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REVIEWS

Phosphoinositide-Dependent Perimembrane Mechanisms of Regulating Cellular Processes

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Abstract—Phosphoinositides are minor phospholipids of cytosolic membrane surface involved in the regulation of vital cellular processes, including membrane trafficking, cytoskeletal dynamics and cell signaling. This regulation lies in their ability to control the subcellular localization and activity of different cytosolic (peripheral) effector proteins bearing phosphoinositide binding domains. However, the detailed molecular mechanisms by which phosphoinositides (and probably other phospholipids) may participate in the regulation of cellular functions remain the subject of debate. This review discusses the general features of the functioning of phosphoinositide system as an organizer and integrator of intracellular events.

Keywords: lipid metabolism, phosphoinositides, membrane traffic, intracellular signaling
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INTRODUCTION

Phosphoinositides – phosphorylated derivatives of phosphatidylinositol – are lipids with various number of phosphate groups in the inositol ring that include mono-, bis-, and trisphosphate derivatives in various combinations. All forms of phosphoinositides are minor components of cell membranes and differ by intracellular localization and functions.

A dramatic change in the phosphoinositide (and phosphatidic acid) metabolism in pancreas slices upon stimulation with acetylcholine was the first indication of the role of lipids as regulatory intermediates [1]. However, the fact that the change in lipid metabolism is tightly related to Ca^{2+} signalization was established only 20 years later [2]. Further studies revealed a specific role of the products of the phospholipase-C-catalyzed hydrolysis of phosphatidylinositol-4,5-bisphosphate – diacylglycerol and inositol-1,4,5-triphosphate – in the regulation of protein kinase C and intracellular Ca^{2+} concentration [3]. Besides, it was found that phosphoinositides are involved not only in signaling processes but also in membrane trafficking and cytoskeleton reorganization [4–7].

The aim of this review is not to cover all the aspects of regulatory functions of phosphoinosidides but rather to survey common molecular events occurring at the surface of cellular compartments (that is, at the perimembrane area of organelles) and mediating the regulation of cellular functions by phosphoinositides.

DISTRIBUTION OF LIPIDS IN ORGANELLE MEMBRANES AND LIPID ASYMMETRY OF THE BILAYER

Lipid synthesis is accomplished in different locations of a eukaryotic cell, and lipids are further delivered to cellular compartments by various ways; this explains a non-uniform distribution of lipids in organelle membranes. A major site of the lipid synthesis de novo is endoplasmic reticulum (ER), where most of phospholipids are synthesized, as well as cholesterol and its esters, triacylglycerides, and sphingolipids, which are the precursors of ceramides [8].

Synthesis of sphingmyelins, glucosyl- and lactosylceramides, and glucosphingolipids takes place in Golgi complex [9]. Phosphatidylethanolamine is formed by phosphatidylcholine decarboxylation in the same organelle [10], and the final stage of the phosphatidylcholine synthesis can be carried out by the enzymes localized both in Golgi complex and in ER [11].

About half of phospholipids of the mitochondrial membrane are synthesized in this organelle. First of all, it is phosphatidic acid, its lysogenic forms that are utilized in the triglyceride synthesis, as well as phosphatidylglycerol – a precursor of the mitochondrial cardiolipin [12]. As in Golgi, phosphatidylethanolamine is formed from phosphatidylserine by its decarboxylation [10].

Lipids are not synthesized de novo in plasma membrane (PM), in which, however, lipid intermediates are produced in the process of cleavage of polar head group or acyl moiety, as well as owing to phosphoryla-

tion/dephosphorylation of inositol-containing lipids [13, 14].

In cellular membranes, with the exception of ER membrane, which will be considered later, lipids are distributed between monolayers asymmetrically. The outer monolayer of PM features a high content of sphingomyelin, phosphatidylcholine, and glycosphingolipids and the inner monolayer, a higher content of aminophospholipids (phosphatidylserine and ethanolamine), inositol-containing lipids, and phosphatidic acid. Correspondingly, in Golgi and endosome membrane, a cytosolic monolayer is enriched with aminophospholipids and inositol-containing lipids and a luminal monolayer, with sphingomyelin, phosphatidylcholine, and glycolipids [15].

It is known that the lipid asymmetry can be maintained owing to the physico-chemical properties of the lipid molecules. It was shown on model membranes that the rate of spontaneous "flip-flop" translocation of polar lipids depends on the charge and size of the polar head group. For example, phosphatidic acid at low pH is neutral and easily translocates between monolayers; however, when a pH gradient across the membrane is created, phosphatidic acid accumulates at the side where pH is higher [16]. Besides, characteristic lifetime of lipids in the monolayer depends on their structure. For complex glycosphingolipids it can be days; for less complex phosphatidylcholines, hours [17, 18], and for small hydrophobic lipids like ceramides, diacylglycerol and sterols, seconds [19–21].

However, physico-chemical and structural peculiarities of lipid molecules are not the only cause of the lipid asymmetry. The transbilayer (or flip-flop) lipid motion may be mediated by integral proteins. This view is based on the comparison of the rates of the lipid flip-flop motion in biogenic membranes (with autonomous production of lipids) and non-biogenic membranes. It was shown that a characteristic time of the flip-flop translocation in artificial bilayers [22, 23] and non-biogenic membranes [24, 25] ranges from several hours to several days, while in biogenic membranes this time is of the order of seconds [26–28]. These data suggest the existence of specialized membrane proteins that mediate the lipid motion from one monolayer to the other and thus participate in the creation and maintenance of lipid asymmetry of the natural membranes. Nowadays, at least three groups of membrane proteins are classified as lipid translocases; among them are flippases (P4-ATPases), floppases (ABC transporters), and scramblases [15, 29].

Flippases catalyzing ATP-dependent transfer of lipids from exoplasmic to cytosolic monolayer of the membrane are grouped into the P4 family of the P-type ATPase superfamily, which in mammalians includes 14 members [30, 31]. P4-ATPases are identified in the membranes of eukaryotic cells only; in contrast to other P-type ATPases, they translocate phospholipids but not cations [30, 32]. Flippase activity of the P-type ATPases was demonstrated in reconstructed proteoliposomes containing Drs2p (yeast P4-ATPase) that exhibited ATP-dependent transfer of fluorescent analog of phosphatidylserine, while in the proteoliposomes with catalytically inactive form of this protein the transfer was absent [31].

ABC transporters are also involved in the maintenance of the membrane lipid asymmetry; these transporters belong to a large superfamily of evolutionary conservative proteins that are able to translocate various substances, including sugars, peptides, xenobiotics, lipids, etc. [33–35]. In higher organisms the ABC superfamily includes 49 members that are classified into 7 families (A–G). By means of genetic methods it was shown that about half of the ABC transporters can actively translocate lipids between monolayers [34, 36]; however, direct proofs in vitro were obtained only for some of them.

ABC transporters, or floppases, consuming the ATP energy, translocate lipids from the cytosilic monolayer into the luminal, that is, they operate in the direction opposite to that of flippases. This was shown both for naturally occurring membranes [37–39] and for the artificial membranes with reconstructed ABC proteins [40]. For example, ABCA1 and ABCA7 translocate cholesterol and phosphatidylserine [41–43], ABCA4 is a high-affinity transporter of phosphatidylserine [37], and ABCA1 translocates a broad range of lipids, including glycerophospholipids, sphingolipids, and platelet activation factor [34, 37].

In contrast to the membranes of other organelles, the ER membranes have a symmetric lipid composition of the outer and inner monolayers [44]. Lipid synthesis occurs at the cytosolic leaflet of ER; so, half of the lipids synthesized has to get into the luminal leaflet. In ER, the rate of the lipid migration between the monolayers is orders of magnitudes higher than the rate of their spontaneous flip-flop between the monolayers [23, 28, 45]; this suggests an existence of a special mechanism ensuring a symmetric distribution of lipids. This mechanism is termed *scrambling* and, accordingly, the proteins accomplishing this process were named scramblases. It is assumed that they translocate lipids from one monolayer to the other with equal probability and, in contrast to flippases and floppases, without energy consumption. Although this phenomenon is substantiated by the reconstruction of the ER protein extract into the liposomes [46], the particular proteins ensuring the lipid symmetry in ER have not been identified yet [47].

The asymmetry of the lipid bilayer is tightly related with cellular functions. It was shown that the platelet activation induced by the blood vessel injury is accompanied by the appearance of phosphatidylserine in the outer monolayer, and this is required for further reactions of the blood clotting [48]. Induction of apoptosis in lymphocytes also leads to the loss of the lipid asymmetry, appearance of phosphatidylserine in the outer monolayer, and activation of phagocytosis [49, 50]. Artificial redistribution of aminophospholipids

between monolayer affects the cell cycle and cytokinesis [51]. Besides, convincing evidences have been accumulated, which point to the impact of the lipid asymmetry dynamics on membrane trafficking [52], formation and activity of protein signaling complexes [4, 53], as well as on the activity of receptors, channels, and transporters [54]. A pivotal role in all these processes belongs to phosphoinositides – phosphorylated derivatives of phosphatidylinositol.

SUBCELLULAR DISTRIBUTION AND METABOLISM OF PHOSPHOINOSITIDES

Phosphatidylinositol (PI) can be reversibly phosphorylated in position 3, 4, and 5 of the inositol ring, which gives seven derivatives – mono-, di-, and triphosphatidylphosphates, or phosphoinositides: PI(3)P, PI(4)P, PI(5)P, PI(3,4)P_2, PI(3,5)P_2, PI(4,5)P_2, and PI(3,4,5)P_3 (Fig. 1). The relative content of phosphatidylinositol with respect to other lipids in membranes of eukaryotic cells is several percent, and the amount of its phosphorylated derivatives is one order of magnitude lower.

A peculiar feature of the phosphoinositide biogenesis in a cell is that in ER, the main place of the lipid synthesis de novo, only their precursor – phosphatidylinositol – is produced. All its phosphorylated derivatives are synthesized in other cellular compartments with the participation of phosphoinositide-specific kinases and phosphatases. After its synthesis in ER, phosphatidylinositol is delivered to cellular compartments by means of membrane trafficking. Phosphorylation of PI in position 4 with the formation of PI(4)P mainly occurs in Golgi complex and plasma membrane. In Golgi complex PI(4)P plays an important role in biogenesis of transport vesicles [55, 56], and in plasma membrane it is utilized to form PI(4,5)P_2 – a precursor of secondary messengers of lipidic origin [57, 58]. Early endosomes are the main location of PI(3)P involved in binding of a wide range of cytosolic proteins that control membrane trafficking [59, 60]; in late endosomes, PI(3)P is phosphorylated to PI(3,5)P_2, which influences the migration of these organelles [61].

The available data suggest an existence of the predominant forms of phosphoinositides in various organelles; in particular, PI(4,5)P_2 dominates in PM; PI(4)P, in Golgi complex; PI(3)P, in early endosomes, and PI(3)P and PI(3,5)P_2, in late endosomes. Nowadays, the question of functional significance of such uneven distribution of phosphoinositides is actively discussed. It is believed that the spatio-temporal distribution of phosphoinositides in the cell creates a system of signposts (beacons) that control cellular processes, and the role of the controlling mechanism is played by a targeted association (recruitment) of cytosolic proteins with phosphoinositide local sites on the cell membrane surface [4, 62, 63].

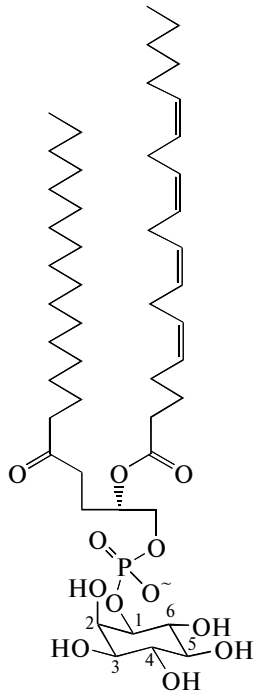

Fig. 1. Chemical structure of phosphatidylinositol. Positions 3, 4, and 5 of the inositol ring are reversibly phosphorylated in the metabolic processes, yielding seven phosphorylated species of phosphatidylinositol (phosphoinositides): PI(3)P, phosphatidylinositol-3-phosphate; PI(4)P, phosphatidylinositol-4-phosphate; PI(5)P, phosphatidylinositol-5-phosphate; PI(3,4)P_2, phosphatidylinositol-3,4-bisphosphate; PI(3,5)P_2, phosphatidylinositol-3,5-bisphosphate; PI(4,5)P_2, phosphatidylinositol-4,5-bisphosphate, and PI(3,4,5)P_3, phosphatidylinositol-3,4,5-trisphosphate.

Why such an important role is ascribed to phosphoinositides? First, they are multicharged (up to –4 at pH 7), and owing to the relatively low content as compared to other lipids, they can form local sites with a strong electrostatic charge on the membrane surface, which in turn gives an advantage for the interactions with positively charged protein groups [64]. Second, within the recent decade, more than 10 of protein structural domains specific to different phosphoinositide species have been recognized; these domains are commonly present in the cytosolic proteins involved in membrane trafficking, cellular signaling, and cytoskeleton remodeling (effector proteins) [4]. And, finally, a dynamic control of the phosphoinositide content in the membranes, accomplished by phosphoinositide-specific kinases and phosphatases, ensures flexible cell responses to stimuli [6, 65, 66].

PHOSPHOINOSITIDE-SPECIFIC PROTEIN DOMAINS

Many cytosolic proteins function when they are associated with the membrane. Their association with the surface of the membrane occurs through structural

protein modules (domains) with specificity to certain lipids [4]. Several types of protein modules are known that are able to bind with phosphoinositides; they are designated as PH, PX (Phox), FYVE, ANTH, FERM, GOLPH3, PDZ, PTB, ENTH, PROPPIN, and some other. The origin of the names of these protein domains and their abbreviations can be found in a number of reviews [4, 67–69].

PH domain. PH domains are the best characterized structural modules of the phosphoinositide-binding proteins [70]. The first PH domain identified in the beginning of the 1990th received its name from two homological regions of pleckstrin, the main substrate of the platelet protein kinase C [71, 72]. Since then, this structural module has been found in 275 human proteins involved in cell signaling, membrane trafficking, cytoskeletal reorganization, and lipid metabolism [4].

Canonical PH domain is a conservative three-dimensional structure formed by 120 amino acid residues. Most of PH domains exhibit a low specificity to phosphoinositides; however, 20% have a high specificity to PI(3,4,5)P_3, PI(4,5)P_2, and PI(3,4)P_2. Among the well defined proteins containing PH domains, there are such proteins as ARNO, Btk, Gap1, and Grp1, which bind with PI(3,4,5)P_3 [73, 74]. PH domain of phospholipase C (PLCδ1) is specific to PI(4,5)P_2, and domains of proteins TAPP1, centaurin β2, PEPP1, and FAPP1 prefer PI(3,4)P_2, PI(3,5)P_2, PI(3)P, and PI(4)P, respectively [70, 75]. PH domains are found in guanine nucleotide exchange factors (ARNO, FGD2, Grp1, etc.) and in the small GTPase activating proteins (centaurin β2, Gap1), in phospholipases (PLCδ1, PLD1, and PLD2), lipid-transfer proteins (FAPP2), cytoskeletal proteins (dynamin, β-spectrine), kinases (Btk/Itk, CERK, PKB), phosphatases (PHLPP1), and in other proteins involved in almost all important cellular processes, including cell growth, proliferation, migration, membrane trafficking, signaling, immune response, and apoptosis.

PH domains contain sites composed of lysine and arginine residues that can create electrostatic contacts with negatively charged groups of lipids on the membrane surface. Spatial structure and high positive potential of these sequences favor specific binding of PH domains with phosphoinositides; however, non-specific interactions with other lipids are also possible [70, 76–78]. In particular, using the method of single molecule fluorescence, it was shown that PH domain of protein Grp1 can interact with phosphatidylserine polar group, and this contact facilitates its binding with PI(3,4,5)P_3 [79]. Besides, it was shown that hydrophobic loops of PH domains of various proteins (ARNO, DAPP1, Fapp1, Grp1, PLCδ1, and TAPP1) can insert into the lipid bilayer [77, 78, 80].

FYVE domain. Structural domain, first identified in proteins Fab1, YOTB, Vac1, and EEA1, and named FYVE by the first letters of these proteins, is a conservative spatial structure formed from 70 amino acid residues. Nowadays, this protein module is found in 28 human proteins [68]. By its topology, FYVE belongs to a known protein family with zinc-binding fingers and predominantly interacts with PI(3)P. PI(3)P is a dominating phosphoinositide of endosomes, multivesicular bodies, and phagosomes [81–83]. It was shown that many proteins containing FYVE domains (EEA1, FENS-1, FYCO1, Hrs, Rabip4, and WDFY2, as well as yeast Vac1p and Vps27p) regulate docking and fusion of transport vesicles with endosomes; other proteins (Hrs, SARA, FGD2) play an important role in signaling, cytoskeletal reorganization (EhFP), or apoptosis (Phafin1 and 2) [84]. Some kinases (Fab1 and PIKfyve) and phosphatases (MTMR3 and MTMR4) also contain domain FYVE, and its binding with PI(3)P determines both localization of these proteins in the cell and their enzymatic activity [84].

FYVE-domains predominantly interact with PI(3)P. Their association with the membrane surface, as in the case of PH domains involves non-specific factors, such as electrostatic contacts with phosphatidylserine or phosphatidic acid [85, 86] or insertion of hydrophobic loops into the bilayer [85–87]; in some cases, dimerization of FYVE domains is required [88, 89].

PX domain. Structural domain PX found in 47 signaling and regulatory proteins of mammals is comprised of about 130 amino acids [67]. The domain got its name from two subunits of the phagocyte NADPH-oxidase – $p40^{phox}$ and $p47^{phox}$, in which it was found initially [90]. The main target of PX domains is PI(3)P (proteins KIF16B, $p40^{phox}$, PXK, Vam7p), although binding with other forms of phosphoinositides is also possible (proteins Bem1, CISK, CPK, FISH, NOXO1, $p47^{phox}$, PI3K-C2α, PLD1, and SNX) [91, 92]. Nexins (SNX) regulating membrane trafficking between endosomes and Golgi complex comprise the largest group of proteins that carry PX domain [67].

As in the case of the FYVE and PH domains, association of PX domains with the membrane surface involves non-specific electrostatic contacts with negatively charged lipids, as well as the insertion of the hydrophobic loop into the bilayer [93–95].

Other phosphoinositide-specific domains. The list of the phosphoinositide-binding domains has been extended recently and nowadays, in addition to domains PH, FYVE, and PX described above, it also includes ANTH, C2, ENTH, FERM, GOLPH3, PDZ, PROPPIN PTB, and Tubby. Domain ANTH and a related one, ENTH, specifically bind PI(4,5)P_2 of the plasma membrane [69]. C2 domains more commonly associate with phosphatidylserine and phosphatidylcholine, although some of them prefer PI(3,4,5)P_3 and PI(4,5)P_2 [96]. Domain FERM is an effector of PI(4,5)P_2; GOLPH3 binds PI(4)P in Golgi complex, and PDZ recognizes PI(4,5)P_2 of the plasma membrane [97–100]. Domains PROPPIN of human and yeast proteins bind PI(3,5)P_2 in the membranes of endosomes, lysosomes, and vacuoles [101], and in Drosophila they interact with PI(3,5)P_2 and PI(3)P [4]. Domain PTB, which usually binds phosphoty-

rosine residues, interacts with $PI(4,5)P_2$ and PI(4)P, and Tubby associates with plasma membrane through binding with $PI(4,5)P_2$ [70, 102, 103].

Modules described above have no similarity at the primary sequence level; however, the mechanisms of their association with the membrane surface have much in common. First, they all have phosphoinositide-specific pocket containing three to six positively charged amino acid residues and a histidine residue; for example, three positive residues and one histidine residue in domain ANTH [103], or six positive residues and one histidine in domains ENTH and PH [104]. Second, the process of the association of structural modules with the membrane proceeds in several steps; apart from the phosphoinositide-specific binding, it includes non-specific electrostatic contacts with the membrane surface and the insertion of the hydrophobic loops into the bilayer. Besides, in some cases the association of proteins with the membrane surface can be intensified owing to dimerization of the phosphoinositide domains [4, 13].

PHOSPHOINOSITIDES REGULATE MEMBRANE TRAFFICKING, CELL SIGNALING, AND CYTOSKELETAL DYNAMICS

Until recently, it was believed that phosphoinositides just play the role of the precursors of the lipidic second messengers. Now phosphoinositides are also regarded as regulatory molecules that, together with small G-proteins of superfamily Ras, control membrane trafficking and cytoskeletal dynamics [105]. Numerous investigations show that a unique combination of various forms of phosphoinositides and small G-proteins that, like phosphoinositides, exhibit organelle-specific properties, can be a basis of the mechanism controlling the time and place of the targeted association of the effector proteins with the organelle membranes [106].

In naturally occurring membranes phosphoinositides are in the process of interconversions. Short-lived signal molecules $PI(3,4)P_2$ and $PI(3,4,5)P_3$ are produced in response to extracellular stimuli [107]. The content of PI(3)P, PI(4)P, $PI(3,5)P_2$, and $PI(4,5)P_2$ in the membranes is relatively stable but can be regulated by stimuli and thus influence intracellular processes [105].

$PI(4,5)P_2$ OF PLASMA MEMBRANE

$PI(4,5)P_2$ of plasma membrane can play the role both of the precursor of secondary messenger and of the anchor, by which cytosolic proteins attach to the membrane surface. In the eukaryotic cells, secondary messengers of lipid origin are produced by PLCδ and phosphatidylinositol-3-kinase of type I (PI3KI). It is noteworthy that the PLC-dependent signaling process is found in all eukaryotes, while PI3KI-mediated signaling is a distinctive feature of multicellular organisms [108].

In the "canonical" signaling process involving PLCδ, two secondary messengers – diacylglycerol and inositol-3-phosphate (IP_3) – are formed from $PI(4,5)P_2$ [109]. IP_3 activates Ca^{2+} channels of the intracellular stores, through which Ca^{2+} is released into the cytoplasm, thus conveying a signal. Diacylglycerol remains in plasma membrane and in a Ca^{2+}-dependent manner activates protein kinases of the PKC and PKD families, as well as a number of ion channels [110, 111]. A feature peculiar to this process is that $PI(4,5)P_2$ plays the role not only of the PLCδ substrate but also of an anchor connecting the enzyme with plasma membrane through its PH domain. PLC-dependent signaling process regulates such physiological functions as cellular motility, fertilization, sensory transduction, and is also involved in the control of cell growth, proliferation, and differentiation [112, 113].

$PI(4,5)P_2$ can also be phosphorylated by PI3KI to phosphatidylinositol-3,4,5-triphosphate [107, 114, 115]. This secondary messenger of the lipidic origin in minor quantities is present in quiescent cells, but its level can considerably (by an order of magnitude) rise upon stimulation by growth factors. The mechanism of the $PI(3,4,5)P_3$ action is that it recruits to the plasma membrane various cytosolic proteins-effectors, such as guanine nucleotide exchange factors; small GTPase activating proteins; phosphoinositide-dependent protein kinase 1 (PDK1) and protein kinase B (PKB/Akt) that regulate many signaling targets [107, 114]. It was shown that $PI(3,4,5)P_3$ regulates cell proliferation and migration, chemotaxis, phagocytosis, cell differentiation, and other processes [107, 114, 115]. Dephosphorylation of $PI(3,4,5)P_3$ is performed by phosphatases of two types and leads to different results. Dephosphorylation in position 3 by PTEN "switches off" the signal [116], while dephosphorylation by 5-phosphatases results in the formation of $PI(3,4)P_2$ that is able to prolong the effect of the $PI(3,4,5)P_3$ signal to a certain extent [117].

A stable activation of both PLCδ- and PI3KI-dependent signaling processes requires the replenishment of the $PI(4,5)P_2$ pool in plasma membrane; this is accomplished by the successive phosphorylation of phosphatidylinositol by kinases PI4K and PI4P5K. That is why the identification of these enzymes is essential for the understanding the regulation mechanisms of intracellular signaling [5]. It was shown in a number of studies, however, that depending of a cell type or physiological stimuli, PI4P5K isoforms, which directly supply PLC with the substrate, may vary. In particular, the hydrolysis of $PI(4,5)P_2$ by PLCδ in stimulated platelets was maintained by isoforms PI4P5Kα and PI4P5Kβ but not by PI4P5Kγ [118], while in mast cells PLCδ hydrolyzed $PI(4,5)P_2$ generated by isoform PI4P5Kγ [119, 120].

$PI(4,5)P_2$ is not only a precursor of secondary messengers but also functions as an anchor, with the help

of which many cytosolic proteins attach to the PM surface with different selectivity [62, 121]. Quite frequently, as, for example, in the case of PLCδ, it is ensured by means of the previously mentioned structural domains; however, in a number of cases cytosolic proteins attach to the membrane surface by means short polybasic amino acid sequences [122]. An interaction of $PI(4,5)P_2$ with such sequences of actin-binding proteins and small GTPases has been demonstrated [122, 123]. As small GTPases regulate different cellular processes, including signaling, membrane trafficking, and cytoskeleton reorganization, it is assumed that they can interact with different $PI(4,5)P_2$ pools of PM.

In PM local microdomains enriched with $PI(4,5)P_2$ are found; their origin, however, remains unclear: some investigations point to the association of $PI(4,5)P_2$ with detergent-resistant membrane regions – "rafts", while other data suggest the immobilization of $PI(4,5)P_2$ by proteins termed "phosphoinositide modulins", or PIP modulins [64, 124]. Protein MARCKS (myristoylated alanin-rich C-kinase substrate) is a well-known example of PIP modulins [124]. Considering that one molecule of PIP modulin can bind several $PI(4,5)P_2$ molecules and that these proteins are quite abundant, theoretically they are able to immobilize in local sites the entire PM pool of $PI(4,5)P_2$ [64, 125, 126]. Lateral compartmentalization of phosphoinositides in rafts or local sites by means of immobilization with PIP modulins are not mutually excluding events, as, on the one hand, many PIP modulins, including MARCKS, are located in rafts, and on the other hand, $PI(4,5)P_2$ itself is involved in the raft formation [127]. Although proteins with functions of PIP modulins have not been found in yeast and plants yet, these data suggest an existence of spatially separated microdomains (pools) of $PI(4,5)P_2$ in PM and put forward a question of their functional similarity.

Available data suggest that different pools of $PI(4,5)P_2$ in PM can be sustained by different phosphoinositide kinases. For example, in stimulated mast cells, PIP5KIβ and PIP5KIγ provide the $PI(4,5)P_2$ pools that are involved, respectively, in regulation of Ca^{2+} and in the production of IP_3 catalyzed by PLC [119, 120]. This implies the existence of the mechanisms of the targeted delivery of phosphoinositide kinases to certain/specific sites of PM. It is believed that the mechanisms of the targeted delivery can vary but in any case they are determined by the lipid composition of the membrane surface and peripheral proteins that assist the kinases to attach to the membrane [128, 129]. In mammals and yeast, these proteins are small GTPases, and the membrane-associated protein complexes containing appropriate lipid-metabolizing enzymes and small GTPases are regarded as a mechanism involving phosphoinositides in cell regulation [130, 131].

Phosphorylation/dephosphorylation of phosphoinositide kinases and phosphatases offers new regulatory options. This was illustrated by altering the metabolism of $PI(4,5)P_2$ by protein kinase Cdk5 and protein phosphatase calcineurine that regulate the activity of both PI4P5Kγ and phosphoinositide phosphatase synaptojanin 1 in synapses [132, 133]. There are many examples of the regulation of the PI4P5K activity by protein kinases and phosphatases in cells of mammals, yeast, and plants [134, 135].

Mammalian cells contain six small GTPases of the Arf family; based on the primary sequence, these proteins are divided into three classes [136]. They all can activate PIPKI and PLD, which leads to the change of the content of $PI(4,5)P_2$ and phosphatidic acid, respectively, in the lipid bilayer [137, 138]. Small GTPase Arf6 is associated with PM, where it is involved in the membrane trafficking and the remodeling of actin cytoskeleton [139, 140]. It was shown that Arf6 is co-localized with PIPKI, activates it and thus stimulates the production of $PI(4,5)P_2$, which is required for the targeted association of PLD with PM [141, 142]. Phosphatidic acid produced by PLD stimulates in its turn the PIPKI activity [142, 143]. Such combined loops of positive feedback increase the levels of both $PI(4,5)P_2$ and phosphatidic acid in the membrane; this determines the role of Arf6 in the activation of proteins ensuring membrane trafficking and rearrangements of the cytoskeleton [63, 139, 140].

In the membrane trafficking, Arf6 regulates Ca^{2+}-dependent exocytosis [140, 141]. Expression of an active mutant protein Arf6 in neuroendocrine cells results in translocation of this protein, as well as $PI(4,5)P_2$ and PIPKIγ from plasma membrane to endosomes. The authors suggested that the Arf6-induced depletion of $PI(4,5)P_2$ in PM results from the translocation of PIPKIγ from PM to endosomes that, in turn, leads to the inhibition of Ca^{2+}-dependent exocytosis. The fact that Arf6 physically associates with PIPKIγ and stimulates PIPKIγ-dependent exocytosis in neuroendocrine cells can be regarded as a direct proof of the Arf6-mediated regulation of PIPKIγ [141].

The ability of $PI(4,5)P_2$ to regulate cellular processes by means of PLCδ/PI3KI signaling or by recruiting into the PM of cytosolic proteins containing lipid-binding structural domains (PLCδ) or specific polybasic amino acid sequences (small GTPases) is apparently an evolutionary feature of the eukaryotic cells. Most of the biochemical data, however, have been obtained on mammalian cells and yeast, and only a few proteins containing phosphoinositide-specific domains were found in plants. Nevertheless, the studies of the *Arabidopsis* genome predict the existence of numerous proteins with characteristic lipid-binding domains [144].

PI(3)P OF EARLY ENDOSOMES

PI(3)P mediates targeted association of cytosolic proteins containing FYVE and PX domains with the membrane of early endosomes [145–147]. A number

of such proteins regulate membrane trafficking and membrane dynamics of these organelles. Good examples of these proteins are early endosome antigene-1 (EEA1), kinase substrate Hrs, and a family of sorting proteins nexins (SNX).

EEA1 regulates docking and fusion of vesicles with the endosome membrane. Successful docking requires both association of EEA1 (through the FYVE domain) with the endosome PI(3)P and interaction of EEA1 with active form of small GTPase Rab5, even though EEA1 was shown to possess a low affinity to Rab5 [88, 148]. It is assumed that Rab5 helps EEA1 to bind with the membrane and also regulates its further functions. For example, when interacting with such membrane fusion proteins (SNARE) as syntaxin-6 and syntaxin-13 in early endosomes, Rab5 acts as a regulator of the membrane trafficking. When Rab5 recruits Vps34 (PI3-kinase, generating PI(3)P from phosphatidylinositol), it plays the role of the regulator of the endosome membrane dynamics [149, 150]. In other words, the mechanism of recognition of the endosome membranes by means of PI(3)P and small GTPase Rab5 is in essence the mechanism controlling the time and place of the association of cytosolic proteins with the membrane.

In contrast to EEA1, association of Hrs (through domain FYVE) with the early endosome membrane occurs independently of Rab5 [151]. Protein Hrs participates in sorting of ubiquitinated proteins of endosomes [152]. It also associates with the phagosome membrane in a PI(3)P-dependent manner, which is necessary for the phagosome maturation and their fusion with late endosomes [153].

Yet one more group of cytosolic proteins employing PI(3)P as an anchor is a family of sorting nexins (SNX) containing PX domains in their structure [154]. However, not all PX domains of the proteins from the SNX family specifically interact with PI(3)P. For example, SNX9 exhibits quite a wide specificity to phosphoinositides [155].

PI(3)P can be dephosphorylated by different phospholipid phosphatases. In particular, phosphatase Sac1 can dephosphorylate PI(3)P, PI(4)P, and $PI(3,5)P_2$, although its role in the regulation of the endosomic pool of PI(3)P in vivo remains questionable [156, 157]. Phospholipid phosphatases of the MTM/MTMR family, containing 15 proteins, dephosphorylate both PI(3)P and $PI(3,5)P_2$ in the endosome membranes [158, 159]. It is established that in early endosomes phosphatase MTM1 can form complexes with kinases Vps34 and Vps15 [160]. It is not clear whether Vps34/Vps15 and MTM1 within the complex are active simultaneously, but it is likely that a tight association of the lipid kinase and phosphatase is essential for the control of the endosomic pool of PI(3)P.

$PI(3,5)P_2$ OF LATE ENDOSOMES

The pool of $PI(3,5)P_2$ in late endosomes depends on the functional state of two phosphoinositide kinases. In yeast, PI3 kinase Vps34 [161] and PI3P5 kinase Fab1 [162] are found that are responsible for the production of $PI(3,5)P_2$ in late endosomes. Kinase Fab1 contains domain FYVE, through which it interacts with PI(3)P and associates with the membrane of late endosomes [162, 163]. PIKfyve, the Fab1 homologue in mice, also binds with PI(3)P and localizes in the endosome–lysosome system [164].

It was shown that yeast Fab1 kinase (or PIKfyve in mice) is a part of a complex based on protein Vac14 (or ArPIKfyve in mice). Another partner of Vac14 in this complex is $PI(3,5)P_2$-specific phosphatase Fig4 [165, 166]. It is proposed that by means of combination of kinase and phosphatase in one complex, the endosomic pool of $PI(3,5)P_2$ can be controlled [165–167]. It should be noted that metabolism of $PI(3,5)P_2$ can also be controlled by phosphatases of the MTM family. In contrast to Fig4, however, that acts as 5-phosphatase, these phosphatases dephosphorylate $PI(3,5)P_2$ in position 3 of the inositol ring with the formation of PI(5)P [168].

Apparently, $PI(3,5)P_2$ of late endosomes is involved in a number of cellular processes. In yeast, one of the effector of $PI(3,5)P_2$ is Atg18 that participates in the retrograde transport of vesicles from vacuoles to Golgi complex [169]. Besides, this protein interacts with myosin adaptor Vac17 and, possibly, regulates interactions between the cytoskeleton and membrane [170].

Other effectors of the endosomic $PI(3,5)P_2$ are epsins containing domain ENTH [171]. In vitro experiments showed that Ent3 and Ent5 can bind with $PI(3,5)P_2$ through their ENTH domain and interact with protein Vps27, which is able to recognize ubiquitinated cargo proteins; however, the physiological role of these complexes is not quite clear [172].

PI(4)P OF GOLGI MEMBRANES

Golgi membranes are enriched in PI(4)P; besides, minor pools of $PI(4,5)P_2$ and $PI(3,4,5)P_3$ are found in this organelle [127, 173]. PI(4)P is formed from phosphatidylinositol with the help of two lipid kinases: PI4KIIα and PI4KIIIβ. It is proposed that these two enzymes generate spatially separated and, possibly, functionally different PI(4)P pools of Golgi complex [173].

PI4KIIα has a lipid modification, which is important for its association with the membrane; it is unclear, however, how PI4KIIα binds with Golgi membranes [174]. It is shown that this kinase is predominantly located in rafts and is recruited not only by Golgi complex but also by other organelles, such as PM, endosomes, and ER [174, 175].

Targeted association of PI4KIIIβ with Golgi membrane occurs in the complex with an activated form of small GTPase Arf1 and protein NCS-1 – calcium sen-

sor involved in the modulation of Ca^{2+}-dependent processes [176, 177]. Although the function of NCS-1 is not fully established, it is known, however, that this protein interacts with ion channels and G-protein-coupled receptors [178]. The activity of PI4KIIIβ is regulated by protein kinases PKD1 and PKD2 that phosphorylate PI4KIIIβ, which is subsequently recognized by the proteins of the family 14-3-3 stabilizing the active form of PI4KIIIβ [179, 180]. A similar mechanism operates in yeast; the only difference is that in contrast to mammalian proteins, yeast proteins of family 14-3-3 regulate not the activity but the redistribution of kinase Pik1 (ortolog of PI4KIIIβ) between the cytosole, nucleus, and membrane of Golgi complex [181].

Several phosphoinositide phosphatases can associate with Golgi membranes. These are 5-phosphatases OCRL1 and INPP5B, predominantly using $PI(4,5)P_2$ as a substrate; INPP5E, cleaving 5-phosphate from $PI(3,4,5)P_3$ and $PI(3,5)P_2$, and 4-phosphatase Sac1.

It was established that OCRL1 contains N-terminal PH domain and associates both with the *trans*-Golgi network and with endosome membranes in various cells. Targeted association of OCRL1 with *trans*-Golgi is controlled by small GTPases Rab1 and Rab6; Rab5 participates in the association of OCRL1with endosomes [105].

The amino acid sequences of INPP5B and OCRL1 exhibit 45% homology. Like OCRL1, INPP5B is located in Golgi complex and endosomes and interacts with Rab5 [182]. Besides, INPP5B can interact with other proteins of the Rab family (Rab1, Rab2, Rab6, and Rab9) and, in contrast to OCRL1, is located in earlier compartments of Golgi complex. Lipidic 5-phosphatase INPP5E is also found in Golgi but its localization is controlled by small GTPase Rab20 [183].

Phosphoinositide phosphatase Sac1 is an integral transmembrane protein found in ER and Golgi complex both in yeast and mammalians [184]. In vitro substrates of Sac1 are PI4P, PI3P, and $PI(3,5)P_2$; however, yeast strains with mutant form of this phosphatase have a high level of PI4P, suggesting that in vivo Sac1 prefers PI4P to other substrates. A human homologue of Sac1 is cloned, which is fully analogous to the yeast protein by substrate specificity and localization [185].

There is a permanent flow of membrane and protein structures going on through Golgi complex; this requires specialized controlling mechanisms preventing their random migration. PI(4)P gradient increasing in the direction ER–*cis*-Golgi–*trans*-Golgi is considered to be a possible mechanism [186]. It is believed that this gradient is maintained by phosphatase Sac1 that migrates between the ER and Golgi compartments [184, 187]. Although the cellular function of the PI(4)P gradient is still to be clarified, it is known that the Sac1 deficiency leads to obvious disarrangement of all Golgi compartments, structural anomalies, and impaired localization of enzymes in this organelle [186].

Three functionally different groups of PI(4)P effectors can be distinguished in Golgi complex: coat adaptors, lipid-transfer proteins, and regulators of G-proteins. Association of these effectors with the membrane generally occurs either by means of PH or ENTH/ANTH domains, as well as through the interaction of the polybasic amino acid sequences with PI(4)P.

As known examples of PI(4)P-dependent coat adaptors of Golgi complex, clathrin adaptor protein complex 1 (AP-1) can be mentioned [56], as well as proteins of the Arf family (GGA) binding small GTPases [188], protein of clathrin-coated vesicles EpsinR, and adaptors of family Vps74/GOLPH3 involved in vesicle formation and membrane trafficking from Golgi to PM [98, 99].

AP-1 and proteins of family GGA participate in the cargo delivery from *trans*-Golgi network to endosomes and lysosomes. For their targeted association with Golgi membrane, an activated small GTPase Arf1 and PI(4)P are needed [56, 188]. A decrease in the PI(4)P level owing to the inhibition of the kinase PI4KIIα activity by interfering RNA blocks the association of AP-1 and GGA proteins with Golgi complex [56, 188]. EpsinR – one more adaptor protein functioning between *trans*-Golgi and endosomes – binds with PI(4)P, AP-1, and clathrin [189, 190]. PI(4)P-effector proteins of the GOLPH3/Vps74 family connect Golgi membrane with cytoskeleton, participate in the membrane trafficking from Golgi to plasma membrane, and in the control of localization of Golgi glycosylating enzymes [98].

Another group of the Golgi PI(4)P effectors are lipid-transfer proteins involved in non-vesicular lipid motion. These are proteins of families CERT, FAPP, and OSBP that are featured by N-terminal PH domain and C-terminal lipid-transferring domain. Members of these protein families are able to associate with Golgi membrane; an important role in this association is played by the activated small GTPase Arf1 [191, 192]. OSBP, CERT, and FAPP can transfer sterols, ceramides, and glycosylceramides, respectively, between the membranes in the in vitro conditions. Ceramide and cholesterol synthesis is completed in ER, but steady intracellular distribution of the derivatives of these lipids is characterized by low content in ER and high content in *trans*-Golgi compartments. Thus, a possible role of proteins CERT and OSBP is the supply of Golgi membranes with these lipids [193, 194]. A similar situation for the family FAPP proteins: glycosylceramides are synthesized on the cytosolic side of *cis*-Golgi but are metabolized to complex glycosphingolipids in late Golgi, and this process requires FAPP2 as a co-factor [195]. Besides, CERT, FAPP, and OSBP are involved in the events of membrane trafficking between ER, Golgi, and PM; this suggests that these proteins not only can participate in lipid

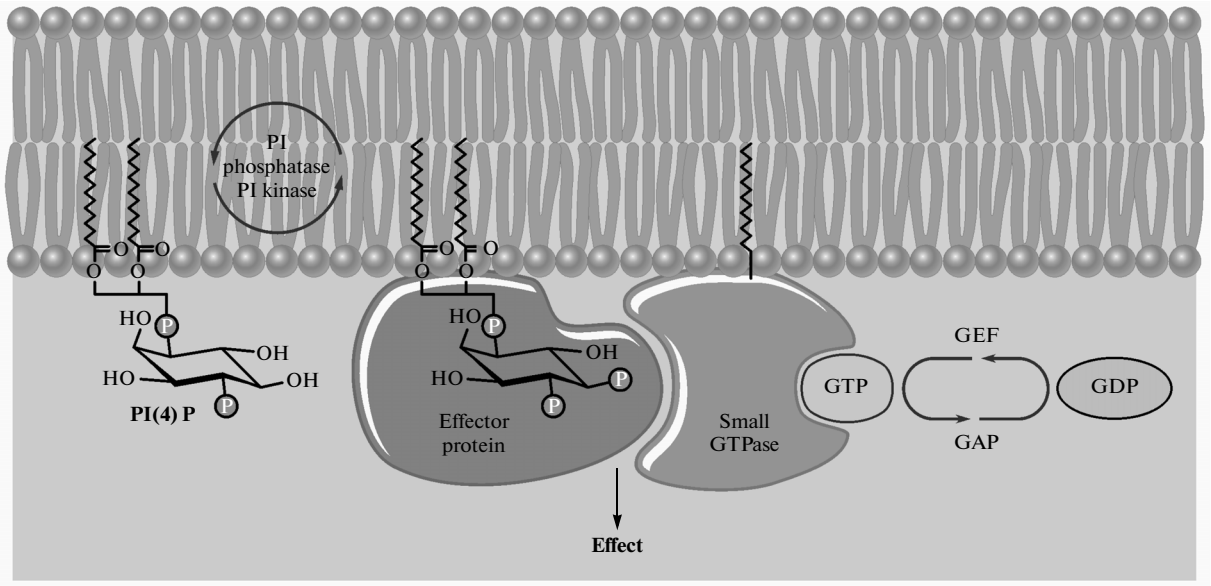

Fig. 2. General features of perimembrane protein complexes controlling cellular processes. Cytosolic effector proteins possess structural domains that are able to bind with phosphoinositides of the membrane surface. In the perimembrane state effector proteins probably exist in complexes with phosphoinositide-specific kinases and phosphatases, as well as with small GTPases that are necessary for targeted association of the effectors with organelle membranes. Regulation of the activity of the effectors in the perimembrane complex depends on the rate of either activation/inactivation cycles of small GTPases by means of GEF and GAP or phosphorylation/dephosphorylation cycles of phosphoinositides by means of phosphoinositide kinases and phosphatases. Designations: PI(4)P, phosphatidylinositol-4-phosphate; GEF, guanine nucleotide exchange factor; GAP, GTPase-activating protein.

sorting but also accomplish PI(4)P-dependent control of the membrane lipid composition in points of intersections of vesicular and non-vesicular lipid transfer.

CONCLUSIONS

In eukaryotic cells, membranes of organelles, except for ER, are characterized by asymmetric distribution of lipids between the cytosolic and luminal monolayer. Lipid asymmetry is generated by ATP-dependent lipid translocases (P4-ATPases and ABC transporters) and is maintained owing to physicochemical properties of certain lipids.

Bilayer lipid asymmetry is related with cellular functions. These relations are shown for a number of phospholipids, including phosphatidylserine, phosphatidic acid, and phosphoinositides. However, molecular mechanisms through which the membrane lipids can participate in the regulation of cellular processes remain unascertained. Most of the researches that addressed these questions concern the role of phosphoinositides in the regulation of cell signaling and membrane trafficking.

Phosphoinositides are minor components of the cytosolic surface of cellular membranes. They bear a considerable (compared to other phospholipids) negative charge, which provides them an advantage in interactions with positively charged protein moieties. Phosphorylation/dephosphorylation of the inositol ring is accomplished by cytosolic enzymes – phosphoinositide kinases and phosphatases. Various phosphoinositide-specific isoforms of these enzymes are associated with different organelles, and therefore the membranes of organelles are enriched with appropriate phosphoinositides. It is proposed that the uneven distribution and precise spatial-temporal control of the content of certain phosphoinositides in cellular compartments are the factors underlying the mechanism of the control of cellular processes.

Cytosolic effector proteins regulating cell signaling, membrane trafficking, and cytoskeletal dynamics frequently contain phosphoinositide-specific structural domains mediating targeted association of these proteins with membrane surface of cellular compartments (Fig. 2). This process is often controlled by organelle-specific small G-proteins (small GTPases and their regulators) that recognize membrane surface by means of polybasic amino acid sequences. Thus, perimembrane protein complexes controlling cellular processes contain not only effector proteins but also small G-proteins and possibly organelle-specific isoforms of phosphoinositide kinases and phosphatases. Regulation of the activity of the effector proteins in such complexes will depend on the rates of cycles both activation/inactivation of small GTPases and phosphorylation/dephosphorylation of phosphoinositides. In this model, a strong lipidic specificity of a effector protein seems a less important factor than its interaction with lipid-modifying enzymes and suggests the

existence of fast feed-backs enabling a cell to flexibly respond to stimuli.

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