This PI(3)P pool serves to recruit SNX9 to late-stage CCPs. Whether PI(3,4)P_2 to PI(3)P conversion can occur at the plasma membrane *in vivo* is uncertain. INPP4A has been localized to endosomes (25) rather than CCPs. Moreover, a re-engineered class III-like PI3KC2α mutant with wild-type PI(3)P-synthesizing activity *in vivo* (26), but unable to make PI(3,4)P_2, fails to rescue defective CME in the absence of the endogenous enzyme (15). These data suggest that PI(3,4)P_2 rather than PI(3)P is required for CCP maturation and SNX9-mediated membrane constriction. Future studies are needed to provide a better understanding of the mechanisms that control PI(3,4)P_2 hydrolysis by INPP4A/B and possibly other enzymes in time and space within the early endocytic pathway.

PI(3)P defines endosomal membrane identity and is key to endosome function

PI(3)P is a hallmark of the endosomal system and is of key importance for endosome function. Within the endosomal system, cargo may be recycled to the cell surface, trafficked retrogradely to the TGN, or sorted to multivesicular bodies (MVBs)/late endosomes for lysosomal degradation. Although conversion of plasma membrane-derived PI(3,4)P_2 to PI(3)P by INPP4A/B and possibly other PI phosphatases contributes to endosomal

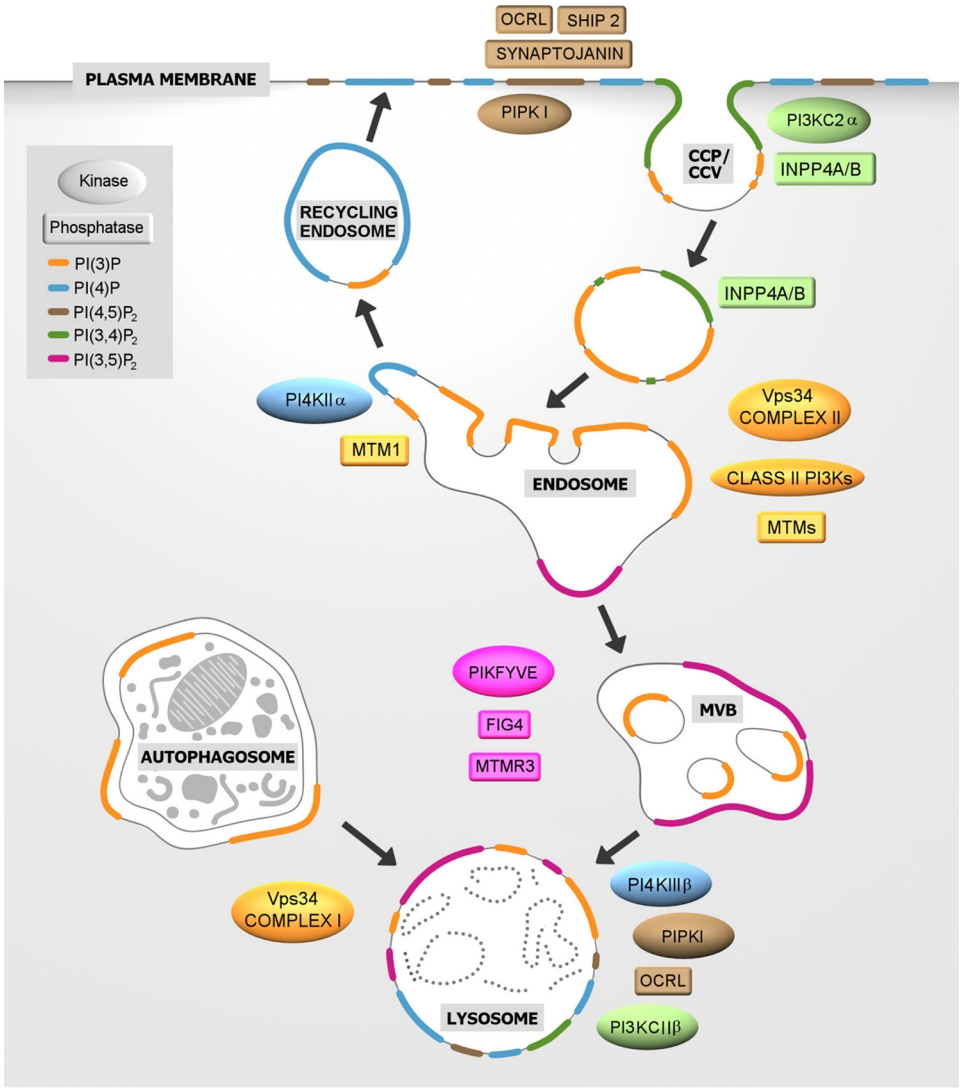

Figure 2. PI conversion in CME and in the endolysosomal system. Clathrin-mediated endocytosis requires plasma membrane PI(4,5)P_2, which is a substrate for the PI 5-phosphatases OCRL, synaptojanin, and SHIP1/2. Class II PI3Kα generates a plasma membrane pool of PI(3,4)P_2 necessary for CCP maturation and formation of free clathrin-coated vesicles (*CCV*). PI(3)P, an essential feature of early endosomes, is generated primarily by the class III PI3K Vps34 complex II with a possible contribution of class II PI3Ks (*encircled* by *dashed line*), either by direct PI(3)P synthesis or indirectly via PI(3,4)P_2 hydrolysis by the PI 4-phosphatases INPP4A/B. Endosomal recycling to the cell surface requires PI(3)P hydrolysis by myotubularin phosphatases (*MTMs*) such as MTM1 and the concomitant generation of PI(4)P by PI4KIIα to enable exocytosis. As endosomes mature into late endosomes/MVBs, the PI(3)P 5-kinase PIKFYVE converts PI(3)P into PI(3,5)P_2. PI(3,5)P_2 turnover at MVBs and/or lysosomes is mediated by MTMs together with the PI(3,5)P_2 5-phosphatase Fig4. Lysosomal membranes contain several PIs such as PI(3)P, PI(4)P, and PI(4,5)P_2. PI(3)P can be produced by class III PI3K/Vps34 directly at the lysosome or is obtained by fusion with autophagosomes, where PI(3)P is produced by VPS34 complex I. PI(4)P is generated by PI4KIIIβ, and PI(4,5)P_2 is hydrolyzed by OCRL. PI(4)P can be converted to PI(3,4)P_2 by the class II PI3KC2β.

PI(3)P levels, the majority of PI(3)P at endosomes is generated by class III PI3K Vps34 and to a minor extent class II PI3Ks (*e.g.* PI3KC2α) (Fig. 2) (26, 27). Class III PI3K forms two distinct complexes that function in autophagy (complex I) and endosomal sorting (complex II). The endosomal complex II consisting of Vps34, p150/Vps15, Beclin 1/Vps30, and UVRAG/Vps38 (28) assembles at early endosomes via association of Vps15/p150 with Rab5-GTP to generate PI(3)P from phosphatidylinositol. Endosomal PI(3)P recruits various downstream effectors, including early endosome autoantigen 1 (EEA1), the ESCRT component hepatocyte growth factor-regulated tyrosine kinase substrate (Hrs, termed Vps27 in yeast), and endosomal SNXs. These proteins directly bind to PI(3)P, *e.g.* via FYVE or PX domains, to regulate endosomal fusion, intralumenal vesicle formation, tubulation, and maturation (29–31).

PI(3)P production at endosomes by class III PI3K/Vps34 is counteracted by the myotubularin family PI 3-phosphatases MTM1, MTMR1, and MTMR2. MTM1 and MTMR2 can associate with the WD40 domain of the Vps15 subunit of the class III PI3K complex II on endosomes. Interestingly, the association of the class III PI3K complex with MTM1 or with endosomal Rabs is mutually exclusive (32, 33). How PI(3)P production by class III PI3K/Vps34 and hydrolysis by MTM1/MTMRs are reciprocally regulated is unknown, but regulation of Rab GTPases, possibly in conjunction with PI signals, is likely of crucial importance. For example, it has recently been shown that Sbf, the *Drosophila* ortholog of the PI 3-phosphatase MTMR13, recruits active MTM to promote PI(3)P turnover at endosomes and functions as a GEF to activate the Rab5 family member Rab21 (34), a regulator

Table 1
Examples of PI-metabolizing enzymes implicated in human genetic diseases

Gene	Catalytic activity	Kinase–phosphatase complexes	Disease
FIG4	PI (3,5)P_2 5-phosphatase	PIKFYFE	Charcot-Marie-Tooth type 4J Yunis-Varon syndrome
MTM1	PI (3)P, PI (3,5)P_2 3-phosphatase	PI4KIIα, VPS34 complex II	Centronuclear X-linked myopathy
MTMR2	PI (3)P, PI (3,5)P_2 3-phosphatase	VPS34 complex II	Charcot-Marie-Tooth type 4B1
MTMR5	Pseudophosphatase	Phosphatase-active MTMs	Charcot-Marie-Tooth type 4B3
MTMR13	Pseudophosphatase	Phosphatase-active MTMs, PI3KCIIα	Charcot-Marie-Tooth type 4B2
MTMR14	PI (3)P, PI (3,5)P_2 3-phosphatase		Centronuclear X-linked myopathy
OCRL	PI (4,5)P_2 5-phosphatase		Lowe syndrome Dent-2 disease
PIKFYVE	PI, PI (3)P 5-kinase	with Fig4	Fleck corneal dystrophy
PIP5K1C	PI (4)P 5-kinase		Lethal congenital contracture syndrome type 3
INPPL1 (SHIP2)	PI (3,4,5)P_3, PI (4,5)P_2 5-phosphatase		Opsismodysplasia
INPP5E	PI (4,5)P_2 5-phosphatase		Joubert syndrome MORM syndrome
SYNJ1	PI (3,4,5)P_3, PI (4,5)P_2 5-phosphatase		Parkinson's disease

of integrin trafficking (35). These results show that PI(3)P levels and endosomal sorting are controlled by a complex interplay between PI kinases, phosphatases, and Rab GTPases. Similar modules may control PI turnover and conversion at other subcellular locations.

PI(3)P to PI(4)P conversion is required for endosomal exocytosis

The crucial importance of PI(3)P for endosomal identity raises the question how cargo can exit the endosomal system, for example during endosomal recycling, which delivers integrins and other receptors to the cell surface. Recent data suggest that surface delivery of endosomal cargo requires loss of endosomal identity by hydrolysis of PI(3)P mediated by MTM1, a PI 3-phosphatase mutated in X-linked centronuclear myopathy patients in humans (Table 1). PI(3)P turnover during endosomal exocytosis is accompanied by PI 4-kinase IIα (PI4KIIα)-dependent generation of PI(4)P (Fig. 2) (36, 37). MTM1 and PI4KIIα are part of a PI(3)P-to-PI(4)P conversion module that involves complex formation of PI4KIIα with MTM1 and with the PI- binding exocyst tethering complex that mediates fusion with the plasma membrane enriched in PI(4)P and PI(4,5)P_2 (36). Membrane recruitment of MTM1 depends on PI4KIIα and is associated with Rab conversion from Rab5 to Rab11, a GTPase switch required for endosomal recycling (38). These data link defective PI conversion at endosomes to muscle and non-muscle defects in patients suffering from X-linked centronuclear myopathy due to mutations in the *MTM1* gene (36). Importantly, defects caused by mutations in MTM1 can be partially reversed by pharmacological inhibition of PI(3)P production by Vps34 (36) or genetic ablation of the class II PI3K PI3KC2β in zebrafish or mouse models (39). These exciting observations may thus pave a way for treatment of this otherwise fatal human disease.

PI(3)P to PI(3,5)P_2 conversion promotes endosomal maturation and degradative sorting

Endosomal maturation is accompanied by Rab conversion from early endosomal Rab5 to late endosomal Rab7 (40, 41) and active cargo sorting into intraluminal vesicles by the ESCRT complex, two processes that critically depend on PI(3)P. PI(3)P activates the Rab7 GEF Mon1-Ccz1 (40–42) and facilitates recruitment of Hrs/Vps27, a subunit of ESCRT-0, and of ESCRT-II, a complex that mediates inward vesicle budding (43). Furthermore, PI(3)P promotes the microtubule-dependent translocation of late endosomes and lysosomes to the cell periphery. This pathway involves membrane contacts between late endosomes/lysosomes and the endoplasmic reticulum (ER) via the kinesin adaptors protrudin and FYCO1 (44, 45). These findings reveal an unexpected connection between endolysosomal PI 3-phosphates and contact site formation with the ER to regulate membrane dynamics and cell signaling (as discussed below).

Endosomal maturation along the late endosome/lysosome pathway is accompanied by conversion of PI(3)P to PI(3,5)P_2 at the limiting membrane of late endosomes (Fig. 2). This process depends on PIKFYVE, a phosphatidylinositol and PI(3)P 5-kinase, which binds to PI(3)P via its FYVE domain and uses the same lipid as a substrate to form PI(3,5)P_2. PI(3,5)P_2 serves several important functions at late endosomes/lysosomes. It is crucial for the sorting of degradative cargo into MVBs (46) and may aid intralumenal vesicle formation by associating with the ESCRT-III component Vps24 (47). Consistently, acute inhibition of PIKFYVE has been shown to block late endosomal protein sorting or turnover as well as retroviral budding, a process that depends on the cellular ESCRT machinery (48). Furthermore, lysosomal PI(3,5)P_2 regulates Ca^{2+} release from the lysosome lumen and is required for acidification by the v-ATPase (49, 50). How precisely PI(3,5)P_2 acts in these pathways and which effector proteins directly bind to PI(3,5)P_2 remain to be elucidated.

Given the multiple functions of PI(3,5)P_2 at late endosomes/lysosomes, the activity of PIKFYVE must be tightly regulated. The PIKFYVE kinase is in fact part of a larger protein complex, including the PI 5-phosphatase Fig4, that is held together by the scaffold protein Vac14. Loss of either PIKFYVE, Fig4, or Vac14 causes a similar depletion of PI(3,5)P_2 in models that range from yeast to mammals (51–53). Active cell signaling along the PI3K-Akt pathway promotes PIKFYVE activity via its direct phosphorylation by Akt (54). A similar activation of PIKFYVE by AMPK-mediated phosphorylation is observed during muscle contraction (55). Much remains to be learned about the physiological roles of PI(3,5)P_2, the regulation of its synthesis and turnover by extracellular signals, and its interplay with other PI lipids.

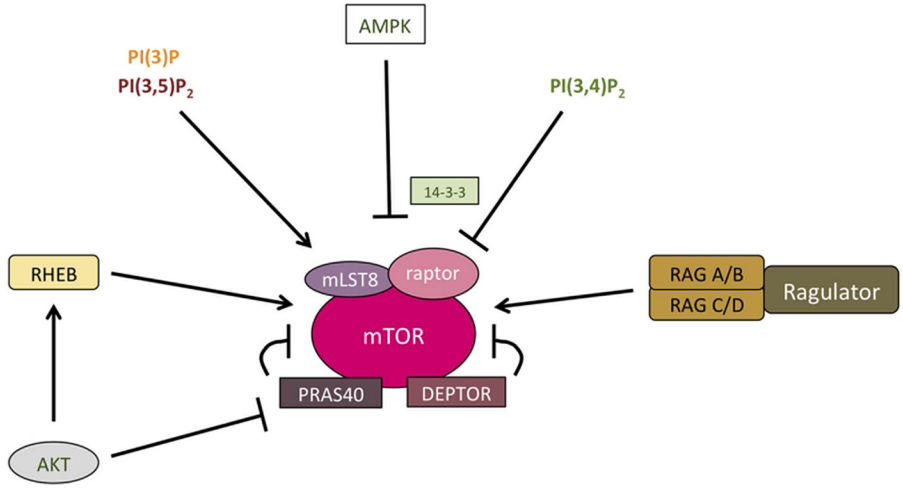

Figure 3. Subunit composition and regulation of mTORC1. mTORC1 consists of the mTOR kinase, the complex defining subunit raptor mLST8, and the two negative regulators PRAS40 and DEPTOR. Activation of mTORC1 depends on recruiting the complex to its place of activation–the lysosomal surface– by Ras-related GTP-binding protein (Rag) GTPases. mTORC1 is fully activated by the RHEB GTPase downstream of AKT-dependent pathways. Furthermore, lysosomal PI(3)P and PI(3,5)P_2 play roles in mTORC1 activation. AMPK and PI(3,4)P_2 can inhibit mTORC1 activity by regulating the binding of raptor to inhibitory 14-3-3 proteins.

PI 3-phosphates are required for autophagosome formation and maturation

Autophagy is a major pathway for the degradation of cytoplasmic material that eventually is targeted to the lysosome lumen for proteolysis. It is initiated by the engulfment of bulk cytosol (containing protein aggregates) and/or organelles by a cup-shaped double-membrane sheet known as the phagophore. The phagophore closes on itself to form the autophagosome, which delivers its contents to the lysosome for degradation by lysosomal hydrolases (56). Autophagy is reciprocally regulated by class I and class III PI3Ks (57). Receptor-mediated class I PI3K signaling activates the mechanistic target of rapamycin complex 1 (mTORC1) during times of nutrient abundance. Active mTORC1 phosphorylates and thereby inactivates the serine/threonine kinase ULK1 (termed Atg1 in yeast) (57) and the acetyltransferase p300 (58), resulting in suppression of starvation-induced autophagy. The serine/threonine kinase ULK1 together with ATG13, FIP200, and ATG101 form part of a multiprotein complex, which plays a pivotal role in the earliest steps of the autophagy process (56). ULK1 stimulates the class III PI3K–Vps34 complex I to generate a local pool of PI(3)P on autophagic membranes (59). This pool of PI(3)P then serves to recruit various proteins needed for the formation of the phagophore (60). A prominent example are WIPIs, PI(3)P-binding proteins with β-propeller folds, that mediate the recruitment of the ATG12–ATG5–ATG16L1 E3 ligase complex to the phagophore membrane to promote LC3 conjugation and autophagosome formation (61, 62).

In addition to PI(3)P, which is essential for early steps of autophagosome formation, PI(3,5)P_2 may regulate later stages of the autophagy/lysosomal pathway (Fig. 2). Defective autophagy/lysosome-mediated protein turnover has been observed under conditions of either impaired PI(3,5)P_2 synthesis via PIKFYVE (63) or defective PI(3,5)P_2 turnover in the absence of MTMR8/9 (64) and related MTMRs (65) or the 5-phosphatase INPP5E, mutations in which cause Joubert syndrome (Table 1), a human ciliopathy (66). Hence, cytoplasmic turnover via the autophagy/lysosomal pathway require spatiotemporally controlled formation and conversion of PI 3-phosphates, the mechanisms of which remain poorly understood.

PI 3-phosphates regulate nutrient signaling

The lysosome acts as a central metabolic regulator by directing the cell into either an anabolic or catabolic state. A central element in this metabolic control hub is mTORC1 (Fig. 3), which integrates extracellular growth factor signals with the cellular nutrient and energy status to elicit downstream signals that directly impinge on autophagy, protein and lipid synthesis, and cell growth (67, 68). Disruptions in mTORC1-mediated lysosomal signaling are implicated in diseases such as diabetes, cancer, and neurological disorders (69, 70). Recruitment of mTORC1 to the lysosome depends on the cellular and lysosomal amino acid status and is mediated by the Rag small GTPases (71–73). The activation status of lysosomal mTORC1 is under further control by growth factor signals, most notably class I PI3K-mediated synthesis of plasma membrane PI(3,4,5)P_3, which activates mTORC1 via its effector Akt (Fig. 3) (74, 75).

Recent data have identified additional PIs other than PI(3,4,5)P_3 as important regulators of mTORC1-mediated nutrient signaling. For example, amino acids stimulate class III PI3K/Vps34-mediated PI(3)P synthesis (76). PI(3)P facilitates lysosomal recruitment of phospholipase D1 (PLD1) via its PX domain, resulting in the formation of phosphatidic acid, which then triggers dissociation of the inhibitory DEPTOR subunit from mTORC1 (77). Similarly, PI3KC2α and PIKFYVE have been implicated in mTORC1 activation via the sequential phosphorylation of phosphatidylinositol to generate PI(3,5)P_2, which activates mTORC1 by associating with the WD40 domain of its Raptor subunit (Fig. 3) (78). In yeast, an additional role of PI(3,5)P_2 in nutrient signaling via recruitment of the mTORC1 substrate Sch9 (the homolog of mammalian ribosomal S6 kinase, S6K) has been proposed (79).

In addition to affecting nutrient signaling via mTORC1 directly, PI(3,5)P_2 may regulate nutrient signaling indirectly via

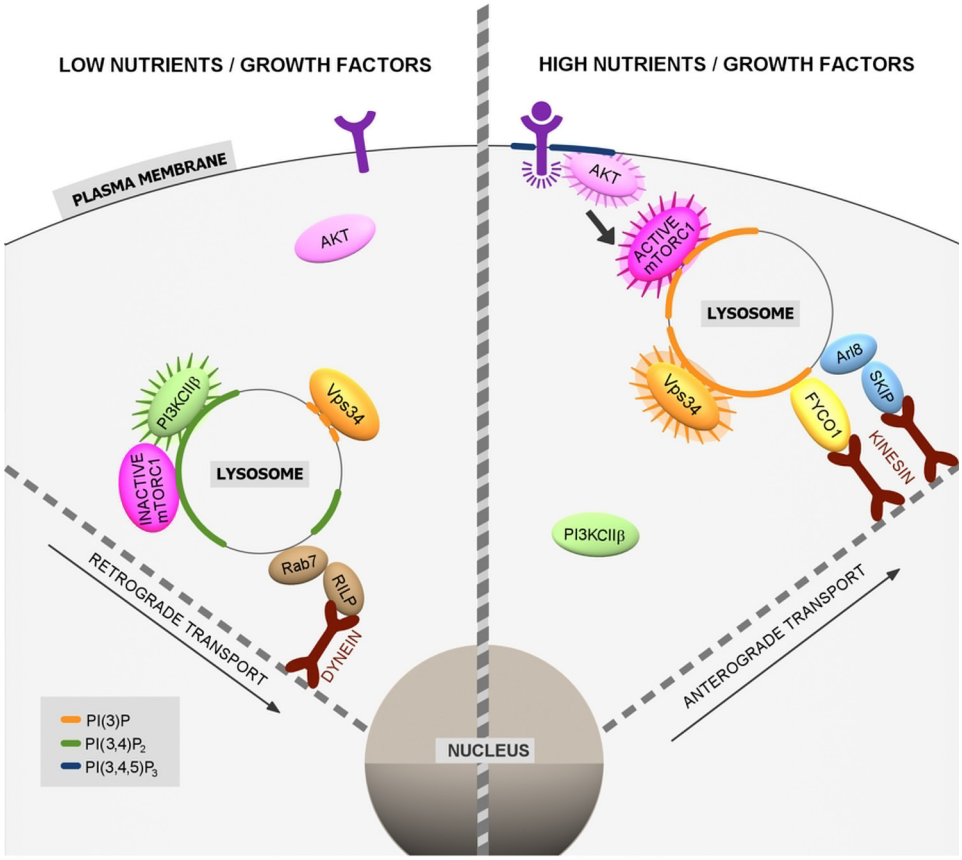

Figure 4. Interplay between lysosome position and function and nutrient signals regulated by PIs. In the absence of nutrients and growth factors, lysosomes are transported retrogradely via dynein motors linked via Rab7/RILP. Growth factor deprivation also triggers PI3KC2β recruitment to lysosomes, where it generates PI(3,4)P_2 to suppress mTORC1 activity, and facilitates perinuclear clustering of lysosomes. High nutrient and growth factor conditions cause activation of the class III PI3K Vps34. Vps34-mediated synthesis of PI(3)P results in lysosomal recruitment of the kinesin 1 adaptor FYCO1, which may supply kinesin motors to the Arl8-associated adaptor SKIP/PLEKHM2. Lysosomes undergo anterograde transport resulting in their dispersion to the cell periphery and in activation of mTORC1, *e.g.* by growth factor-derived Akt signaling.

activating lysosomal TRPML Ca^{2+} channels (49). Blockade of lysosomal Ca^{2+} release has been suggested to inhibit mTORC1 signaling (80). Moreover, it has been shown that lysosomal Ca^{2+} release via TRPMLs triggers activation of calcineurin, resulting in the activation and nuclear translocation of transcription factor EB (TFEB) to up-regulate the expression of autophagy/lysosomal genes (81, 82). These data suggest a possible role for PI(3,5)P_2 in both anabolic (mTORC1 activation) and catabolic pathways (induction of autophagy via TFEB). Future studies will need to address how PI lipids, including PI(3,5)P_2, couple mTORC1 regulation to lysosomal ion homeostasis and protein turnover via the autophagy/lysosome pathway.

PI(3)P and PI(3,4)P_2 play opposing roles in the coupling of nutrient signaling to lysosome position

Recent studies have revealed a surprising but mechanistically poorly understood link between lysosomal position and function and nutrient signaling via mTORC1. Peripheral lysosomes display a more alkaline pH due to reduced v-ATPase function and elevated mTORC1 activity compared with perinuclear lysosomes (83, 84). Lysosomal distribution is largely controlled by the small GTPases Rab7 and Arl8A/B (83). Rab7 links lysosomes via its effector RILP to retrograde dynein motors (85), whereas Arl8A/B directly (in the case of kinesin 3) or indirectly (via the kinesin 1 adaptor SKIP/PLEKHM2 (86)) couples lysosomes to different kinesins 1 and 3 (87), which promote their peripheral dispersion (Fig. 4). Rab7 can also interact with and recruit the late endosomal/lysosomal kinesin 1 adaptor FYCO1, a protein associated with PI(3)P (45), and with PLEKHM1, a negative regulator of Arl8/SKIP/PLEKHM2-mediated lysosomal dispersion (88). Thus, lysosome position and dynamics are determined by a complex interplay between competing GTPases and their associated motors that likely are regulated by multiple factors, including lysosomal Ca^{2+} signals and PIs.

Under conditions of ample nutrient supply, activation of Vps34-mediated PI(3)P synthesis facilitates the recruitment of the PI(3)P-associated kinesin-1 adaptor FYCO1 to late endosome/lysosome contact sites with the ER. There the motor protein kinesin-1 is transferred from the PI(3)P-binding integral ER membrane protein protrudin to lysosome-associated FYCO1 (45). FYCO1-kinesin–mediated anterograde traffic of lysosomes to the cell periphery causes the concomitant activation of mTORC1 (44). Conversely, growth factor deprivation has recently been shown to cause the late endosomal/lysosomal recruitment of class II PI3Kβ (PI3KC2β), which locally produces a lysosomal pool of PI(3,4)P_2 that facilitates perinuclear clustering of lysosomes and suppresses mTORC1 activity (89). These data argue that lysosomal PI con-

tent is subject to nutrient regulation; conversely, lysosomal PIs, including PI(3)P and PI(3,4)P_2, couple lysosome position to the regulation of nutrient signaling via mTORC1 (Fig. 4). How local pools of PI(3)P or PI(3,4)P_2 couple lysosome position to the activity status of mTORC1 is essentially unknown. Late endosomal/lysosomal PI(3,4)P_2 has been shown to facilitate recruitment of 14-3-3 proteins to the mTORC1 subunit Raptor (89), but whether 14-3-3 proteins regulate lysosome position is unknown. Therefore, it is conceivable, if not likely, that some of the factors that control lysosome position are regulated by the local PI content. Conceivably, PIs may also modulate the association of mTORC1 components with the lysosomal transport machinery, such as the recently described interaction between the Arl8-activating BLOC1-related complex and the mTORC1-associated ragulator–LAMTOR complex (90).

So far, little is known about the PI phosphatases that control lysosome position and nutrient signaling. The PI(3)P 3-phosphatase MTMR3 has recently been found to interact with mTORC1, and overexpression of this enzyme inhibits mTORC1 activity (91), further supporting the view that PI(3)P facilitates nutrient signaling via mTORC1. Moreover, as lysosomes contain a variety of other PI lipids, including PI(4)P (92) and PI(4,5)P_2 (93), the question arises whether and how these lipid pools may be subject to nutrient regulation or, conversely, contribute to the coupling of lysosome function and position to nutrient signals and, possibly, to membrane contact site formation with other organelles (94). Given the key functions that lysosomes execute in cell physiology, the answers to these questions may impact our understanding of lysosome-related disease and be of relevance for the treatment of diseases related to lysosome function.

Conclusions and perspectives

Although the role of PIs as signposts of membrane identity and as important signaling factors is well established (1–3), we are only now beginning to appreciate the complex pathways that mediate the spatiotemporally regulated turnover of PI identity that underlies membrane dynamics and signaling in eukaryotic cells and tissues. Recent years have also witnessed the identification of new functions of PIs, for example in the formation of membrane contact sites between various organelles, including the plasma membrane, the ER, and the endolysosomal system, that likely contribute to cellular lipid homeostasis and couple membrane dynamics to signaling pathways (95). Among the major obstacles to resolve these mechanisms are the limited tools to visualize and manipulate PIs in live cells and with nanoscale resolution in real time. As novel techniques ranging from chemical genetic and optogenetic systems to pharmacological inhibitors of PI kinase and phosphatase function, as well as chemical tools (*e.g.* photocaged PI derivatives) (96), and new sensors are being developed (97), we should soon be able to obtain a more complete picture of the mechanisms that govern PI conversion in endocytosis and the endolysosomal system. Such knowledge may turn out to be crucial when it comes to developing new treatment avenues for the growing list of human genetic disorders of PI metabolism (98).

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