Phosphoinositide Signaling: New Tools and Insights

Phosphoinositides constitute only a small fraction of cellular phospholipids, yet their importance in cellular processes is fundamental and cannot be overstated.

Phosphoinositides are important structural elements of eukaryotic cellular membranes and participate in intercellular communication, although the same lipids can also have signaling roles within the cell. Phosphoinositides are known as lipid compounds for intercellular communication, although the same lipids can also have signaling roles within the cell. Phosphoinositides are best examples of how phospholipids, namely phosphatidylinositol (PtdIns), can be utilized as a scaffold to generate by phosphorylation a variety of compounds that control a whole range of cellular functions. It is important to distinguish the small amount of phosphoinositides in the cell to maintain its structural integrity. Each membrane compartment has its unique lipid composition.

Phospholipids are very important structural elements of all eukaryotic cellular membranes that undergo constant metabolic changes according to the need of the cell to maintain its structural integrity. Each membrane compartment has its unique lipid composition: for example, the plasma membrane (PM) has high phosphatidylinositol (PtdIns) and phosphatidic acid (PtdA) in response to stimulation of some cell surface receptors.

Increased turnover of PtdIns and phosphatidic acid (PtdA) in response to stimulation of some cell surface receptors was the first indication that these lipids could serve as regulatory molecules. These early observations opened research areas that ultimately clarified the plasma membrane role of phosphoinositides in Ca++ signaling. However, research of the last 10 years has revealed a much broader range of processes dependent on phosphoinositides. These lipids control organelle biology by regulating vesicular trafficking, and they modulate lipid distribution and metabolism more generally via their close relationship with lipid transfer proteins. Phosphoinositides also regulate ion channels, pumps, and transporters as well as both endocytic and exocytic processes. The significance of phosphoinositides found within the nucleus is still poorly understood, and a whole new research concerns the highly phosphorylated inositols that also appear to control multiple nuclear processes.

The expansion of research and interest in phosphoinositides naturally created a demand for new approaches to determine where, within the cell, these lipids exert their effects. Imaging of phosphoinositide dynamics within live cells has become a standard cell biological method. These new tools not only helped us localize phosphoinositides within the cell but also taught us how tightly phosphoinositide control can be linked with distinct effector protein complexes.

The recent progress allows us to understand the underlying causes of certain human diseases and design new strategies for therapeutic interventions.
Figure 1. Phosphoinositides regulate multiple membrane-associated molecular events

A: A signaling complex of kinases (Rho, Akt, PKC), phospholipases (PLC, PLD), and exchange factors or GAP proteins.

B: PM phosphoinositide signaling enzymes: PLC, PI3-kinase, Akt/PKB, Btk, PKC.

C: Phosphoinositides control vesicular traffic and ion channels. Phosphoinositides also regulate the activity of a number of membrane-associated molecular events: the endoplasmic reticulum (ER) alone is a sufficient signal to activate a Ca2+ influx pathway (129), yet the molecular mechanism of ER luminal Ca2+ sensing and its coupling to a PM Ca2+ influx pathway were discovered only very recently (96). The tremendous expansion of the inositol lipid research field has inevitably led to its fragmentation, and now a review could be written about each of the special aspects of regulation by phosphoinositides. This review will not attempt to cover all of these areas in great detail. Instead, it will try to highlight new developments in the respective areas and find common principles that govern regulatory processes. Finally, it will discuss some of the methodological advances that allowed the gathering of new information on these important lipid regulators.

D: Phosphoinositides regulate multiple molecular events: a signaling complex of kinases (Rho, Akt, PKC), phospholipases (PLC, PLD), and exchange factors or GAP proteins. Phosphoinositides control vesicular traffic and ion channels. Phosphoinositides also regulate the activity of a number of membrane-associated molecular events: the endoplasmic reticulum (ER) alone is a sufficient signal to activate a Ca2+ influx pathway (129), yet the molecular mechanism of ER luminal Ca2+ sensing and its coupling to a PM Ca2+ influx pathway were discovered only very recently (96). The tremendous expansion of the inositol lipid research field has inevitably led to its fragmentation, and now a review could be written about each of the special aspects of regulation by phosphoinositides. This review will not attempt to cover all of these areas in great detail. Instead, it will try to highlight new developments in the respective areas and find common principles that govern regulatory processes. Finally, it will discuss some of the methodological advances that allowed the gathering of new information on these important lipid regulators.
Cellular Processes Regulated by Inositides

It might be easier at this time to list the processes that are not regulated by inositides in a eukaryotic cell than those that are clearly inositol dependent (Figure 1). The following sections will outline some research areas where phosphoinositides have high profile and discuss some current questions that the authors feel are important and unresolved. This selection is not exhaustive, and the readers are encouraged to review the recent literature on this topic. These important phosphoinositide converting enzymes. Such summaries can be found in several other recent reviews (5, 8, 10, 103, 147).

Questions still unanswered about the classical canonical signaling roles of phosphoinositides

The general concept of signal transduction utilizing PM phosphoinositides is clearly established for Ca2+-mobilizing hormones when they activate their cell surface receptors. These receptors activate phosphoinositide-specific phospholipase C (PLC) enzymes to hydrolyze phosphatidylinositol 4,5-bisphosphate [PtdIns(4,5)P2] of the PM. The PLC enzymes that come in a variety of forms (133) can be activated by heterodimeric G-protein subunits (PLCβ) enzymes, in the case of G-protein-coupled receptors) or via tyrosine phosphorylation (PLCε enzymes in case of receptor tyrosine kinases) as well as by small GTP binding proteins (PLCδ enzymes, in a variety of mostly G-protein-coupled receptors) or via tyrosine phosphorylation (PLCε enzymes in case of receptor tyrosine kinases) as well as by small GTP binding proteins.

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Given the high PtdIns4P content of the Golgi, it is also plausible that PtdIns4P reaches the PM in the form of vesicular transport between the Golgi and the PM. Indeed, there are several findings that suggest that the P4KIIIs mechanism is not the only way cells can get their PM PtdIns4P. Patch-clamp studies have shown that many ion channels require PtdIns(4,5)P2 for proper function (55) (also see below). In one of these studies, the restoration of the channel activity, presumably by resynthesis of PtdIns(4,5)P2, is insensitive to inhibition of type III P4Ks, suggesting an alternative mechanism of PtdIns4P resynthesis (184). Yet another study using a similar experimental paradigm, but a different PtdIns(4,5)P2-sensitive channel, reported that the calcium-binding regulatory protein NCS-1 can regulate the restoration of the PtdIns(4,5)P2 pools that regulate the channel (54). NCS-1, on the other hand, has been shown to regulate the Golgi-localized P4KIIIb enzyme (71, 180) as first demonstrated in yeast (72). These data would be compatible with a role of Golgi PtdIns4P being the ultimate source of PM PtdIns4P. What makes some of these experiments difficult to evaluate is the long time period required for either overexpression or downregulation of the P4Ks or their regulators. During these prolonged periods, the trafficking of channels or other proteins could drastically change, making it hard to determine the primary cause of the defect. The use of subtype-specific PI kinase inhibitors or the acute regulation of lipid levels on specific compartments (see below) may be a better approach to revisit these questions.

The question of what enzyme regulates the plasma membrane PtdIns(4,5)P2 pools appears less complicated in the case of the PIP5K enzymes that are most often found associated with the PM, although some of their specific forms can also be specially localized in other cellular compartments, such as the focal adhesions or the nuclei (39). Nevertheless, it has been shown that the 87-kDa splice form of PIP5K type b is primarily responsible for the synthesis of the agonist-sensitive PtdIns(4,5)P2 pools in HeLa cells (178). On the other hand, PIP5K1a (human terminology) is recruited to the PM by the Bruton’s tyrosine kinase in B-cells, which in turn enhances Ca2+ signaling (139). This suggests that PIP5K1a can also generate PtdIns(4,5)P2 that is accessed by PLCγ.

A third important component of the mechanism by which the PM is supplied with PtdIns(4,5)P2 is comprised of the PtdIns transfer proteins (PITPs) that transfer PtdIns from the site of its synthesis in the ER to the PM (28). These proteins will be discussed in greater detail below together with the roles of phosphoinositides in lipid transport processes.

Why is almost every ion channel and transporter affected by phosphoinositides?

Early research has already suggested that phosphoinositides can directly affect various ion transport pathways. A direct effect of membrane PtdIns(4,5)P2 on the activity of the PM Ca2+ pump was found as early as 1981 [117], and the sarcoplasmic reticulum Ca2+ ATPase was reported to contain tightly associated phosphoinositides (171). However, it was the Na+ /Ca2+ exchanger (NCX1) and the K$_{ATP}$ potassium channels studied in giant excised patches of the heart where the phosphoinositide regulation of ion channels/transporters has first been clearly postulated (74). This was followed by a large number of studies showing PtdIns(4,5)P2 requirement for proper activity of several ion channels (55, 75). In almost all of these studies, the question was raised whether PtdIns(4,5)P2 is merely a requirement for the optimal functioning of the channels or the channels are actually regulated by PtdIns(4,5)P2 changes that occur during activation of PLC-coupled receptor mechanisms. In some cases, such as the M-current (77, 156) or the cold- and menthol-sensitive TRPM8 channels (33, 135), the channel activity clearly follows very closely the changes in PM PtdIns(4,5)P2. However, in other examples, PtdIns(4,5)P2 has both inhibitory and stimulatory effects on channel activity (108). The TRPV1 channels, for example, are sensitized by PLC-coupled agonists presumably via reduction of PtdIns(4,5)P2 (26), whereas the same lipid is required for their recovery from desensitization (99, 100). More detailed discussion on the inositol regulation of ion channels can be found in excellent recent reviews [55, 134, 155].

The mechanism by which inositol lipids can regulate ion channels remains elusive. Almost all ion channels and transporters contain clusters of basic residues in their membrane-adjacent regions facing the cytosol or within their COOH-terminal tails. Lipid regulation of TRP channels was mapped to the TRP domains of their cysteinal tails (118, 135). However, an interesting and common feature of the PtdIns(4,5)P2 regulation of many potassium and TRP channels is that the lipid alters the interaction of the channels with other specific regulators such as calmodulin (87), β-subunits (78), or ligands such as ATP (16). In addition, indirect regulation of channels via inositol-binding channel-interacting proteins is also possible, as shown by the recent identification of Pirt, a molecule that interacts with TRPV1 channels and also binds phosphoinositides (84).

**Phosphoinositides regulate vesicular trafficking**

The presence of phosphoinositide kinases and phosphatases in various membrane fractions of fractionated cells or tissues has been noted by early studies on these enzymes (111). However, the general notion was that these findings either indicated PM contamination of the other membranes or represented intermediate membrane compartments as phosphoinositides were on their way to the PM. Some of the earliest indications that phosphoinositides may be important for membrane fusion or fission events came from studies on dense core vesicles. The best recognized example is the role of phosphoinositides for priming the docked vesicles for their complete fusion at the PM when a receptor (e.g., the fibroblast growth factor receptor) is activated. The role of an already docked vesicle was first suggested by the observation that antibodies that can inhibit the activity of the Na+/Ca2+ exchanger (NCX1) and the K$_{ATP}$ potassium channels studied in giant excised patches of the heart where the phosphoinositide regulation of ion channels/transporters has first been clearly postulated (74). This was followed by a large number of studies showing PtdIns(4,5)P2 requirement for proper activity of several ion channels (55, 75). In almost all of these studies, the question was raised whether PtdIns(4,5)P2 is merely a requirement for the optimal functioning of the channels or the channels are actually regulated by PtdIns(4,5)P2 changes that occur during activation of PLC-coupled receptor mechanisms. In some cases, such as the M-current (77, 156) or the cold- and menthol-sensitive TRPM8 channels (33, 135), the channel activity clearly follows very closely the changes in PM PtdIns(4,5)P2. However, in other examples, PtdIns(4,5)P2 has both inhibitory and stimulatory effects on channel activity (108). The TRPV1 channels, for example, are sensitized by PLC-coupled agonists presumably via reduction of PtdIns(4,5)P2 (26), whereas the same lipid is required for their recovery from desensitization (99, 100). More detailed discussion on the inositol regulation of ion channels can be found in excellent recent reviews [55, 134, 155].

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on dense core granule (DCV) exocytosis, where it was recognized that PtdIns(4,5)P₂ synthesis is important for priming the exocytic vesicles (43, 70). DCVs containing their cargo undergo maturation that increases their competence to dock and ultimately fuse with the PM when a rapid rise in Ca²⁺ concentration triggers the fusion process. Some of the mature granules under the membrane will dock to the PM, but these pre-docked vesicles still have to undergo "priming" to become the "readily releasable pool" that is first to be fused upon stimulation (31). Furthermore, the mammalian PM also contains a PtdIns(4,5)P₂ isomerase showing of factors necessary for the priming process (69, 76). The question of whether PtdIns(4,5)P₂ is necessary at the PM membrane or at the surface of the exocytic vesicle has not been clarified for a long time, but recent studies on the Ca²⁺-sensitive phosphoinositide-binding regulatory protein of exocytosis, CAPS (176), suggested that PtdIns(4,5)P₂ is needed on the PM site (81). This conclusion was also reached using single cell studies on PC12 cell exocytosis (112). However, in addition to exocytosis, endocytosis is also regulated by inositol lipids. The tetrameric clathrin adaptor protein AP-2, a key component of the clathrin-mediated endocytic machinery, was identified as a "receptor" for InsP₆ (175), although the natural binding site of this protein turned out to be PtdIns(4,5)P₂ (53, 136). These studies gave strong support to the already growing evidence that phosphoinositides might be crucial regulators of endocytosis. Identification of the PtdIns(4,5)P₂ 5-phosphatase, synaptojanin (105), and PIP₅Kα (60, 182) as crucial regulators of synaptic vesicle cycling has put phosphoinositides in their place in the endocytic pathway also depends on phosphoinositide-mediated endocytic machinery, was identified as a "receptor" for InsP₆ (175), although the natural binding phosphoinositides are key lipid signals that control the sorting and recycling of the endocytic machinery to the PM regardless of the type of endocytosis, it appears that PtdIns(4,5)P₂ has to be dephosphorylated by 5-phosphatase enzymes in order for the exocytic vesicle to find its destination whether recycling to the PM or sorted via the sorting endosome (190). Thus a high rate of phosphorylation and dephosphorylation of the five-position in PtdIns(4,5)P₂ is crucial for proper membrane cycling at the PM. The sequence of the type II PI4Ks in endosomal membranes (10, 113) suggest that PtdIns4P also has an important role in determining the fate of the endocytosed membranes. A critically important step in recognizing the relevance of phosphoinositides in intracellular vesicular trafficking was the discovery that the yeast Vps34p protein, an essential element of vacuolar sorting, was a PtdIns3-kinase (142). This was followed by a whole set of studies that unraveled protein modules capable of recognizing PtdIns4P (22, 149) as well as the enzymes that convert these lipids to PtdIns(3,5)P₂ (56, 148) or back to PtdIns (123, 161) and the signaling complexes that are regulated by them. Even a superficial summary of the advances made in these respective research fields would exceed the scope of this review. Excellent reviews can be found in all of these topics. We will only discuss some general questions that come to mind when trying to understand the underlying principles common to all of these processes. The current understanding of how inositides contribute to these complex membrane fusion or fission steps is that these lipids interact with protein modules present in the proteins that regulate these processes. Many such inositol binding modules have been identified and characterized. Pleckstrin homology (PH) domains, phox-homology (PX) domains, FYVE-domains, ENTH-domains FERM-domains are the best known ones but others certainly also exist (12, 91). Typical proteins that contain such domains include both tetrameric and monomeric clathrin adaptors, CMEAP- and GAP proteins of small GTP binding proteins belonging to various classes (Arfs, Rabs, Rho/Rac, and Ras), sorting nexins, protein and lipid kinases, as well as various phospholipases, and probably many others (91). In many instances, the inositol binding to the domain is only a partially effective signal, and it needs an additional input, which in most cases is an active small GTP-binding protein (FIGURE 2A). This mode of operation is often referred to as coincidence detection (25).

Initially, it was believed that the lipid binding domains was to recruit the protein to the site where it needs to function. Although this certainly is the case for many proteins, it is increasingly recognized that membrane localization is often determined by other parts of the molecule, and the inositol binding domain serves as a lipid-dependent regulatory module rather than a localization signal. Another adjustment in our view of the inositol binding domains is concerned with their specificities. Initial examples suggested that these modules show a high degree of specificity in their inositide recognition. And indeed, in many cases, PH domains, PX domains, or FYVE domains show a high degree of inositide binding specificity. However, an increasing number of such modules have proven to be rather promiscuous in their phosphoinositide binding (187) and even showed no lipid binding at all. Often, these modules also have protein binding partners, which is not surprising, since PH domains, for example, have a very similar folding structure to phosphotyrosine binding.
monophosphorylated inositides populate the internal membranes whether on endocytic or exocytic routes, and the polyphosphoinositides [PtdIns(4,5)P$_2$ or PtdIns(3,4,5)P$_3$] are located in the plasma membrane. Monophosphorylated inositides on internal membranes that border a sequestered "outside" compartment such as the duplicating organelle, the lysosome). During evolution, phosphoinositide kinases appear together with internal membranes and hence, their functions are likely linked to the identification and fate-determination of the internal membranes. In this context, it is relevant that PtdIns(4,5)P$_2$ is mostly found in the PM (although in small amounts it was detected in intracellular membranes in EM studies) (186). The important roles of phosphoinositide 5-phosphatases, such as synaptojanin and the OCRL 5-phosphatase as well as the PIP 5-kinases at the internalization and recycling of vesicles from and into the PM, indicate that PtdIns(4,5)P$_2$ is a functional identifier of the PM. A delicate balance between PtdIns4P and PtdIns(4,5)P$_2$ in the PM-endosomal interface is critical both to the fusion (recycling) and the fission (endocytic) processes. The Golgi contains the largest amount of PtdIns4P, and the various intracellular vesicular compartments contain either PtdIns3P or PtdIns4P and perhaps PtdIns5P, although the distribution of the latter as well as the major route of its production is still not fully understood. Although PtdIns4P has long been viewed only as a precursor of PtdIns(4,5)P$_2$ in the PM, it has become clear in the last decade that PtdIns4P is a major regulatory lipid in the Golgi, the TGN, and some endosomes (35). PtdIns4P working together with Arf1 proteins is important to recruit clathrin adaptors such as AP-1 (179), AP-3 (29), or GGAs (177) to their respective target membranes. It is noteworthy that all of the PH domains that recognize PtdIns4P are part of proteins that transport lipids (see below), revealing a pivotal role of PH kinases as master regulators of structural lipid distribution within the cell. Whether PtdIns4P made in the Golgi contributes to the phosphoinositide supply of PM is still an important open question discussed above.

Membrane dynamics, actin cytoskeleton, and phosphoinositides

The plasma membrane of most metazoan cells shows enormous plasticity and undergoes shape changes driven by actin polymerization. This process is very critical for both physiological processes such as chemotaxis, cell adherence, or morphogenesis and to pathological ones such as invasive cancer metastasis or the spreading of pathogens from one cell to another. In all of these instances, a very active membrane deformation is elicited by polymerized actin. We have discussed above the phosphoinositide-binding domains (FERM, ENTH) found in a large number of proteins that interact with the actin cytoskeleton. However, in addition, proteins that regulate actin polymerization have their own phosphoinositide-binding motifs (122). One such motif is the profilin/WH2 (Arabidopsis) domain, which is present in a large number of proteins that interact with the actin cytoskeleton (37). Mo...
The internal components of cytocytic routes, such as PtdIns(4,5)P₂, are sequestered in the dumping of vesicles (80). The formation of these vesicles is relevant to the understanding of the internal processes that are linked to nuclear components. This is also true for the internalization of the actin cytoskeleton (128). The Arp2/3 complex is regulated by members of the Wiscott-Aldrich Syndrome proteins (WASP). Some of these, like N-WASP, have putative phosphoinositide binding motifs (122). Our knowledge on the dynamics of actin polymerization has been greatly enriched by studies on the intracellular movement of the bacterium Listeria monocytogenes with a strong connection to phosphoinositides (89).

The endless complexity of PI3-kinase signaling

Research on PI3-kinases has exploded in the last 20 years, and the progress cannot be summarized even in a limited way in this review. The roles of the various PI3-kinase isoforms have been clarified using recombinant enzymes. These developments have been summarized in (10). The roles of the enzymes Ipk1 and Ipk2, which are identified the enzymes Ipk1 and Ipk2, which are PI3Ks, are critical components of immune cell signaling (3, 88, 103). This clear functional separation has generated enormous interest in developing subtype-specific PI3-kinase inhibitors as adjuvant treatment in cancer and as anti-inflammatory or immunosuppressive agents, respectively (103). Progress in understanding the functions of the Class II and Class III PI3-kinases has been somewhat slower since these enzymes have more complex roles in vesicular trafficking that in many cases might be redundant with other phosphoinositide regulatory enzymes. These developments have been summarized in a number of excellent reviews (e.g., Ref. 47) and will not be further discussed here.

Nuclear processes and phosphoinositides

Several lines of evidence suggest that the nucleus has its own phosphoinositide signaling system (36), although its significance has only recently been emerging (80). Nuclear phosphoinositide changes separate those detected in the cell membranes from those detected in the cell membrane with the help of confocal (164). PtdIns(4,5)P₂ binding to the actin binding proteins, on the other hand, has been described in IGF-stimulated Swiss 3T3 cells (37). Moreover, several of the enzymes known to process inositol-glycerol linked to a PIP₅-kinase Isx, which was recently found to associate with and regulate the activity of a mRNA polyadenylation enzyme STAR (5). The most recent addition to the class of PI3-kinase inhibitors as adjuvant treatment in cancer and as anti-inflammatory or immunosuppressive agents, respectively (103). Progress in understanding the functions of the Class II and Class III PI3-kinases has been somewhat slower since these enzymes have more complex roles in vesicular trafficking that in many cases might be redundant with other phosphoinositide regulatory enzymes. These developments have been summarized in a number of excellent reviews (e.g., Ref. 47) and will not be further discussed here.

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Lipid transport regulated by phosphoinositides

One of the most exciting recent developments in phosphoinositide research is the finding of a link between sphingolipid metabolism and phosphoinositides (66). Earlier analysis of the phosphoinositide-binding specificities of various PH domains revealed that all of the proteins whose PH domains specifically recognized PtdIns4P were lipid transport proteins. These included the oxygen binding protein (OSBP) [95] and its yeast homologs Osh1 and Osh2 [93, 138, 187], the ceramide-binding protein CERT [67, 94], as well as the FAPP1 and FAPP2 proteins [40]. These findings have already forecasted that lipid transfer function and PH-kinasess would be intimately interconnected. This was elegantly demonstrated with the discovery of the CERT protein that transfers ceramide between the site of its synthesis in the ER to the trans side of the Golgi, where ceramide is then flipped and converted to sphingomyelin (SM) in the Golgi lumen. In addition to the ceramide binding module, CERT also contains a PH domain that is important to dock the protein to the Golgi and a mutation within the PH domain that eliminates PtdIns4P renders the CERT protein completely dysfunctional (67). What is remarkable about the PtdIns4P regulation of CERT transport function is that it appears to require the type IIbeta PH-kinate enzyme (159), even though all of the four PH-kinate enzymes can produce PtdIns4P in some parts of the Golgi (8). Based on recent observations, a regulatory loop is emerging in which Golgi DAG levels regulate the recruitment/activity of PKD enzymes that phosphorylate and activate PIK3CIII (68) but also phosphor- ylate CERT, but this phosphorylation decreases CERT binding to PtdIns4P (52).

Less is known about the inositol regulation of the oxygen transport function of the OSBP protein or its homologs. However, several clues suggest that phosphoinositides and oxytore binding proteins are functionally coupled. Deletion of one of the yeast oxytore binding proteins, Kex1p, bypasses the Sec14 (a yeast PI- transfer protein) defect [45, 97], and recent structural and functional data suggest that the sterol transfer function of the Kex1p (and perhaps other OSBP homologs) are regulated by phosphoinositides [78, 131]. There exists an important connection between cholesterol metabolism and SM synthesis: oxytore treatment or cholesterol depletion strongly stimulates CERT-mediated ceramide transport and Golgi SM production, and this effect requires the OSBP protein (125).

The biology of the FAPP1 and FAPP2 proteins has been more controversial. FAPP1 was initially identified as a protein that contains a PH domain with specific PtdIns4P recognition (four-phosphate adaptor protein) [46]. A highly homologous protein, FAPP2, however, was also shown to contain a glycolipid transfer protein homology (GLTP) domain (174), and it has been proposed that FAPP2 transfers glycosylceramide (GlcCer) between membranes in a PtdIns4P-regulated manner (32). It is not clear yet between which membranes this transfer occurs. GlcCer is synthesized on the outer surface of the cis-Golgi, but its conversion to lactosyl ceramide and the more complex glycosphin- golipids (GLSL) occurs in the lumen at the trans-Golgi (64). One report suggested that FAPP2 is needed to transfer GlcCer from the cis- to the trans-Golgi in a nonvesicular transport step (32), whereas another study proposed that FAPP2 transferred GlcCer from the cis-Golgi back to the ER, where its flipping to the lumen was found most efficient (64). Regardless of the route (s) of transport FAPP2 supports, the protein was found critical for the transport of apical cargos to the membrane in polarized cells and for the formation of cilia in the apical membrane, presumably because of the need for organization of glycolipid-rich membrane domains (173). More studies are expected to clarify this role of the FAPP2 protein as well as to determine which of the PI4-kinasess are critical for supporting its PH domain membrane interactions.

The importance of phospholipid transport proteins in the Golgi to PM secretion process has long been established in S. cerevisiae. Here, the Sec14p protein that encodes a phosphatidylinositol-phosphatidylcholine (PP/PC) transfer protein was found to be critical to the yeast secretion process (14). The function of the Sec14p is to supply the Golgi with PtdIns and PtdCho, ultimately to maintain the levels of DAG in this organelle. DAG is an important regulator of a number of Golgi-associated kinases containing cystein-rich Zn2+ finger motifs, such as some PKC isofoms (90) and PKD (15). In addition, PtdIns4P is also a key organizer of the Golgi to PM vesicular secretion process via recruitment of adaptor proteins (179), and Sec14p can also supply PtdIns for the Golgi-localized PI4-kinasess (65, 141). Moreover, there are several Sec14 homologs in yeast that have a role in transferring lipids between the various yeast membranes, and it was recently shown that PtdIns4P synthesis is important in some of these transport functions (137). One of these Sec14 homologs was also identified as a component of the synthesis of the aminophospholipid phosphatidylethanolamine (PtdEtN) via decarboxylation of ER-derived phosphatidylethanolamine (PtdSer) in Golgi membranes (162). For this, PtdSer has to be transferred to the Golgi membranes to be decarboxylated, and genetic studies have shown that Stt4p (the yeast homolog of PKH23[162]) is a regulatory component of this process at the Golgi (but not at the mitochondrial) site (162). It is not yet understood why the Stt4p kinase is needed for the lipid transfer and whether it acts at the donor or acceptor membrane site. Also, it has yet to be seen whether a similar regulation of aminophospholipid synthesis by PI4-kinasess or by other phosphoinositides is present in higher organisms.

Interestingly, mammalian PI(3,4,5)P3 show only low sequence homology to Sec14, although Sec14 homologs are also found in mammalian cells, and they also transport α-tocopherol and PtdIns(4,5)P2 (38), and they may be involved in the transport of aminophospholipids (140). Mammalian PI(3,4,5)P3 transport is required cell proliferation (18), and it is also important for ciliogenesis, and cilia in the apical membrane, presumably because of the need for organization of glycolipid-rich membrane domains (173). More studies are expected to clarify this role of the FAPP2 protein as well as to determine which of the PI4-kinasess are critical for supporting its PH domain membrane interactions.

Methods

Most of our initial observations were made using either 3H- or 14C-labeled lipids, followed by separation of extracted lipids using thin-layer chromatography (TLC) (145). The separation of extracted lipids was performed using either 3H- or 14C-labeled lipids, followed by separation of extracted lipids using thin-layer chromatography (TLC) (145). The separation of extracted lipids was performed using either 3H- or 14C-labeled lipids, followed by separation of extracted lipids using thin-layer chromatography (TLC) (145). The separation of extracted lipids was performed using either 3H- or 14C-labeled lipids, followed by separation of extracted lipids using thin-layer chromatography (TLC) (145). The separation of extracted lipids was performed using either 3H- or 14C-labeled lipids, followed by separation of extracted lipids using thin-layer chromatography (TLC) (145). The separation of extracted lipids was performed using either 3H- or 14C-labeled lipids, followed by separation of extracted lipids using thin-layer chromatography (TLC) (145). The separation of extracted lipids was performed using either 3H- or 14C-labeled lipids, followed by separation of extracted lipids using thin-layer chromatography (TLC) (145). The separation of extracted lipids was performed using either 3H- or 14C-labeled lipids, followed by separation of extracted lipids using thin-layer chromatography (TLC) (145). The separation of extracted lipids was performed using either 3H- or 14C-labeled lipids, followed by separation of extracted lipids using thin-layer chromatography (TLC) (145). The separation of extracted lipids was performed using either 3H- or 14C-labeled lipids, followed by separation of extracted lipids using thin-layer chromatography (TLC) (145). The separation of extracted lipids was performed using either 3H- or 14C-labeled lipids, followed by separation of extracted lipids using thin-layer chromatography (TLC) (145). The separation of extracted lipids was performed using either 3H- or 14C-labeled lipids, followed by separation of extracted lipids using thin-layer chromatography (TLC) (145). The separation of extracted lipids was performed using either 3H- or 14C-labeled lipids, followed by separation of extracted lipids using thin-layer chromatography (TLC) (145). The separation of extracted lipids was performed using either 3H- or 14C-labeled lipids, followed by separation of extracted lipids using thin-layer chromatography (TLC) (145). The separation of extracted lipids was performed using either 3H- or 14C-labeled lipids, followed by separation of extracted lipids using thin-layer chromatography (TLC) (145).
which membrane transport operations are also transport lipophilic compounds such as the α-tocopherol binding protein [101]. However, mammalian PITPs are functionally similar to Sec4 proteins [28], and they help maintain DAG levels in the Golgi complex. PITPs are regulated by inositol phosphates, and rather than being transported to the ER to the PI4-kinasas [141] and the PI4-specific PtdIns(3,4,5)P3 in Golgi, DAG in this form takes anywhere between 4 h and several days to achieve the desired effects. During this period, the primary affected process (such as the release of an adapter protein) initiates a whole sequence of events, leading to changes from which it is hard to deduce what processes have been primarily linked to the lipid changes. The optimal solution to these problems is to use specific inhibitors to evoke acute changes in inositol levels. This is best demonstrated by the enormous boost that discovery of PI3-K kinases brought to the field of PI3-kinases [4]. Unfortunately, there are no good specific inhibitors for many of the inositol converting enzymes. This problem has prompted us [169] and others [156] to design alternative strategies by which inositol levels can be acutely changed in specific membrane compartments. This is based on the regulated recruitment into defined membrane compartments of inositol kinase or phosphatase enzymes that are stripped of their own localization mechanisms. This method relies on the assumption that the enzymes resulting in the cytosol have limited impact on the formation of inositol lipid domains.
on the membrane-bound lipids, but this is changed dramatically once the enzyme is recruited to the membrane where its substrate resides. The recruitment is based on the heterodimerization of the FRB domains of mTOR and the FRBIP12 protein (17) in the presence of rapamycin or an appropriate analog, so fuising the enzyme to one of these domains and targeting the other partner to the desired membrane compartment allows a regulated recruitment process. This method was successfully used to change the PtdIns(4,5)P2 levels in the plasma membrane and assess its consequences on ion channel activity (156, 169) as well as on the membrane binding of clathrin adapters and their roles in the endocytic process (1, 191). This approach can be extended to other enzymes and compartments and will be interesting to study the roles of phosphoinositides in other cellular locations. The fact remains that we have fundamental gaps in our research tools and developing more specific inhibitors should help us better answer the open questions. Although this approach can be extended to other enzymes and compartments and will be interesting to study the roles of phosphoinositides in other cellular locations.

Concluding Remarks

Even from this limited overview, it should be obvious that phosphoinositides have an enormous impact on any membrane-associated signaling processes. Extensive research on these lipids over several decades has led to great advances in our understanding of cell signaling. However, the plethora of information available on these lipids is hard to comprehend. The fact remains that we have fundamental gaps in our understanding of the functions of phosphoinositides and the mechanistic details of how they control membrane dynamics. Improving our research tools and developing more specific inhibitors should help us better answer the open questions. Although this field of research was mostly driven by the curiosity of scientists conducting basic research, the achievements of the field have served the goals of public health very aptly. Ten years ago, PI3-kinase inhibitors were only thought about as research tools, and today they are in clinical trials in targeting diseases such as cancer, autoimmune, allergy, and metabolic disorders. Right now, “translational research” does not appear to be why we need to develop inhibitors of PIP5-kinases or PI4-kinases. However, this can change in a heartbeat. As a good example, PI4KIIIs has just emerged as a critical factor in the assembly of the Hepatitis C virus in the liver as reported in three separate recent studies (18, 157, 163). This finding will suddenly make this enzyme a desirable drug target. There is ample reason to expect that an expanded investment in phosphoinositide research will bring a payback in real medical terms.

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References

several as "nucleolus disorder" does not appear to be a term used in biological or medical literature. It may be a misprint or a typo.


The lipid connectivity

The lipid connectivities (LCCs) are used to describe the connectivity of membrane lipids. LCCs are represented as graphs where nodes represent lipid species and edges represent the connectivity between these species. This approach allows for the visualization and analysis of lipid biogenesis, turnover, and remodeling processes in living cells.


The importance of the EGF receptor

The EGF receptor is a transmembrane protein that plays a critical role in cell growth, proliferation, and survival. It is activated by binding to its ligand, EGF, which leads to the activation of downstream signaling pathways. The EGF receptor's activation is crucial for a wide range of cellular responses, including apoptosis, cellcycle regulation, and differentiation.


The role of oxysterol-binding protein

Oxysterol-binding protein (OXPBP) is a lipid-binding protein that plays a critical role in lipid transport and metabolism. It is involved in the clearance of reactive oxygen species, the regulation of membrane fluidity, and the modulation of lipid signaling.


The significance of polyphosphoinositides

Polyphosphoinositides (PPIs) are a class of lipid molecules that are involved in a variety of cellular processes, including membrane trafficking, vesicle budding, and signal transduction. They are synthesized and degraded by specific enzymes, which play a critical role in the regulation of these processes.


The role of phosphoinositide 3-kinase

Phosphoinositide 3-kinase (PI3K) is a lipid kinase that plays a critical role in various cellular processes, including cell growth, proliferation, and survival. It is activated by binding to specific lipid second messengers, which trigger the phosphorylation of phosphatidylinositol 4-phosphate (PtdIns(4)P) to generate phosphatidylinositol 3,4,5-trisphosphate (PtdIns(3,4,5)P3).


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