OPINION

Coordination between RAB GTPase and phosphoinositide regulation and functions

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Abstract | Membrane trafficking relies on dynamic changes in membrane identities that are determined by the regulation of distinct RAB GTPases and phosphoinositides. RABs and phosphoinositides both act to spatiotemporally recruit effectors of membrane remodelling, including sequential RAB and phosphoinositide activities. New ideas on coordinated regulation of specific RABs and phosphoinositides, achieved by direct physical and functional interactions between their regulatory enzymes, are emerging as a central mechanism to ensure precision and fidelity of membrane trafficking.

Membrane trafficking, the process by which cellular constituents are transported in vesicles, is fundamental to cellular physiology[1]. The organelles and vesicles that are involved in trafficking form discrete membrane domains, which must be dynamically specified to direct numerous intersecting trafficking pathways in the cell. Specific combinations of RAB GTPases and phosphoinositides are responsible for maintaining and coordinating this dynamic 'membrane code'[2,3] (FIG. 1a; TABLE 1).

RAB GTPases and the related ADP-ribosylation factors (ARFs) comprise the largest family of small GTPases, with over 60 members found in humans. Phosphoinositides, which are phosphorylated forms of phosphatidylinositol (PtdIns), are interconverted by large families of enzymes that are encoded by more than 50 genes in humans[4] (BOX 1). Both RAB and phosphoinositide regulators are conserved from yeast to humans, highlighting their fundamental cellular roles and additional adapted roles in metazoan cells. Whereas many aspects of the individual regulation of phosphoinositides or RABs are known, the new idea of direct interactions between phosphoinositide and RAB regulators is emerging as a mechanism to coordinate specific phosphoinositide and RAB functions that are crucial for membrane dynamics.

Although RABs and phosphoinositides are biochemically distinct, there are many striking similarities in both their regulation and essential functions in membrane trafficking. Signalling and scaffolding roles of phosphoinositides that are unlikely to involve RAB GTPases are not described here, nor are the detailed functions of individual RABs and phosphoinositides, which have been reviewed elsewhere[1–3]. This Opinion article focuses on the intriguing parallels and crucial coordination between RABs and phosphoinositides, and highlights specific examples of their crosstalk in membrane trafficking. We also discuss the mechanisms underlying RAB and phosphoinositide co-regulation. We propose that the direct coordination between RABs and phosphoinositides is a regulatory mechanism that is used more broadly than previously appreciated and ensures fidelity during membrane trafficking.

Common themes

RABs and phosphoinositides are both membrane associated and have central roles in membrane trafficking, with their regulation and functions characterized by common themes. Some of these themes are not exclusive to phosphoinositides and RABs (for example, cyclical regulation); however, it is the parallel occurrence of phosphoinositide and RAB effector recruitment, cyclical enzymatic regulation and cascading pathways that together promote specific and dynamic membrane functions.

Roles in effector recruitment. Both RABs and phosphoinositides serve as platforms for the recruitment of effector proteins to membranes[5]. RAB and phosphoinositide effectors perform similar functions: they mediate many key steps of membrane trafficking, including cargo selection, vesicle budding and transport, as well as membrane tethering and fusion. To exert these specialized functions, distinct RABs and phosphoinositides localize to specific membrane compartments or organelles, or they can be restricted to membrane microdomains[6,7] (TABLE 1). How exactly RABs and phosphoinositides acquire precise localizations is not completely understood, but both clearly depend on activating and inactivating enzymes.

Spatiotemporal control by cyclical regulation. RABs and phosphoinositides are both controlled by cyclical regulation in which they are turned 'on' and 'off' by antagonistic enzymes. RABs are localized and stimulated by specific guanine nucleotide exchange factors (GEFs) that convert the inactive GDP-bound RAB to an active GTP-bound form. Subsequently, GTPase-activating proteins (GAPs) deactivate RABs by promoting GTP hydrolysis and restoration of the inactive GDP-bound form[8] (FIG. 1b). As in RAB regulation, phosphoinositides are interconverted by competing phospho-regulatory enzymes. The D-*myo*-inositol head group of PtdIns can be modified at the hydroxyl groups at the 3′ position, 4′ position and/or the 5′ position to generate seven different possible phosphorylated species (BOX 1). This network of phosphoinositide species is controlled by dedicated phosphoinositide kinases and phosphatases[4] (FIG. 1c). As both the phosphoinositide substrates and products can elicit specific responses, this cyclical regulation is not merely an on and off switch, but a switch between distinct biochemical states.

The competing action of specific GEFs and GAPs or of specific kinases and

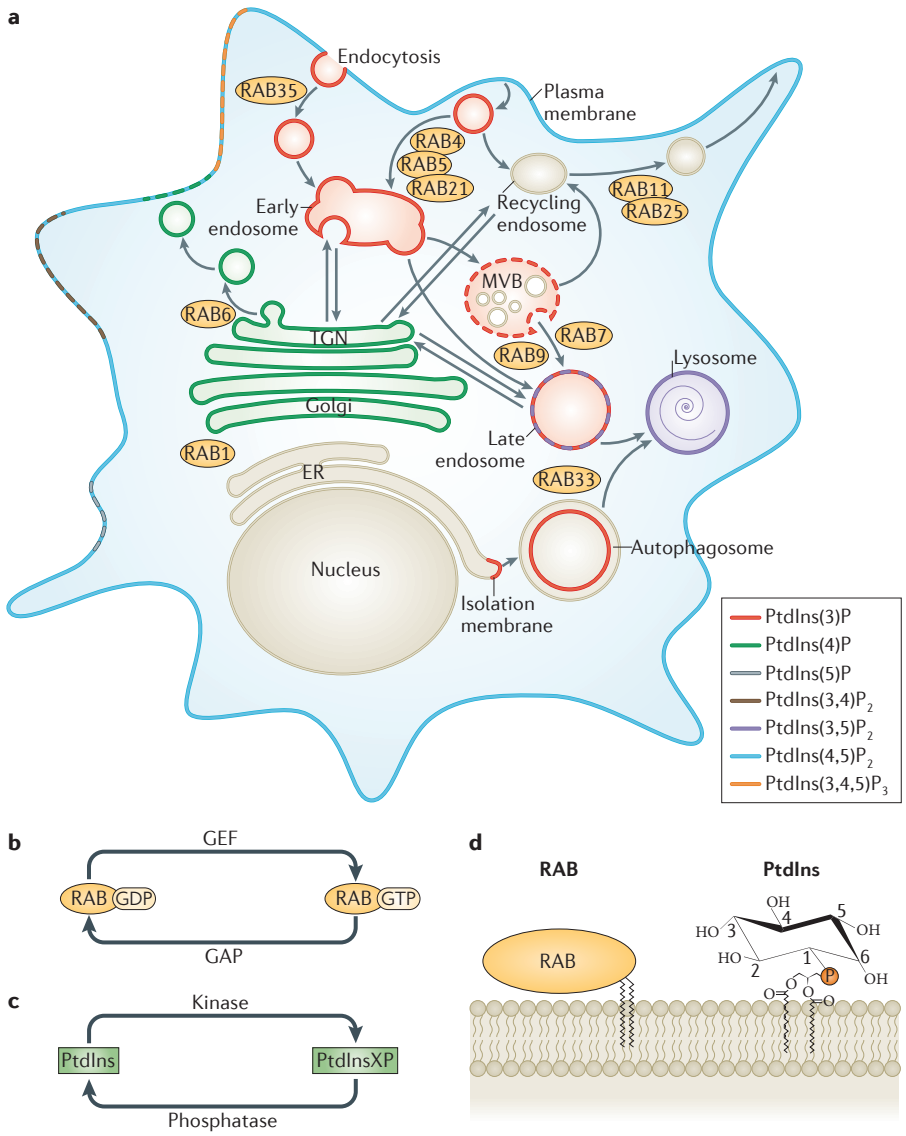


Figure 1 | **Common themes between RAB and phosphoinositide localizations, regulation and functions.** **a** | The cytoplasmic leaflets of cellular membranes are defined by specific combinations of RAB GTPases and phosphoinositides that are under spatiotemporal control of enzymatic regulators. For clarity, only a subset of RAB GTPases and distinct subpools of phosphoinositides are shown. Sites of alternative RAB and phosphoinositide distributions typically of lower abundance, including those on trafficking vesicles, endoplasmic reticulum (ER) and the nucleus, are not shown. See TABLE 1 for a more complete list. **b** | The RAB GTPase cyclical activity is under the control of guanine nucleotide exchange factors (GEFs) and GTPase-activating proteins (GAPs). Typically, GTP-bound RABs stimulate effector recruitment and function. **c** | Phosphoinositide cyclical regulation is controlled by phosphoinositide kinases and phosphatases (the different phosphorylation state of **phosphatidylinositol** (PtdIns) is denoted as XP). These enzymes generally exhibit dedicated selectivity for phosphates at either the 3′ postion, the 4′ position or the 5′ position of the inositol ring, only within specific phosphorylated forms. Both substrates and products can serve to recruit effectors and mediate local membrane roles. **d** | Both RAB and phosphoinositide regulators are cytosolic with protein domains that typically exhibit low affinity for the membrane and reversible membrane recruitment. By contrast, the distinct mechanisms of RAB prenylation at Cys residues at the carboxyl terminus and phosphatidylinositol fatty acid tails retain RABs and phosphoinositides at membranes, respectively. MVB, multivesicular body; TGN, *trans*-Golgi network.

phosphatases spatiotemporally restricts the membrane site for a given RAB or phosphoinositide activity, respectively. These regulators are typically cytosolic proteins that contain protein domains with low affinity for the membrane and can be recruited to the membrane in a reversible manner. By contrast, RABs and phosphoinositides are retained at membranes through the distinct mechanisms of post-translational RAB prenylation versus the incorporation of fatty acid tails as molecular building blocks in PtdIns (FIG. 1d). Thus, transient membrane associations of the enzymatic regulators are key to generating sharp and dynamic boundaries between different RAB or phosphoinositide forms. This is especially crucial for the formation of precise microdomains within a membrane compartment or for vesicle fusion and fission dynamics, both of which are needed at endosomes or the Golgi for cargo entry, sorting and exit. In addition to the regulators discussed here, other cofactors (such as members of the GDP dissociation inhibitor (GDI) family and other enzymes (such as phospholipases in branching phosphoinositide metabolic pathways) can also determine the abundance and cellular distribution of specific RABs or phosphoinositides.

The partially overlapping selectivity of RAB and phosphoinositide regulatory enzymes expands the specific regulatory possibilities at different membrane sites. For example, RAB5 can be activated by multiple GEFs (for example, RABEX5, RIN or GAPEX5) and, conversely, interactions can occur between a specific GEF and the multiple RAB5 subfamily members (such as RAB5, RAB21, RAB22 and RAB31). Such overlap could permit specialized regulation of RAB5 functions to meet localized trafficking demands in different cell types. Likewise, large families of phosphoinositide regulators show overlapping but distinct selectivity for multiple phosphoinositide forms, thereby permitting separable regulation of a specific phosphoinositide species in different cellular contexts. For example, three different classes of phosphoinositide 3-kinase (PI3K) (termed class I, class II and class III) exhibit overlapping capacity to synthesize phosphoinositide 3-phosphate forms: class I produces phosphatidylinositol-3,4,5-trisphosphate ($PtdIns(3,4,5)P_3$); class I and class II produce $PtdIns(3,4)P_2$; and class II and class III produce PtdIns(3)P. Moreover, different protein subcomplexes that contain class III PI3K can direct PtdIns(3)P synthesis in different contexts, such as during endocytosis or during autophagy[9,10]. RABs and phosphoinositides are thus highly spatiotemporally controlled and balanced by regulated, localized actions of competing regulators.

Cascading pathways. A third emerging theme in both RAB and phosphoinositide regulation is their assembly into cascading pathways. Cargo transport through successive membrane compartments, which is achieved

via membrane maturation or through ongoing vesicle fusion and fission, requires a coordinated progression (or switch) of membrane identities[11,12]. This can be visualized as a directional cargo hand-off between specific compartments[7]. One way to control membrane maturation and cargo routing is through RAB- or phosphoinositide-directed recruitment of the correct downstream RAB or phosphoinositide, respectively. In addition, RAB and phosphoinositide activities can lead to the recruitment of enzymes that inactivate their own function, which ensures a coordinated switch (FIG. 2).

The appropriate hand-off mechanism between different RABs, which occurs via direct protein–protein interactions, can be specified through the recruitment of RAB GEFs and GAPs as RAB effectors (FIG. 2a). The maturation of membrane compartments through cascading RAB pathways is established in both exocytosis and endocytosis[3]. For example, in yeast the Rab switch for trafficking through the late Golgi is ensured by the coordinated inactivation of Ypt1 and activation of Sec4 by the functional RAB GTPases Ypt31 and Ypt32 (REFS 13,14). A similar Rab regulatory cascade occurs during early-to-late endosomal maturation, which is regulated by Rab5-mediated recruitment of a Mon1–Ccz1 complex, which functions as a Rab7 GEF[11,15]. In addition to feedforward RAB cascades, there are positive feedback pathways through RAB effectors that retain the RAB GEF activity, as seen with RABEX5 at endosomes[16] and Sec2 at secretory vesicles[3].

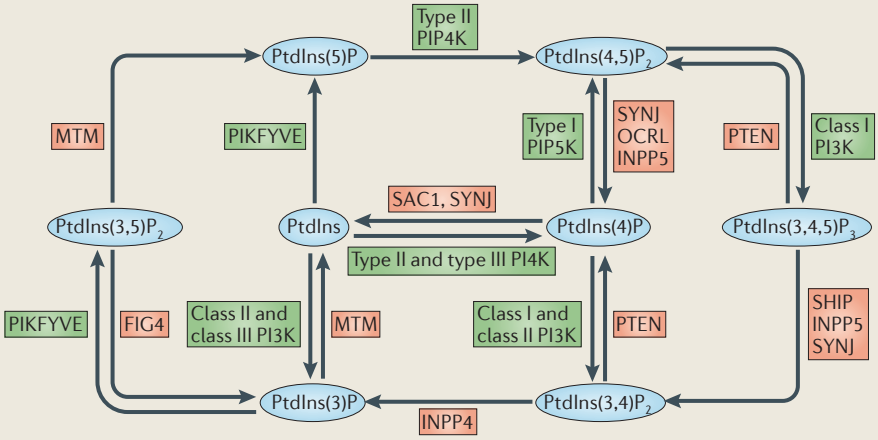
Membrane maturation also occurs through cascading phosphoinositide pathways. Dedicated phosphoinositide kinases and phosphatases can direct either cyclical (reversible) or sequential phosphoinositide regulation (FIG. 2b). Phosphoinositide-metabolizing enzymes can also be recruited as phosphoinositide effectors. For example, VPS34-mediated synthesis of PtdIns(3)P at endosomes assists in the recruitment of the phosphatidylinositol 3-phosphate 5-kinase PIKFYVE (in mammals) and FAB1 (in yeast) through a PtdIns(3)P-binding FYVE domain. FAB1, in turn, synthesizes PtdIns(3,5)P_2 on the late endolysosome (in mammals) or vacuole (in yeast)[17]. Another intriguing emerging mechanism for the control of phosphoinositide conversion is the use of a common scaffold protein that is shared between a specific kinase and phosphatase pair. This was first demonstrated for the VAC14 (vacuole morphology and inheritance mutant 14) adaptor, which directly couples FAB1 kinase and FIG4 phosphatase in PtdIns(3)P–PtdIns(3,5)P_2 regulation in vacuole homeostasis and neurodegenerative disease[17,18]. More recently, in addition to the p110 catalytic subunit, the p85α regulatory subunit of class I PI3K was discovered to bind and regulate the phosphatase PTEN (phosphatase and tensin homologue) to control PtdIns(4,5)P_2–PtdIns(3,4,5)P_3 cycles. Interestingly, p85 and PTEN are linked to tumour suppressor function, supporting the proposed scaffold role of p85 for this phosphatase[19,20]. Similarly, the myotubularins MTM1 and MTMR2, which are phosphoinositide 3-phosphatases, were found to directly interact with the VPS15 kinase, which functions as a scaffold for the class III PI3K VPS34 in cells[21]. Recently, the pseudophosphatase SBF, which

Table 1 | **Subcellular localizations of phosphoinositides and RAB GTPases**

Cellular localization	RABs	Phosphoinositides		
		Major	Minor	Refs
Plasma membrane	RAB5, RAB21, RAB35	PtdIns(4,5)P_2	PtdIns(3,4,5)P_3*,‡ PtdIns(3,4)P_2*,‡ PtdIns(5)P PtdIns(3)P	47–51
Early endosomes	RAB4, RAB5, RAB10, RAB14,RAB15, RAB17, RAB21,RAB22, RAB23, RAB35	PtdIns(3)P	PtdIns(3,4,5)P_3 PtdIns(3,4)P_2 PtdIns(4,5)P_2	6,50, 51
Late endosomes, multivesicular body, lysosomes	RAB7, RAB9	PtdIns(3,5)P_2	PtdIns(3,4)P_2*	50,52
Recycling endosomes	RAB11, RAB13, RAB17, RAB25, RAB35, RAB40		PtdIns(3,4,5)P_3 PtdIns (4,5)P_2	53,54
Autophagic compartments				10, 55–57
• Autophagosome precursors			PtdIns(4,5)P_2	
• Phagophore assembly site	RAB24, RAB33,	PtdIns(3)P		
• Autophagosome	RAB7, RAB27, RAB33	PtdIns(3)P		
Endoplasmic reticulum	RAB1, RAB2, RAB24		PtdIns(3)P PtdIns(5)P PtdIns(4,5)P_2 PtdIns(3,4)P_2* PtdIns(3,4,5)P_3*	48–51
Golgi	RAB1, RAB24, RAB33, RAB34, RAB36, RAB39	PtdIns(4)P	PtdIns(3)P PtdIns(5)P PtdIns(3,4,5)P_3*	48,49
Trans-Golgi network	RAB6, RAB8, RAB10, RAB13, RAB14, RAB30, RAB31, RAB40	PtdIns(4)P		34,49
Secretory vesicles	RAB3, RAB8, RAB26, RAB27, RAB37	PtdIns(4)P		28
Nucleus			PtdIns(4,5)P_2 PtdIns(5)P	51,58
Cytokinetic apparatus				59,60
• Cleavage furrow	RAB11, RAB21	PtdIns(4,5)P_2		
• Intercellular bridge	RAB11, RAB35		PtdIns(3)P	

Summary of known phosphoinositide subcellular localizations and relative levels, as determined from various methods. These methods include phosphoinositide labelling followed by cell fractionation and direct measurements, immuno-electron microscopy with specific antibodies against phosphoinositide or GST (glutathione S-transferase)-phosphoinositide binding domain, and/or by live cell microscopy using expressed GFP (green fluorescent protein)-tagged phosphoinositide binding domains. The major and minor phosphoinositide pools reflect current data on the relative abundance of each species within the specified membrane compartment, and not a set threshold amount. The major pool column represents phosphoinositide distributions that, in addition to other measurements, have been observed by light microscopy in live cells and/or in immuno-electron microscopy. PtdIns, phosphatidylinositol. *Indicates that the phosphoinositide localization to a specific compartment is dependent on cell stimulation by, for example, growth factors. ‡Phosphatidylinositol-3,4,5-trisphosphate (PtdIns(3,4,5)P_3) and PtdIns(3,4)P_2 mostly localize at the plasma membrane. RAB localization is presented as described before[3], with some modifications from recent literature.

Box 1 | **Phosphoinositide conversion and enzymes**



Phosphoinositides can be interconverted between their many forms by the action of dedicated kinases and phosphatases (see the figure). Phosphorylation of phosphatidylinositol (PtdIns) can give rise to seven different phosphoinositide forms: three mono-phosphorylated forms (phosphatidylinositol-3-phosphate (PtdIns(3)P), PtdIns(4)P and PtdIns(5)P), three bi-phosphorylated forms (PtdIns(3,4)P_2, PtdIns(3,5)P_2 and PtdIns(4,5)P_2) and the single tri-phosphorylated form, termed PtdIns(3,4,5)P_3. Most forms can be generated through specific kinase (shown in green) or phosphatase (shown in red) activities. For example, PtdIns(4)P can be generated either by type II or type III phosphatidylinositol 4-kinase (PI4K), which convert PtdIns to PtdIns(4)P, or as the product of phosphoinositide 5-phosphatases, such as SYNJ, OCRL (oculocerebrorenal syndrome of Lowe) and INPP5 (inositol polyphosphate 5-phosphatase), that convert PtdIns(4,5)P_2. In some instances, no known enzymes have been identified to direct the synthesis or turnover of certain forms, as indicated by the lack of an opposing arrow. Note that the FYVE finger-containing phosphoinositide kinase PIKFYVE was shown to generate PtdIns(5)P from PtdIns *in vitro*, but it is unclear whether this kinase is involved in the generation of PtdIns(5)P *in vivo*. The enzymes are shown in vertebrate nomenclature. In yeast, PIKFYVE is called Fab1, the only existing myotubularin (MTM) is termed Ymr1, and there are no known class II or class III PI3Ks. Multiple genes encode many of the enzymes shown, but only the family name is given for simplicity. A complete list of enzymes and their encoding genes has been compiled elsewhere[1,2,4]. Certain phosphoinositides can be generated by multiple enzymes with overlapping selectivity. For example, the antagonistic action of class II or class III PI3Ks and MTM 3-phosphatases can cycle PtdIns and PtdIns(3)P, whereas only class I PI3Ks and the 3-phosphatase PTEN (phosphatase and tensin homologue) cycle PtdIns(4,5)P_2 and PtdIns(3,4,5)P_3, respectively. Although the enzymes may exhibit selectivity for multiple phosphoinositide forms *in vitro*, the enzymatic functions may be restricted to distinct subpools and cellular contexts *in vivo*.
PIP4K, phosphatidylinositol 5-phosphate 4-kinase; PIP5K, phosphatidylinositol 4-phosphate 5-kinase; SAC1, suppressor of actin 1; SHIP, SH2 domain-containing protein .

is the *Drosophila melanogaster* homologue of MTMR13, was shown to physically and functionally interact with both MTM (which is the homologue of human MTM1, MTMR1 and MTMR2) and class II PI3K, indicating a scaffold role for SBF in the co-regulation of a specific PtdIns(3)P subpool[22]. These examples suggest that the boundaries for specific phosphoinositide pools are shaped by a tightly controlled balance between phosphoinositide synthesis and turnover. This mechanism could provide the precision that is needed to control different subpools of the same biochemical phosphoinositide species.

Interdependent regulation

Given their intricate localization patterns, it was hypothesized early on that RABs and phosphoinositides might influence each other's regulation[23]. This idea has been strengthened by well-established physical interactions that involve RAB GTPase-induced recruitment of phosphoinositide regulators, or phosphoinositide-mediated recruitment of RAB GTPase regulators, which coordinate RAB and phosphoinositide functions in endocytosis and exocytosis (FIG. 3a).

RAB-mediated recruitment of phosphoinositide enzymes. Pioneering work identified class I p110β and class III VPS34 (via the regulatory subunit p150) as effectors of activated RAB5 (REF. 24). At endosomes, VPS34 phosphorylates and converts PtdIns to PtdIns(3)P, which is required for the efficient recruitment of other endosomal RAB5 effectors[23] (FIG. 3a). Thus, it was proposed that localized RAB5 activity interdependently controls phosphoinositide metabolism to promote targeted endosomal functions, including the recruitment of ESCRT (endosomal sorting complex required for transport) pathway components for cargo sorting, and the recruitment of the HOPS (homotypic fusion and vacuole protein sorting) complex together with RAB7 for endosomal fusion and maturation.

More recently, another RAB5-mediated control mechanism of a different cascade of phosphoinositide enzymes was shown to also increase PtdIns(3)P on endosomes[12]. Following growth factor addition, RAB5 translocates to membrane ruffles and interacts with stimulated p110β, which produces the signalling phosphoinositide PtdIns(3,4,5)P_3. Concomitantly, activated RAB5 on endocytosed vesicles at these sites recruits a phosphoinositide 5-phosphatase and a phosphoinositide 4-phosphatase, which successively convert PtdIns(3,4,5)P_3 to PtdIns(3,4)P_2 and finally to PtdIns(3)P (REF. 12) (FIG. 3a). It was estimated that this cascading process engenders approximately 30% of the PtdIns(3)P that is detected on crude membrane fractions, whereas the other 70% of PtdIns(3)P is synthesized by class II and class III PI3Ks directly on endosomes. The biological significance of the two different PtdIns(3)P pools is not clear, but there may be specific coordinated functions between RAB5 and the distinct phosphoinositides.

Since the discovery of these initial examples of RAB5-dependent regulation of phosphoinositide enzymes, other RAB-dependent regulatory pathways have been identified.These include the feedback regulation of RAB7 on VPS34 function[25], the regulation of the phosphoinositide phosphatase OCRL (oculocerebrorenal syndrome of Lowe) by RAB1, RAB5, RAB6, RAB8 and RAB35 (REF. 26), as well as ARF6-mediated regulation of phosphatidylinositol 4-phosphate 5-kinase (PIP5K)[27], indicating the widespread importance of RAB and phosphoinositide regulatory interdependence.

Phosphoinositide-mediated recruitment of RAB regulators. Conversely, phosphoinositide-mediated regulation can influence a RAB switch. In yeast secretion, PtdIns(4)P provides directionality to a Rab cascade at the Golgi (FIG. 3a). High PtdIns(4)P concentration at Golgi membrane promotes a preferential Sec2–Ypt32 interaction[28], whereas decreased PtdIns(4)P levels following vesicle exit promotes a shift to a Sec15–Ypt32 interaction and the subsequent Sec2 GEF-dependent

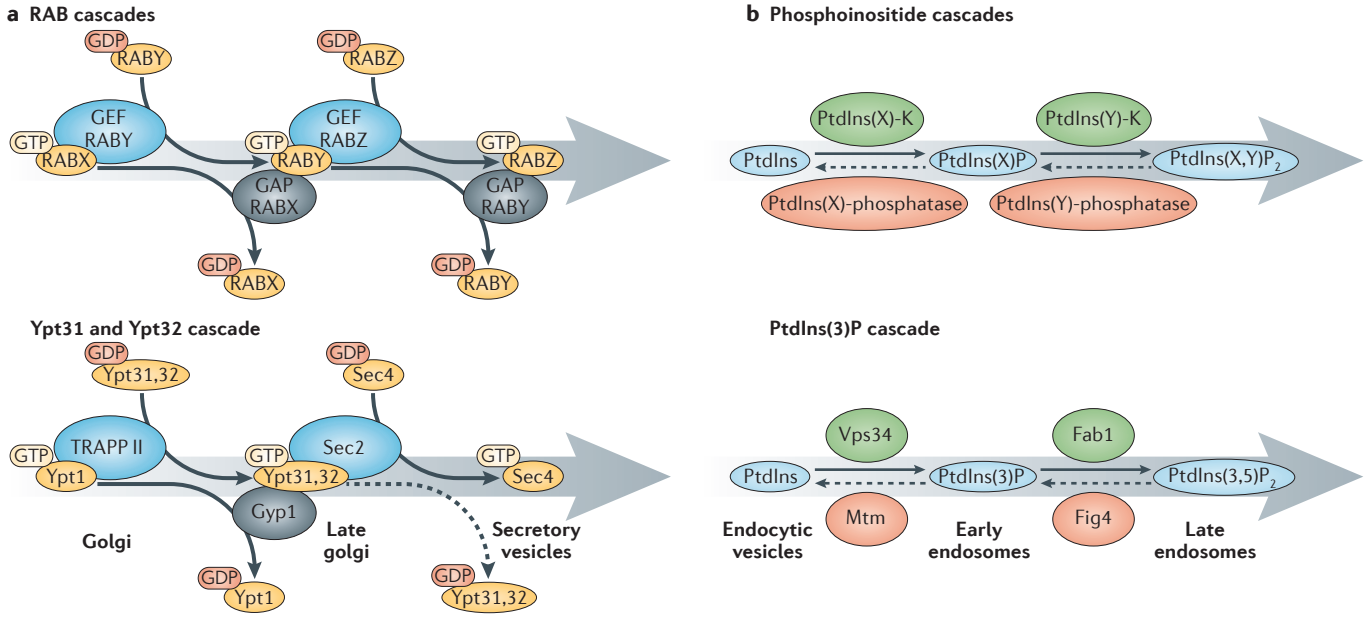


Figure 2 | **Shared themes in cascading RAB and phosphoinositide pathways.** RAB and phosphoinositide cascades control the switches in membrane identities that are needed for the progression in membrane trafficking. **a** | A conceptual diagram of RAB effector recruitment of specific RAB guanine nucleotide exchange factors (GEFs) or GTPase-activating proteins (GAPs) for consecutive RABs that are involved in pathway progression (the different RABs are denoted as X, Y and Z). One example of a Rab cascade is seen in yeast secretion. Golgi maturation occurs with inactivation of the early Golgi-associated Ypt1 by Gyp1, a Ypt1 GAP that is recruited at the late Golgi by Ypt31 and Ypt32 (REF. 13). GTP-bound Ypt31 and Ypt32 also recruit Sec2, which is the GEF for Sec4, to drive secretory vesicle trafficking[14]. In this way, the Rab-mediated switch for trafficking through the late Golgi is ensured by Ypt31–Ypt32-controlled coordination of Ypt1 inactivation with Sec4 activation. **b** | A conceptual diagram of phosphoinositide effector recruitment of specific phosphoinositide kinase and phosphatase functions for cyclical and sequential regulation (the different kinases and phosphatases involved are denoted as X and Y). One example of a phosphoinositide cascade is seen during endosome maturation. Vps34 synthesizes phosphatidylinositol-3-phosphate (PtdIns(3)P) at endosomes, which leads to the recruitment of the phosphatidylinositol 3-phosphate 5-kinase Fab1, through a PtdIns(3)P-binding FYVE domain. Fab1 in turn synthesizes PtdIns(3,5)P_2 on the late endolysosome or vacuole[17]. A subfamily of myotubularin (Mtm) phosphoinositide 3-phosphatases contain a PtdIns(3)P-binding FYVE domain, which is thought to be important for the recruitment of enzymes that mediate PtdIns(3)P turnover. TRAPP II, transport protein particle complex II.

activation of Sec4. Examples of ARF regulation by PtdIns(4,5)P_2 protein binding include HERC1 (HECT domain and RCC1-like domain-containing protein 1)-mediated stimulation of GDP release from ARF6 (REF. 29) and ARF1 activation by ARNO (ARF nucleotide site opener and cytohesin 2)[30], respectively (FIG. 3a). A role for a phosphoinositide regulator in proper RAB localization was shown by the requirement of the *D. melanogaster* PtdIns(4)-kinase FWD (Four wheel drive) to target RAB11 to the midzone during cytokinesis[31]. However, a specific PtdIns(4)P concentration was not required to localize RAB11, as a kinase-dead version of the FWD enzyme could partially rescue *fwd* mutant flies. Together, these examples highlight the intricate relationship between phosphoinositide regulators and RABs.

Coincidence detection

As illustrated by the above examples, the intrinsic complexity of the membrane trafficking network relies on the coordinated regulation between specific RABs and phosphoinositides. A key collaboration between colocalized RAB and phosphoinositide functions also occurs through their shared control of common downstream effectors. This process of 'coincidence detection' describes the co-requirement for multiple factors involved in direct binding of an effector to mediate effector recruitment, a reoccurring theme in membrane trafficking[32] (FIG. 3b).

Why would such a co-requirement be important in membrane trafficking? For one reason, phosphoinositides and RABs often function in more than one location. For example, PtdIns(3)P is found at both endosomes and nascent autophagosomes, and coincidence detection confers the specificity to the effectors that are recruited to either site: PtdIns(3)P and RAB5 jointly specify the recruitment of early endosome antigen 1 (EEA1) to endosomes[23] (FIG. 3b), whereas PtdIns(3)P and autophagy-related protein 2 (ATG2; which is a non-RAB protein) co-recruit ATG18 to autophagosomes[33]. In addition, protein phosphoinositide-binding domains often exhibit promiscuous and weak binding affinities to phosphoinositides that are important for the transient interactions underlying membrane dynamics. However, the presence of simultaneous protein interactions with a phosphoinositide and an additional colocalized factor — either a cargo protein, another lipid, a specific membrane geometry or a specific RAB[32] — could increase local binding strength and ensure target specificity. One can hypothesize that coincidence detection between RAB GTPases and phosphoinositides could increase effector compartmentalization, both across and within membranes.

Coincidence detection at the Golgi.

The pleckstrin homology (PH) domain-containing proteins FAPP1 and FAPP2 localize to the *trans*-Golgi network (TGN), where they are required for Golgi-to-plasma membrane transport. The PH domain of FAPP1 and FAPP2 conveys this Golgi localization[34]. However, it was found that this PH domain has a relatively equal affinity for both PtdIns(4)P and PtdIns(4,5)P_2. How can FAPP1 and FAPP2 harbour such an exclusive localization pattern? The PH domain of FAPP1 and FAPP2 also interacts with

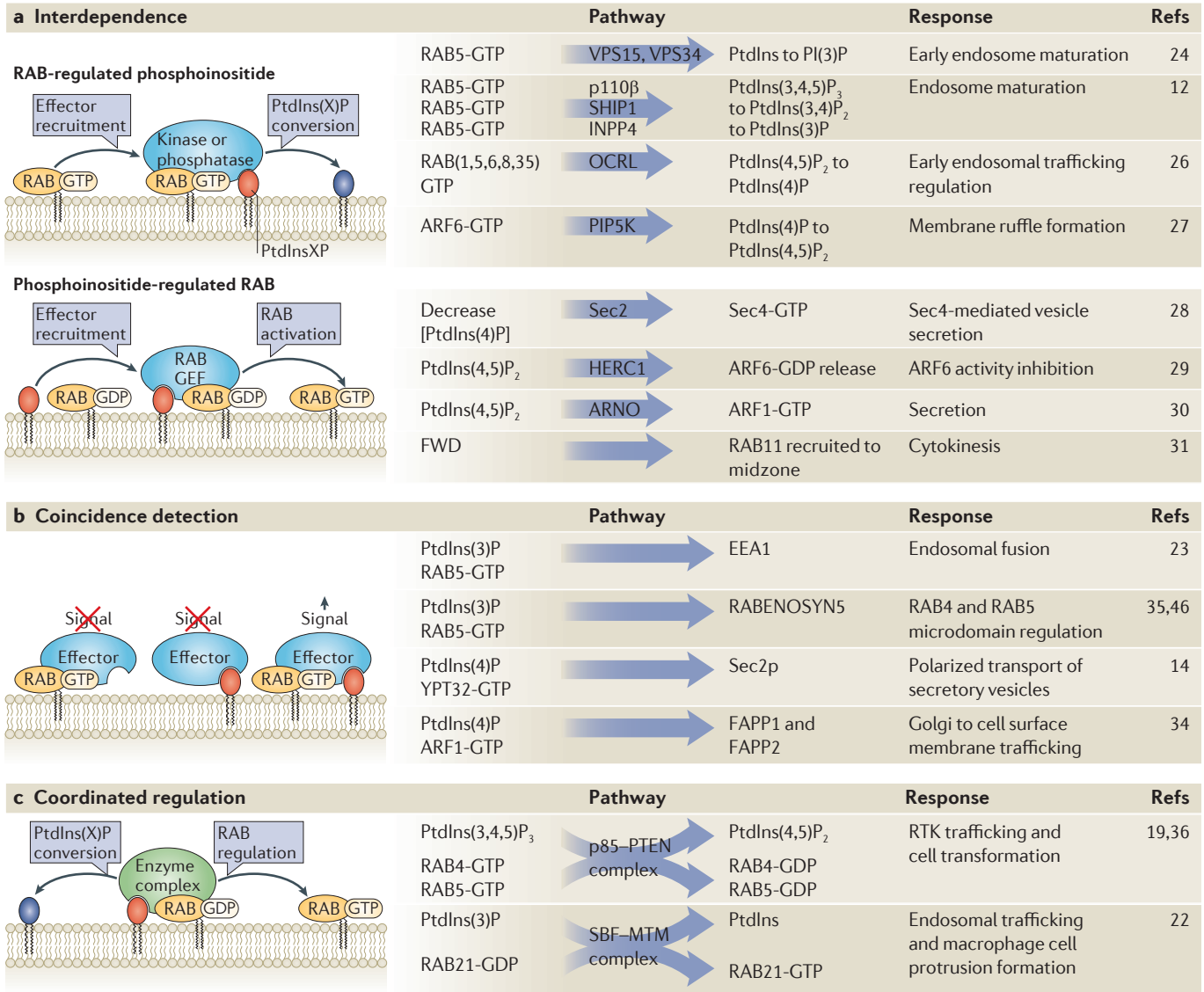


Figure 3 | **Interrelationships between RABs and phosphoinositides.** Three ways that RAB GTPases and phosphoinositides coordinately interact to control membrane trafficking are shown. Specific examples of each type of RAB–phosphoinositide interaction are depicted as pathways and their responses (right). **a** | RABs and phosphoinositides can act in interdependent pathways, in which one acts as an effector and recruits a regulatory enzyme for the other. For example, phosphoinositides can regulate RABs through the recruitment or release of guanine nucleotide exchange factors (GEFs) or GTPase-activating proteins (GAPs) (bottom), and RABs can regulate phosphoinositides through the recruitment of kinases or phosphatases (top). **b** | RABs and phosphoinositides can provide coincidence detection through shared recruitment of common effectors with dual recognition domains. Coincidence detection increases the affinity and therefore the specificity of effector localization, which is particularly important for the specificity in responses to phosphoinositide subpools at distinct membrane compartments. **c** | RABs and phosphoinositides can be under direct, coordinated regulation in order to ensure combined activities and responses that are needed for membrane progression or maturation. Two known examples of dual regulatory roles are the p85–PTEN (phosphatase and tensin homologue) complex that controls a phosphatidylinositol-3,4,5-trisphosphate ($PtdIns(3,4,5)P_3$)–$PtdIns(4,5)P_2$ cycle and RAB5 GAP function, as well as the myotubularin complex SBF–MTM-mediated control of a PtdIns(3)P–PtdIns cycle and RAB21 GEF function. ARF, ADP-ribosylation factor; ARNO, ARF nucleotide site opener; FWD, Four wheel drive; HERC1, HECT domain and RCC1 like domain-containing protein 1; INPP4, inositol polyphosphate 4-phosphatase; OCRL, oculocerebrorenal syndrome of Lowe; PIP5K, phosphatidylinositol 4-phosphate 5-kinase; RTK, receptor Tyr kinase; SHIP1, SH2 domain-containing protein 1.

the Golgi-localized ARF1 GTPase, and the combination of both PtdIns(4)P and ARF1 at the Golgi directs the precise cellular localization of FAPP1 and FAPP2 (FIG. 3b). Another example is the Sec2 GEF coincident interaction with both the late Golgi Ypt32 Rab and PtdIns(4)P in yeast, both of which are essential for Sec2 Golgi localization[28].

Coincidence detection at endosomes. The formation of endosomal microdomains is key for protein sorting. Coincidence detection is one mechanism by which endosomal microdomains are generated. RAB5 and PtdIns(3)P together recruit EEA1 and RABENOSYN5 through coincidence detection[23,35,46] (FIG. 3b). Thus, at these sites, EEA1 directs early endosomal fusion, whereas RABENOSYN5 compartmentalizes RAB5–PtdIns(3)P from RAB4 subdomains. These coordinated functions ensure that newly fused regions of endosomal membranes are segregated from sorted regions that are undergoing membrane efflux and influx for cargo recycling and degradation, respectively.

As the effector identities of many RAB and phosphoinositide functions remain unknown, it is likely that coincident detection is a more widely used mechanism than currently appreciated.

Coordinated regulation

An exceptional aspect of membrane trafficking is the rapidity and selectivity by which cargos can be sorted and trafficked. The examples discussed above illustrate how both RABs and phosphoinositides collaborate to achieve selectivity and directionality in trafficking. The prevalent pairing of different RABs and phosphoinositides at distinct membrane domains raises the question of whether their regulation is directly linked. In this final section, we high light how simultaneous regulation can couple specific RAB and phosphoinositide activities (FIG. 3c), and we discuss in what ways we envision that this mechanism is likely to be of broad significance in membrane trafficking.

PtdIns(3,4,5)P_3 and RAB5 co-regulation. The class I PI3K p85 regulatory subunit has well-appreciated roles in p110 kinase activation for the synthesis of PtdIns(3,4,5)P_3, which is involved in receptor signalling pathways at the plasma membrane. In addition, the BH domain in p85α was found to possess RAB GAP activity[36]. Given that the p85 subunit stays associated with internalized, activated receptors, it was proposed that the RAB GAP function of p85 acts on endosomes to inactivate RAB5 and RAB4 and downregulates receptor signalling. Point mutations in the p85 GAP domain led to increased platelet-derived growth factor receptor (PDGFR) signalling through AKT (also known as PKB) and mitogen-activated protein kinase (MAPK) and result in cell transformation, signifying the importance of the direct regulation of RAB5 and RAB4 by p85 (REF. 37). As described above, p85 was also recently shown to not only recruit p110 but also to bind and regulate the antagonistic 3-phosphatase PTEN for turnover of PtdIns(3,4,5)P_3 to PtdIns(4,5)P_2 (REF. 19). Thus, the recruitment of p85 to activated receptors at the plasma membrane initiates p110 PI3K signalling. Subsequently, p85 can recruit PTEN to downregulate the PtdIns(3,4,5)P_3 signal, and acts as a RAB5 and RAB4 GAP for endocytic receptor down-regulation and receptor degradation (FIG. 3c).

PtdIns(3)P and RAB21 co-regulation. MTMs are a large family of phosphoinositide 3-phosphatases, with half the members of this subfamily being catalytically inactive or 'pseudo'-phosphatases. In mammals, the catalytic MTMR2 and the pseudophosphatase MTMR13 directly interact, and mutations in these proteins are associated with a neuromuscular disease[38]. We demonstrated that *D. melanogaster* SBF has dual roles in MTM-mediated turnover of a PtdIns(3)P subpool and RAB21 activation, and that both functions are required for endosomal progression in a shared pathway for cortical remodelling[22] (FIG. 3c). In addition, SBF interacts with class II PI3K, which co-regulates this PtdIns(3)P pool and other related functions. SBF contains a DENN (differentially expressed in neoplastic versus normal cells) domain, which was shown to broadly act as a RAB GEF in several proteins[39]. The SBF DENN domain specifically interacts with inactive RAB21 (but not with other RABs tested) as a RAB21 GEF, and RAB21 is required for shared cellular functions with MTM and SBF in macrophage remodelling[22]. Interestingly, SBF, MTM, RAB21 and class II PI3K are only found in higher eukaryotes, suggesting that this regulatory module might have evolved to regulate a specialized endosomal trafficking route and cellular roles.

What is the possible significance for such a tight control of RAB21 and PtdIns(3)P co-regulation? On the basis of current knowledge of protein sorting at vesicular-tubular endosomes, this dual RAB–phosphoinositide regulatory mechanism may serve to coordinate key steps of cargo sorting and membrane tubulation and remodelling involved in endocytic recycling. Others have shown that the members of the sorting nexin (SNX) family, which contain PtdIns(3)P-binding PX (phox homology) domains, are recruited to membrane subdomains on vesicular-tubular endosomes, and that specific SNX proteins are essential for receptor protein sorting for both recycling and retromer functions[40]. It has also been shown that the activation of RAB5 and RAB21, coupled with a decrease in PtdIns(3)P on endosomes, can induce tubulation of early endosomal compartments, indicative of membrane exit and trafficking of sorted cargo[41]. In this way, the coordinated regulation of RAB21 and PtdIns(3)P could serve to coordinate endosomal sorting and membrane exit.

Perspective on phosphoinositide and RAB co-regulation. We propose that the direct coordination between RAB and phosphoinositide regulation might be a broad mechanism in membrane trafficking, and we consider two possibilities that have yet to be tested. One involves Connecdenn 1 (DENND1A), which is a DENN domain-containing protein found in association with clathrin coated vesicles (CCVs) and shown to have RAB35 GEF activity[42]. CCVs are associated with PtdIns(4,5)P_2 and several phosphoinositide modifying enzymes. Thus, it is possible that DENND1A and a PtdIns(4,5)P_2-phosphatase act concomitantly to activate RAB35 and hydrolyse PtdIns(4,5)P_2 following endocytic uptake to define a specific subset of endosomes. A second candidate process for RAB and phosphoinositide co-regulation involves APPL1 (adaptor protein containing PH domain, PTB domain and Leu zipper motif 1; also known as DIP13α) and APPL2 (also known as DIP13β) signalling endosomes that appear after vesicle internalization[43]. As RAB5 and RAB21 effectors, APPL1 and APPL2 are thought to assemble MAPK and AKT signalling platforms that remain active before progression to PtdIns(3)P-containing early endosomes. Furthermore, as activated RAB5 also leads to VPS34 recruitment and PtdIns(3)P synthesis, a competition model was postulated, in which APPL proteins are displaced by proteins with RAB5–PtdIns(3)P coincidence detection[43]. Alternatively, another way of generating such endosomes could involve the direct coordination of a RAB5 and a RAB21 GEF with a PtdIns(3)P-phosphatase. In this case, simultaneous RAB5 and RAB21 activation and PtdIns(3)P depletion would favour APPL protein retention and thus endosomal signalling. It is worth noting that co-overexpression of MTMR2 phosphatase and MTMR13 GEF prolonged AKT signalling, which suggests that such a mechanism could occur[44].

The multiple substrate selectivity of certain RAB and phosphoinositide regulatory enzymes further suggests that distinct complexes could direct specific combinations of RAB–phosphoinositide activities at distinct membranes. One example of such modular interactions is seen with the three distinct yeast transport protein particle (TRAPP) complexes that target one common Rab (namely Ypt1, which is the yeast homologue of human RAB1) to different locations during membrane trafficking in secretion and autophagy[45].

Conclusions

RABs and phosphoinositides are appreciated as central coordinators of membrane trafficking. Many examples described in this Opinion article demonstrate how coordinated RAB and phosphoinositide activities function in shared pathways, and how this coordination results in the recruitment of shared effectors. New evidence indicates that there is also direct coordination between RAB and phosphoinositide regulation. Most of the phosphoinositide modifying enzymes are known, whereas only a subset of RAB GEFs and GAPs are currently described.

Thus, future identification of novel RAB regulators and the characterization of the phosphoinositide modifying proteome might reveal other protein modules that are involved in RAB and phosphoinositide co-regulation. The dynamics of membrane trafficking depend on the mechanisms enumerated in this Opinion article. However, we still do not fully understand the regulatory hierarchies and dynamics of RAB and phosphoinositide mechanisms in live cells, especially owing to challenges in the detection of endogenous phosphoinositide pools. It will be important to identify the functional pathways that characterize distinct RAB and phosphoinositide subpools that, for example, can explain the existence of expanded gene families with overlapping enzymatic activities yet non-redundant functions in metazoans. A next challenge will be to determine how RAB and phosphoinositide control mechanisms are regulated and relate to the broader context of organismal development and homeostasis.

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Competing interests statement
The authors declare no competing financial interests.

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