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Phosphoinositide Control of Membrane Protein Function: A Frontier Led by Studies on Ion Channels

Diomedes E. Logothetis,[1] Vasileios I. Petrou,[2] Miao Zhang,[1] Rahul Mahajan,[1] Xuan-Yu Meng,[3] Scott K. Adney,[1] Meng Cui,[1] and Lia Baki[1]

[1]Department of Physiology and Biophysics, School of Medicine, Virginia Commonwealth University, Richmond, Virginia 23298-0551; email: delogothetis@vcu.edu

[2]Department of Physiology and Cellular Biophysics, Columbia University, New York, NY 10032

[3]School for Radiological and Interdisciplinary Sciences (RAD-X), Collaborative Innovation Center of Radiation Medicine of Jiangsu Higher Education Institutions, and Jiangsu Provincial Key Laboratory of Radiation Medicine and Protection, Soochow University, Suzhou 215123, China

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Keywords

phosphoinositides, PI(4,5), P_2, ion channels, PI(4,5), P_2-induced gating, modulation, phosphorylation

Abstract

Anionic phospholipids are critical constituents of the inner leaflet of the plasma membrane, ensuring appropriate membrane topology of transmembrane proteins. Additionally, in eukaryotes, the negatively charged phosphoinositides serve as key signals not only through their hydrolysis products but also through direct control of transmembrane protein function. Direct phosphoinositide control of the activity of ion channels and transporters has been the most convincing case of the critical importance of phospholipid-protein interactions in the functional control of membrane proteins. Furthermore, second messengers, such as $[Ca^{2+}]_i$, or posttranslational modifications, such as phosphorylation, can directly or allosterically fine-tune phospholipid-protein interactions and modulate activity. Recent advances in structure determination of membrane proteins have allowed investigators to obtain complexes of ion channels with phosphoinositides and to use computational and experimental approaches to probe the dynamic mechanisms by which lipid-protein interactions control active and inactive protein states.

INTRODUCTION

PIP: phosphoinositide

PI(4,5)P_2 or PIP_2: phosphatidylinositol 4,5-bisphosphate

Phosphoinositides (PIPs) are inositol phospholipids found in cellular membranes. They are composed of two fatty acid chains linked by a glycerol moiety to a water-soluble inositol head group (**Figure 1**). Specialized lipid kinases and phosphatases respectively add and remove phosphates at specific positions of the inositol ring (**Figure 1**).

In plasma membranes, the most abundant PIP is phosphatidylinositol 4,5-bisphosphate [PI(4,5)P_2, also known as PIP_2], where it composes ~1% of the total phospholipid pool present (1, 2). PIPs bear a net negative charge at neutral pH that allows them to engage in electrostatic

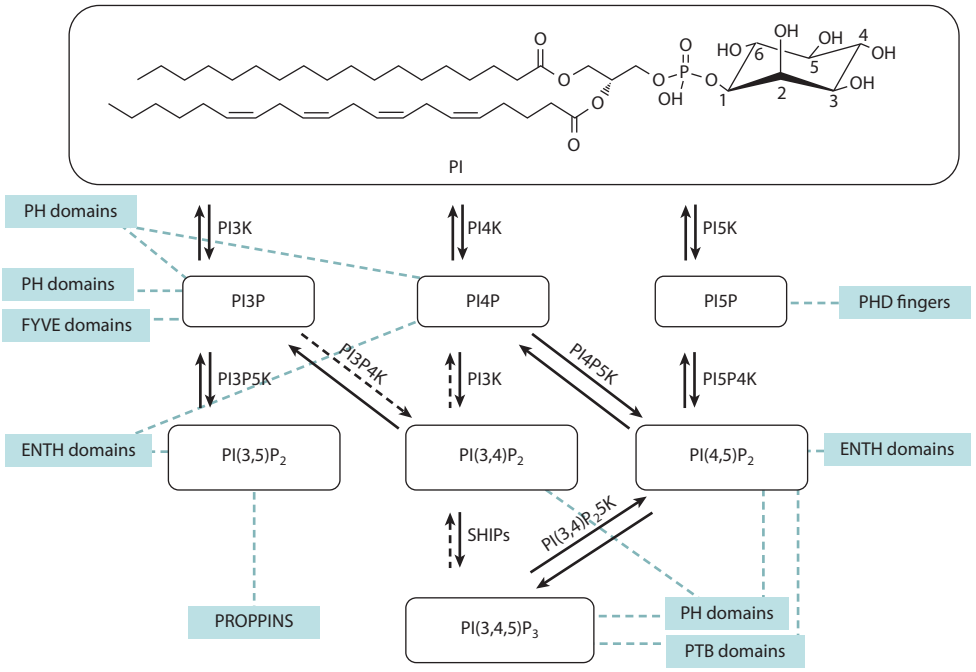

Figure 1

PIPs, their metabolism, and protein domains that bind them. PI is phosphorylated by specific kinases at the 3′, 4′, or 5′ positions to yield monophosphorylated PIPs. PIP kinases recognize the monophosphorylated species and phosphorylate them to yield the diphosphorylated PIPs. PIP_3 can be formed by phosphorylation of PI(4,5)P_2 by PI3K. The reverse reactions are catalyzed by phosphatases acting at the 3′, 4′, or 5′ positions (5′ phosphatase, e.g., SHIPs; 3′ phosphatase, e.g., PTEN). Phospholipid-binding domains that recognize specific PIPs are also shown. Modified from Reference 1. Abbreviations: ENTH domain, epsin N-terminal homology domain; FYVE domain, domain common to the Fab1, YOTB, Vac1, and EEA1 proteins; PH domain, pleckstrin homology domain; PHD fingers, plant homeodomain fingers; PI, phosphatidylinositol; PI(3,4)P_2, phosphatidylinositol 3,4-bisphosphate; PI(3,4)$P_2$5K, phosphatidylinositol 3,4-bisphosphate 5-kinase; PI(3,4,5)P_3, phosphatidylinositol 3,4,5-trisphosphate; PI(3,5)P_2, phosphatidylinositol 3,5-bisphosphate; PI(4,5)P_2, phosphatidylinositol 4,5-bisphosphate; PI3K, phosphatidylinositol 3-kinase; PI3P, phosphatidylinositol 3-phosphate phosphatase; PI3P4K, phosphatidylinositol 3-phosphate 4-kinase; PI3P5K, phosphatidylinositol 3-phosphate 5-kinase; PI4K, phosphatidylinositol 4-kinase; PI4P, phosphatidylinositol 4-phosphate phosphatase; PI4P5K, phosphatidylinositol 4-phosphate 5-kinase; PI5K, phosphatidylinositol 5-kinase; PI5P, phosphatidylinositol 5-phosphate phosphatase; PI5P4K, phosphatidylinositol 5-phosphate 4-kinase; PIPs, phosphoinositides; PROPPINS, β-propellers that bind polyphosphoinositides; PTEN, phosphatase and tensin homolog; PTB domain, phosphotyrosine-binding domain; SHIP, SH2-containing inositol 5-phosphatase.

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interactions with positively charged regions of proteins. Since the 1990s it has been appreciated that, besides the well-established signaling role of their hydrolysis products [e.g., the hydrolysis of $PI(4,5)P_2$ by phospholipase C (PLC) to inositol trisphosphate (IP_3) and diacyl glycerol (DAG)], PIPs interact directly with basic regions of proteins to recruit them to the membrane and control their function.

The number of membrane-associated and transmembrane proteins utilizing changes in PIPs to adjust their conformations and functions is large. Moreover, the biological functions that PIPs modulate are essential to life, and cells depend on PIPs for proper function. In this review, we start by summarizing the roles of PIPs in modulating cell excitability through interactions with membrane-associated and transmembrane proteins. We then focus on PIP regulation of ion channel activity. Ion channels are the most-studied group of transmembrane proteins, and their study promises to offer fundamental insights as to how membrane proteins in general utilize plasma membrane PIPs to adjust their activity levels.

IP_3: inositol 1,4,5-trisphosphate

PI: phosphatidylinositol

PI4P: phosphatidylinositol 4-phosphate

PIP_3 or $PI(3,4,5)P_3$: phosphatidylinositol 3,4,5-trisphosphate

PI5P: phosphatidylinositol 5-phosphate

$PI(3,4)P_2$: phosphatidylinositol 3,4-bisphosphate

$PI(3,5)P_2$: phosphatidylinositol 3,5-bisphosphate

PHOSPHOINOSITIDE METABOLISM AND MEMBRANE LOCALIZATION

Phosphatidylinositol (PI) can be phosphorylated at positions 3, 4, and 5 of the inositol ring to give rise to seven different PIP species (**Figure 1**) that are involved in almost every facet of cell signaling. PI is synthesized in the endoplasmic reticulum and transported to other compartments through vesicles or cytosolic transfer proteins (3, 4). There are major differences in not only the functional significance but the localization and relative abundance of each PIP species; these attributes are intricately connected to the lipid kinases and phosphatases involved in the metabolism of PIP species (3, 5). The localization of different PIPs in the cell, in combination with the localization of small GTPases, is thought to be responsible for providing identity to different organelles (3, 6).

The most abundant PIPs are phosphatidylinositol 4-phosphate (PI4P) and $PI(4,5)P_2$, found predominantly in the plasma membrane, where phosphatidylinositol 3,4,5-trisphosphate [PIP_3, or $PI(3,4,5)P_3$] and PI(3,4)P are also located. $PI(4,5)P_2$ is produced mainly from PI4P through addition of a 5-phosphate by type I PIP kinase [phosphatidylinositol 4-phosphate 5-kinase (PIP5K)]. It can also be produced from phosphatidylinositol 5-phosphate (PI5P) through addition of a 4-phosphate by type II PIP kinase [a phosphatidylinositol 5-phosphate 4-kinase (PIP4K)] (3, 5), but PI5P is much less abundant than PI4P. The exact localization of PI5P is unclear. PIP_3 and phosphatidylinositol 3,4-bisphosphate [$PI(3,4)P_2$] are produced by the addition of a 3-phosphate to $PI(4,5)P_2$ and PI4P, respectively. The enzyme primarily responsible for this action is class I phosphatidylinositol 3-kinase (PI3K) (3, 5).

Early endosomes contain mostly phosphatidylinositol 3-phosphate (PI3P) produced from PI by class III PI3K, but also some $PI(3,4)P_2$. $PI(4,5)P_2$ is cleared during endocytosis from the internalized membranes by 5-phosphatases. Late endosomes are characterized by the presence of phosphatidylinositol 3,5-bisphosphate [$PI(3,5)P_2$], produced from PI3P by PIKfyve kinase. PI3P and $PI(3,5)P_2$ are crucial for the biology of endosomes and their function (3, 5).

The major PIP in the Golgi complex is PI4P. It is produced locally from PI by type IIα phosphatidylinositol 4-kinase (PI4KIIα), an isoform that regulates membrane traffic from the *trans*-Golgi network. Deficiencies in PI4P production affect both the structure and function of the Golgi. Some $PI(4,5)P_2$ has been reported to be present in the Golgi, although the 5-phosphatase OCRL that is located in the *trans*-Golgi network likely converts $PI(4,5)P_2$ back to PI4P rapidly (5).

Finally, some nuclear PIPs have been reported, albeit mostly in nonmembranous locations (7). In mammalian cells, nuclear $PI(4,5)P_2$ is implicated in regulating RNA splicing, histone-mediated transcriptional repression, and chromatin remodeling (8).

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FUNCTIONAL ROLES OF PHOSPHOINOSITIDES

Phosphoinositide Dysregulation Results in Disease

Because PIPs are involved in a number of diverse functions, their aberrant regulation has been implicated in an equivalent diverse number of diseases (reviewed in Reference 9). Briefly, Andersen-Tawil syndrome (ATS), defined by periodic paralysis, cardiac arrhythmia, and dysmorphic features, is caused by mutations in the gene encoding Kir2.1 [an inwardly rectifying K^+ (Kir) channel]. Multiple ATS mutations perturb channel-$PI(4,5)P_2$ interactions. In Kir1.1, loss of function results in hyperprostaglandin E syndrome, a prenatal disease presenting with polyhydramnios, hypokalemic alkalosis, and hypercalciuria. Kir6.2 mutants affecting $PI(4,5)P_2$ binding can be linked to congenital hyperinsulinism, which results from increased β cell excitability. Thyrotoxic hypokalemic periodic paralysis actually results from a gain of function in $PI(4,5)P_2$ binding in Kir2.6, expressed predominantly in skeletal muscle. Outside of the well-characterized Kir channelopathies, KCNQ channels responsible for the slow repolarizing current in cardiomyocytes are subject to mutations affecting channel-$PI(4,5)P_2$ interactions. These mutations are linked to a form of long-QT syndrome, causing debilitating cardiac arrhythmias and the potential for sudden cardiac death (9). Notable examples involving the nervous system are neurodegenerative disorders [such as Alzheimer's disease (AD), in which the oligomeric amyloid-β peptide that accumulates in AD patients decreases $PI(4,5)P_2$ levels (10)], mental retardation [such as Down's syndrome, or trisomy 21, in which the gene coding for the lipid phosphatase synaptojanin is localized to chromosome 21q and is overexpressed (11)], and demyelinating diseases [such as multiple sclerosis, in which the myelin basic protein, an essential component of the myelination pathway, fails to localize to the membrane when its interactions with $PI(4,5)P_2$ are disrupted (12)].

Functional Consequences of Interactions with Membrane-Associated Proteins

The reversible phosphorylation of the inositol group of plasma membrane PIPs provides a robust and flexible system for temporal and spatial regulation of the recruitment of several PIP-binding cytosolic proteins. Such phosphorylation results in the initiation of signaling cascades or the nucleation of multiprotein complexes regulating key cellular functions, such as protein trafficking, actin dynamics, dendritic morphogenesis, and neurotransmission.

Endocytosis/exocytosis. At the presynaptic terminus, PIPs, and specifically $PI(4,5)P_2$, control clathrin-mediated endocytosis by coordinating the biogenesis of clathrin-coated vesicles at multiple steps. Recruitment of coat and fission factors to the membrane depends on the association of the adaptor proteins AP-2 and AP-180 with $PI(4,5)P_2$ (13, 14), whereas fission of clathrin-coated pits involves a $PI(4,5)P_2$-dependent recruitment and activation of the GTPase dynamin I, which polymerizes into rings around the neck of the pits (13, 15). Under conditions of intense neuronal activity, clathrin-mediated endocytosis is largely replaced by activity-dependent bulk endocytosis, which also depends on $PI(4,5)P_2$ (16). Priming of synaptic vesicles for exocytosis also depends on $PI(4,5)P_2$, either indirectly, via its second messenger DAG, or directly through synaptotagmin (17, 18). At the calcium concentrations required for transmitter release, synaptotagmin I binds $PI(4,5)P_2$ and PIP_3 via a polylysine region in its C2 domain, facilitating plasma membrane penetration and exocytosis (17, 19).

Actin dynamics. Precise actin cytoskeleton remodeling and regulation of actin filament assembly and organization are fundamental to many cellular activities, including cellular architecture, adhesion strength, and motility. Actin dynamics are also critical for docking priming and

mobilization of synaptic vesicles at the presynaptic terminus (20), as well as for dendritic spine morphogenesis (21). $PI(4,5)P_2$ binds to and modulates the activity of several actin-associated proteins, which nucleate, cap, cross-link, or dissociate actin filaments or bind to and sequester actin monomers [for a list of such actin-associated proteins and the effects of their binding to $PI(4,5)P_2$, see References 22 and 23]. In general, $PI(4,5)P_2$ upregulates the activities of proteins, such as vinculin, talin, α-actinin, and ezrin, that promote actin polymerization and link actin filaments to each other. In contrast, $PI(4,5)P_2$ inactivates proteins, such as gelsolin, villin, cofilin, and profilin, that inhibit actin assembly or depolymerize actin filaments (23, 24). $PI(4,5)P_2$ is also implicated in the effects of protein kinase C (PKC) on the neuronal cytoskeleton via its association with the PKC substrates MARCKS (myristoylated alanine-rich C kinase substrate) and GAP43 (growth-associated protein 43), which regulate dendrite branching (25), neurotransmission (26), and neurite and growth cone morphology (27).

AKAPs. $PI(4,5)P_2$ also affects postsynaptic structure and function through AKAPs (A-kinase-anchoring proteins). AKAP79/150 is necessary for spine formation (28) and is linked to both structural and signaling proteins at the postsynaptic density. Membrane targeting of AKAP79/150 is achieved through interaction of its domain that is rich in basic and hydrophobic amino acids with $PI(4,5)P_2$, and AKAP79/150 preferentially targets kinases, such as protein kinase A (PKA) and PKC, and phosphatases, such as protein phosphatase 2B, to glutamate receptors, regulating glutamate receptor signaling and synaptic plasticity (29, 30).

Small GTPases. In neurons, small GTPases regulate membrane fusion and neurotransmission (31); dendritic spine morphogenesis and function (32); and axon growth, guidance, and branching (33). PIPs regulate GTPase activity by mediating the recruitment of guanine nucleotide exchange factors and GAPs to membranes (3).

Functional Consequences of Interactions with Transmembrane Proteins

Transmembrane proteins utilize anionic phospholipids in the inner leaflet of the plasma membrane to properly localize their cytosolic and extracellular domains. Since the mid-1990s, it has been appreciated that interactions between the cytosolic domains of transmembrane proteins and anionic phospholipids, $PI(4,5)P_2$ in particular, are critical for maintaining the functional integrity of these proteins.

Ion channels and transporters. Ion channels and electrogenic transporters lend themselves to high-resolution, real-time electrophysiological analysis under conditions in which PIP levels can be manipulated. Ever since they were first reported to functionally depend on $PI(4,5)P_2$ (34), several transporters and most ion channels tested have displayed a strong dependence of their activity on $PI(4,5)P_2$ levels. Several excellent reviews in the past decade have summarized hundreds of original publications (e.g., References 9, 35–46). Most ion transport proteins interact with PIPs to maintain an active state, whereas inhibition of such interactions by either depletion of the phospholipid or nonpermissive changes in protein conformation promotes an inactive state. This rapidly evolving area of PIP regulation of transmembrane protein function continuously provides new fundamental insights that require close monitoring.

Other transmembrane proteins. Regulation of transmembrane proteins by PIPs appears to extend beyond ion channels and transporters to growth factor receptors, such as the epidermal growth factor receptor (EGFR), which topologically utilize a similar protein basic region for interactions with the acidic $PI(4,5)P_2$ (47).

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Mutations in the inner juxtamembrane region of ion channels (near the interface of the last transmembrane domain and the cytosol) and of the EGFR affect the sensitivity of these proteins to PIPs. Because all integral membrane proteins obey the positive inside rule (48) and anionic phospholipids are in general determinants of membrane protein topology (49), these proteins may have evolved in eukaryotic membranes to use the anionic PIPs that are tightly regulated not only for membrane topology but also for modulation of function. Thus, a fundamental understanding of channel-PIP interactions and how they lead to stabilization of an active conformation is likely to underlie a general design of PIP regulation of the activity of eukaryotic transmembrane proteins. Moreover, given the essential roles of ion transport proteins in cellular function, a basic understanding of how PIPs affect the function of these proteins is likely to illuminate modulation of cell physiology.

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MOLECULAR CHARACTERISTICS OF PHOSPHOINOSITIDE BINDING TO PROTEINS

Phosphoinositide Binding to Membrane-Associated Proteins

The molecular characteristics of PIP binding to membrane-associated proteins based on a database of 25 crystallographic complexes have been previously reviewed (42). Certain common folds seem to be used by a variety of proteins to interact with PIPs (e.g., PH, ENTH, PTB, PX, PHD fingers, PROPPINS, FYVE) (**Figure 1**) (1, 42). A number of generalizations can be drawn from the analysis of the 25 complex structures. First, PIP-binding sites (*a*) always contained basic residues with at least one lysine and, in 84% of the structures, at least one arginine; (*b*) always contained at least one residue with an aromatic ring, mostly tyrosine and histidine, although tryptophan and phenylalanine residues could also be found; and (*c*) often contained small polar residues such as serine or asparagine (in 36% of the structures). Second, a total of at least five and at most seven residues were found in a pocket or groove that interacted with PIPs. Third, the affinity of PIP binding may be directly related to the number of specific contacts made between the protein and the PIP. Fourth, in general, specificity is determined by the relative binding affinities of the various PIPs such that a low specificity indicates a low binding affinity as well.

$PI(4,5)P_2$ Binding to Membrane Proteins

Atomic-resolution structures of Kir2.2 (50) and Kir3.2 (51, 52) bound to $PI(4,5)P_2$ (**Figure 2*a***) have identified six basic residues that interact directly with $PI(4,5)P_2$, five of which are common to both channels. The five common residues are the two basic residues in the KWR motif in the M1, outer helix; a basic (mostly lysine) residue on the cytosolic side of the M2, inner helix; and the two lysine residues in the KKR motif in the region linking to the cytoplasmic domain, immediately following the M2 transmembrane domain (**Figure 2*b***). An earlier atomic-resolution structure of a Kir3.1/KirBac1.3 chimera (PDB ID: 2QKS, referred to as the Kir3.1 chimera) showed a nonylglucoside detergent to be cocrystallized with the channel at the position where a $PI(4,5)P_2$ molecule could have been present (**Figure 2*c***) (53).

Structure-function studies in ion channels such as Kir2.1 have implicated ten residues as involved in $PI(4,5)P_2$ sensitivity: five arginines, four lysines, and one histidine (54). The types of residues implicated in PIP sensitivity are consistent with those found to bind PIPs in the complexes with membrane-associated proteins, as discussed above. Residues affecting $PI(4,5)P_2$ sensitivity identified by structure-function studies but not by crystallographic structures likely affect $PI(4,5)P_2$ sensitivity allosterically. Two such key Kir2.1 lysine residues, one in the N terminus (K64) and the

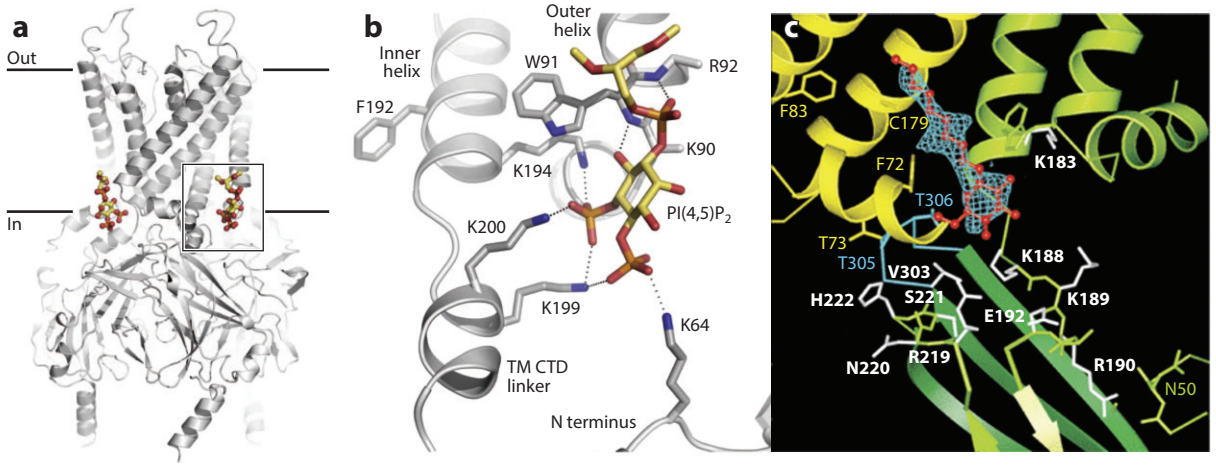

Figure 2

Putative interaction site of Kir channels with $PI(4,5)P_2$. (*a*) $PI(4,5)P_2$ molecules are colored yellow, orange, and red (denoting carbon, phosphorus, and oxygen atoms, respectively). $PI(4,5)P_2$ is shown in the context of the whole GIRK2 channel (*gray*). The thick black lines indicate the approximate boundaries of the plasma membrane, and the black box highlights the region of the close-up view in panel *b*. (*b*) Close-up view of $PI(4,5)P_2$ interactions. The main coordinating residues are shown as sticks. Residues K90 and R92 were modeled as alanines due to a lack of electron density but probably contribute to the positive electrostatics of the binding site. The important gating residue of the inner helix (or helix bundle crossing), F192, is also shown for reference. (*c*) The juxtamembrane Kir3.1 chimera region, where a nonylglucoside (*red*) with its contoured omit map (*cyan mesh*) is cocrystallized with the channel. Channel subunits and selected side chains near the detergent molecule are colored green and yellow, respectively. Residues 303–308 in the G-loop are colored cyan. Side chains colored white alter activation by $PI(4,5)P_2$ when mutated. Among them, side chains of K188, K189, E192, and R219 were disordered and poorly defined in the electron-density map, and residues 67 and 68 on the N-terminal side of the interfacial helix (*yellow*) were omitted for clarity. Abbreviations: CTD, C-terminal domain; $PI(4,5)P_2$, phosphatidylinositol 4,5-bisphosphate; TM, transmembrane. Panels *a* and *b* adapted from Reference 51 with permission from Elsevier. Panel *c* adapted from Reference 53 with permission from the European Molecular Biology Organization.

other in the CD-loop (K219), form a secondary anionic phospholipid-binding site that potentiates the effects of $PI(4,5)P_2$ that binds at the primary site (55, 56).

Use of synthetic water-soluble forms of PIPs with eight-carbon-long acyl chains [e.g., diC8-$PI(4,5)P_2$], whereby the PIPs are perfused from the internal membrane side by using the inside-out mode of the patch-clamp technique, has allowed for assessment of the apparent affinities of different ion channels. Similarly, by using distinct diC8-PIPs in the same patch, one can assess the stereospecificity of a given channel for different PIPs. Research using such approaches has made clear that ion channels, like membrane-associated proteins, display a wide range of apparent affinities and stereospecificities (e.g., Reference 57).

PHOSPHOINOSITIDE CONTROL OF THE ACTIVITY OF ION TRANSPORT PROTEINS

Table 1 lists K^+ channels sensitive to PIPs. Numerous other channels and transporters sensitive to PIPs continue to be discovered (e.g., Reference 9 and **Supplemental Tables 1** and **2**). The functional effect of PIPs and the relative effectiveness of various PIP species tested, when available, are also shown. The various approaches typically used to study PIP regulation of ion channel activity have been reviewed elsewhere (e.g., References 46 and 58) and are not discussed here. Similarly, brief descriptions of the physiology, tissue distribution, and pathophysiology of the channels listed

Supplemental Material

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Table 1 K^+-selective ion channels that exhibit PIP sensitivity

Channel	PIP species	Regulatory effect(s)	References
Inwardly rectifying K^+ channels			
Kir1.1 (ROMK1)	$PI(4,5)P_2 > PI(3,4,5)P_3$	Activation, shifted pH dose response	54, 57, 70, 72, 82, 111–116
Kir2.1 (IRK1)	$PI(4,5)P_2 \gg PI(3,4,5)P_3 \gg PI(3,5)P_2$	Activation	54, 57, 62, 82, 83, 113, 118–120
Kir2.2 (IRK2)	$PI(4,5)P_2 > PI(3,4,5)P_3 \gg PI(3,4)P_2$	Activation	57
Kir2.3 (IRK3)	$PI(4,5)P_2 \approx PI(3,4,5)P_3$	Activation	57, 118
Kir2.4 (IRK4)	$PI(4,5)P_2 \gg PI(3,4,5)P_3 \approx PI(3,4)P_2$	Activation	57
Kir3.2 (GIRK2)	$PI(4,5)P_2$	Activation	82
Kir3.1/3.2 (GIRK1/2)	$PI(4,5)P_2 \approx PI(3,5)P_2 \approx PI(3,4)P_2$	Activation	121
Kir3.1/3.4 (GIRK1/4)	$PI(4,5)P_2 \approx PI(3,4,5)P_3 > PI(3,4)P_2 \approx PI(3,5)P_2$	Activation	57, 82, 119, 122–126
Kir3.4-S143T (GIRK4*)	$PI(4,5)P_2 \approx PI(3,4,5)P_3 > PI(3,4)P_2$	Activation	57, 83, 124
Kir4.1	$PI(4,5)P_2 \gg PI(3,4,5)P_3$	Activation	57
Kir4.2	$PI(4,5)P_2 > PI(3,4,5)P_3$	Activation	57
Kir4.1/Kir5.1	$PI(4,5)P_2$	Activation, shifted pH dose response	86, 117
Kir6.1	$PI(4,5)P_2$	Activation	112
Kir6.2-Δ36	$PI(4,5)P_2 \approx PI(3,4,5)P_3 \approx PI(3,4)P_2$	Activation, reduction of ATP-induced inhibition	57, 112, 127
Kir6.2/SUR1	$PI(4,5)P_2 \approx PI(3,4,5)P_3 \approx PI(3,4)P_2 >$ PI4P > PI ≈ PS	Activation, reduction of ATP-induced inhibition	57, 113, 127–131
Kir6.2/SUR2A	$PI(4,5)P_2 \approx PI(3,4,5)P_3 \approx PI(3,4)P_2 >$ PI4P	Activation	34, 57, 113, 116, 132–134
Kir6.2/SUR2B	PPIs	Activation	113
Kir7.1	$PI(4,5)P_2 > PI(3,4,5)P_3 \gg PI(3,4)P_2$	Activation	57
KirBac1.1	$PI(4,5)P_2$	Inhibition	135
Voltage-gated K^+ channels			
Kv1.1/Kvβ1.1	$PIP \approx PI(4,5)P_2 \approx PI(3,4,5)P_3$	Removal of inactivation	136
Kv1.2	$PI(4,5)P_2$	Activation, shifted *I-V* curve	92
Kv1.3	$PI(4,5)P_2$, $PI(3,4,5)P_3$	Inhibition	137
Kv1.4	$PIP \approx PI(4,5)P_2 \approx PI(3,4,5)P_3$	Removal of inactivation	136
Kv1.5/Kvβ1.3	$PIP \approx PI(4,5)P_2 \approx PI(3,4,5)P_3$	Removal of inactivation	138
Kv3.4	$PIP \approx PI(4,5)P_2 \approx PI(3,4,5)P_3$	Removal of inactivation	136
Kv7.1 (KCNQ1)	$PI(4,5)P_2$	Activation, shifted *G-V* curve	139
Kv7.1 (KCNQ1)/KCNE1	$PI(4,5)P_2$	Activation, shifted *G-V* curve	139, 140
Kv7.2 (KCNQ2)	$PI(4,5)P_2$	Activation, shifted *G-V* curve	139, 141–143
Kv7.3 (KCNQ3)	$PI(4,5)P_2$	Activation, shifted *G-V* curve	142–144
Kv7.2/7.3 (KCNQ2/3)	$PI(4,5)P_2 \geq PI(3,4,5)P_3 \approx PI(3,4)P_2$	Activation, shifted *G-V* curve	139, 141, 143–150
Kv7.4 (KCNQ4)	$PI(4,5)P_2$	Activation, shifted *G-V* curve	139, 142–144
Kv7.5 (KCNQ5)	$PI(4,5)P_2$	Activation, shifted *G-V* curve	139
Kv11.1 (HERG)	$PI(4,5)P_2$	Activation, slowed inactivation	151–153

(*Continued*)

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Table 1 (*Continued*)

Channel	PIP species	Regulatory effect(s)	References
Shaker	$PI(4,5)P_2$	Activation, shifted *G-V* and *Q-V* curves	92, 93
Two-pore-domain K^+ channels			
$K_{2P}2.1$ (TREK1)	PA > PS > PE > $PI(4,5)P_2$ > PI	Activation, shifted *G-V* curve	154–156
$K_{2P}3.1$ (TASK1)	$PI(4,5)P_2$, IP_3 (IP_3 effect contradictory)	Activation	154, 156–158
$K_{2P}4.1$ (TRAAK)	$PI(4,5)P_2$	Activation dependent on mechanical stimuli	156
$K_{2P}9.1$ (TASK3)	$PI(4,5)P_2$, IP_3	Activation	156, 157
Ca^{2+}-activated K^+ channels			
$K_{Ca}1.1$ (SLO1)	$PI(3,4,5)P_3$ > $PI(4,5)P_2$ > PI5P > PI > PS > PC	Activation, left-shifted *G-V* curve	80, 81
$K_{Ca}2.2$ (SK2)	$PI(4,5)P_2$	Activation	74, 159
$K_{Ca}5.1$ (SLO3)	$PI(4,5)P_2$	Activation	160

Abbreviations: IP_3, inositol trisphosphate; PA, phosphatidic acid; PC, phosphatidyl choline; PE, phosphatidyl ethanolamine; PI, phosphatidylinositol; $PI(3,4)P_2$, phosphatidylinositol 3,4-bisphosphate; $PI(3,4,5)P_3$, phosphatidylinositol 3,4,5-trisphosphate; $PI(3,5)P_2$, phosphatidylinositol 3,5-bisphosphate; $PI(4,5)P_2$, phosphatidylinositol 4,5-bisphosphate; PI4P, phosphatidylinositol 4-phosphate; PIP, phosphoinositide; PS, phosphatidyl serine.

Specificity toward PIP species, together with the effect that PIPs have on the channel, is presented where known. Whenever a single PIP species is listed, the effect of other PIP species on the channel has not been tested in most cases. For Kir1.1 (ROMK1), Zeng et al. (111) showed a somewhat different scheme of interaction with PIPs on the basis of the open probability (P_o) of Kir1.1 [$PI(4,5)P_2$ > PI4P (native) and $PI(4,5)P_2 \approx PI(3,4,5)P_3 > PI(3,4)P_2$ (soluble diC_8 analogs)]. Also, MacGregor et al. (112) determined, through a biochemical approach, that the C terminus of Kir1.1 interacts with PIPs in the following way: $PI(3,4,5)P_3 > PI(4,5)P_2 \approx PI4P \gg PI$. PPIs are a mixture of $PI(4,5)P_2$, PI4P (15–20%), PI, PS, and PC that are extracted from the bovine brain (113).

in the tables can be found elsewhere (e.g., Reference 9) and are not discussed further in this review. We comment only that the impressive number of ion channel proteins displaying functional dependence on PIPs underscores that this highly conserved form of regulation of ion channel activity likely reflects a general mechanism of coupling of a channel gate(s) to the PIP sensor(s).

In the course of the past decade, the ever-growing list of ion channels whose activity depends on PIPs shows that channel interactions with PIPs are a fundamental way by which to control the gating (transitions between their active and inactive states) of these transmembrane proteins. Three-dimensional structures of channel proteins in complex with $PI(4,5)P_2$ have confirmed the critical positioning of $PI(4,5)P_2$ relative to that of the channel gates. Kir channels stand out among channel subfamilies in that they have been studied extensively in terms of both their dependence on PIPs (**Table 1**) and their high-resolution structures (**Table 2**). Several studies on Kir channels have demonstrated direct effects of PIPs on single-channel gating (43, 54, 59, 60), strongly suggesting that channel-PIP interactions affect activity by altering protein conformation and gating. An alternative or parallel mechanism by which PIPs may alter activity is through regulating the number of channels by triggering their internalization from or insertion into the plasma membrane (see below).

Molecular dynamics (MD) simulations can provide useful information about transitions of a given protein from one stable state to another. The Kir3.1 chimera structure has been used to study how PIP binding and gating can be coupled, using atomistic simulations and experimental approaches (61). The MacKinnon lab, to obtain high-resolution diffracting crystals, constructed the Kir3.1 chimera (53). Panels *a* and *b* of **Figure 3** show a superposition of two distinct conformations, a constricted conformation and a dilated conformation, obtained from a single-crystal form. The mammalian cytosolic domains, where $PI(4,5)P_2$ and other regulators (such as G

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Table 2 Atomic-resolution Kir channel structures

PDB ID	Name description	Domains[a]	Resolution	State	Reference
1U4F	Kir2.1, mammalian	N, C	2.40 Å	NA	62
1N9P	Kir3.1, mammalian	N, C	1.80 Å	NA	161
1U4E	Kir3.1, mammalian	N, C	2.10 Å	NA	62
2E4F	Kir3.2, mammalian	N, C	2.30 Å	NA	162
1P7B, 2WLL	KirBac1.1, bacterial	N, TM, C	3.65 Å	G-loop, closed	163
2QKS (constricted)	Kir3.1/KirBac1.3 chimera	N, TM, C	2.20 Å	G-loop, closed	53
2QKS (dilated)	Kir3.1/KirBac1.3 chimera	N, TM, C	2.20 Å	G-loop, open	53
3JYC	Kir2.2, mammalian	N, TM, C	3.10 Å	G-loop, open	164
1XL4, 2WL (H–J, M–O), 2X6 (A, B)	KirBac3.1, bacterial	N, TM, C	2.6–4.2 Å	G-loop, closed	68
1XL6, 2WLK, 2X6C	KirBac3.1, bacterial		2.8 Å 2.7 Å	Conductive (all gates open)	68
3SP (C, G, H, I, J)	Kir2.2, mammalian	N, TM, C	2.45–3.31 Å	G-loop, open	50
3SYO, 3SYC	Kir3.2, mammalian	N, TM, C	3.54 Å 3.41 Å	G-loop, closed	51
3SYP, 3SYA	Kir3.2, mammalian	N, TM, C	3.12 Å 2.98 Å	G-loop, open	51
3SYQ	Kir3.2, mammalian	N, TM, C	3.44 Å	G-loop, open; HBC, half-open	51

Kir channels or channel parts for which atomic-resolution structures have been obtained are included. The only structures in which both G-loop and helix bundle crossing (HBC) gates are open are designated as "all gates open." NA denotes the unknown state of the gate as the crystallized structure is incomplete.

[a]Domains are abbreviated as N (N terminus), C (C terminus), and TM (transmembrane).

proteins) exert their effects, revealed differences between the two conformations (highlighted by superposition of closed and open structures in **Figure 3*a*,*b***) in the cytoplasmic G-loop. Kir channels possess a long pore that utilizes the cytoplasmic portion of the channel to extend the transmembrane portion of the pore (**Figure 3*a–c***). Accumulating evidence has implicated the G-loop as a $PI(4,5)P_2$-sensitive cytoplasmic gate (**Figure 3*c***) (62, 63). Moreover, disease-causing mutations of the G-loop in Kir2.1 (Andersen's syndrome, loss of function) and Kir6.2 (DEND syndrome, gain of function) further underscore the importance of the functional integrity of the G-loop (64–66). The Kir3.1 chimera structure showed that in the constricted conformation, the sulfur atoms of M308 line the pore, occluding the passage of even a dehydrated K^+ ion. In contrast, in the dilated conformation, the oxygen atoms of Y306 and G307 line the pore, allowing even a hydrated K^+ ion to pass through (53). The G-loop gate is located in series to the two other gates that also operate in many other channels that do not possess cytosolic extensions of their pores: the inner helix bundle [or helix bundle crossing (HBC)] gate and the selectivity filter (SF) gate (**Figure 3*c***). Leal-Pinto and colleagues (67) showed that the Kir3.1 chimera requires internal $PI(4,5)P_2$ for reconstitution into planar lipid bilayers, showing Mg^{2+}-dependent inward rectification and a diC8-$PI(4,5)P_2$ EC_{50} of ~17 μM. Thus, the Kir3.1 chimera offers a good model for studying structure and function relationships for $PI(4,5)P_2$ gating of Kir channels.

Colleagues from the Gulbis lab obtained 11 bacterial crystal structures mainly of the KirBac3.1 channel and presented several critical interdomain correlations within the channel (68). Two distinct arrangements of the cytoplasmic interface between two subunits (involving the N terminus

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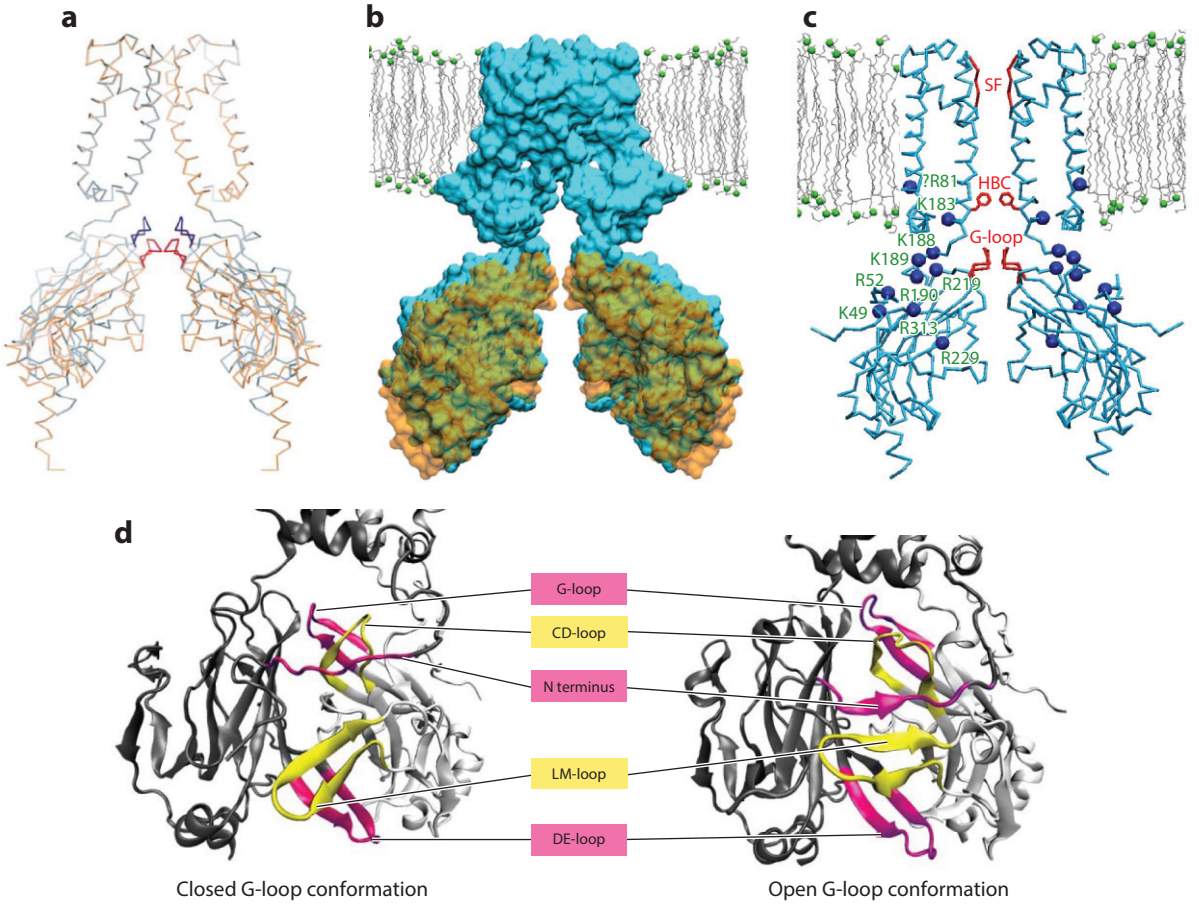

Figure 3

Structure of the Kir3.1 chimera and its activation by phosphatidylinositol 4,5-bisphosphate [PI(4,5)P_2]. (*a*) Superposition of two crystallized conformations of the chimeric crystal. For clarity, only two opposing subunits of the homotetramer are shown. The cytoplasmic pore in one channel (*blue*) is dilated, whereas the other (*red*) is constricted. Residues 302–309 in the G-loop are highlighted. Panel *a* adapted from Nishida et al. (53). (*b*) Surface representations of the subunits shown in panel *a*, showing the movement of the cytosolic domains as the G-loop gate transitions from a constricted (closed, *orange*) to a dilated (open, *blue*) conformation. (*c*) Similar snapshot as in panel *a*, with all three gates labeled (in *red*). The blue dots represent the position of residues that, when mutated, alter the channel's PI(4,5)P_2 sensitivity (see text). Abbreviations: HBC, helix bundle crossing; SF, selectivity filter. (*d*) Summary of the major results of Nishida et al. (53) and Meng et al. (61). Transitioning from closed to open, the secondary structure elements switch their close interactions from adjacent elements on one side to the elements on the other side. PI(4,5)P_2 stabilizes the conformation by direct interactions with the CD-loop and N terminus. Ethanol and G$\beta\gamma$ act by lodging themselves in the DE-LM cleft to stabilize the LM-loop interactions with the N terminus. Panel *d* adapted from Mahajan et al. (84).

in one subunit and the βM sheet in the other), termed latched and unlatched conformations, were closely correlated to the constriction and dilation of the G-loop gate, described by Nishida and colleagues (53) (see below). Transition from the latched to the unlatched conformation occurs through a staged path for each of the four interfaces, and only when all four interfaces adopt unlatched conformations can the channel reach the open state. Moreover, a twisted motion of the cytoplasmic domain relative to the transmembrane domain was directly related to the ion distribution in the SF of the channel.

Experimental and modeling studies have identified a number of key channel regions that are likely involved in the $PI(4,5)P_2$-induced gating process. These regions are the N terminus (K49, R52); the slide helix (R66, K79, R81); the C-linker, which connects the C terminus and the inner transmembrane helix (K183, K188, K189, R190); the CD-loop (R219, R229); and the end of the G-loop gate (R313). It is likely that these channel regions are intimately involved in the gating process and that $PI(4,5)P_2$ (and other signals listed in the next section) stabilizes the open (or closed) conformation of the channel by enhancing specific intra- and intersubunit interactions with the channel gates (G-loop, HBC, and SF; **Figure 3*c***).

Meng and colleagues (61) simulated, in the presence of $PI(4,5)P_2$, an intermediate state between the closed (or constricted) conformation and the open (or dilated) conformation of the G-loop gate (53). In going from the closed conformation to the open conformation, the LM-loop makes a large upward movement to interact closely with the N terminus to form the βA-βM interaction. The CD-loop, in contrast, switches from (*a*) close interactions with the N terminus in the closed state to (*b*) preferential interactions with the G-loop to stabilize its conformation in the open state. These movements are grossly visible in the structures of the two conformations of the G-loop gate (**Figure 3*d***). A recent review focused on additional MD studies that have contributed to our structural understanding of Kir channel gating by PIPs (55).

MODULATORY SIGNALS THAT TARGET THE PHOSPHOINOSITIDE-INDUCED GATING MECHANISM

Here we again use Kir channels as the example for modulation by cholesterol, phosphorylation, G proteins, intracellular Na^+ ions, and pH to illustrate the convergence with the $PI(4,5)P_2$-controlled gating mechanism. Indeed, multiple cellular signals regulating Kir channel activity may converge to affect channel-$PI(4,5)P_2$ interactions and exert their effects (39, 44).

Rosenhouse-Dantsker and colleagues (69) set out to identify the molecular determinants accounting for differences between two Kir2 channels, Kir2.1 and Kir2.3. They identified critical residues that accounted for differences in cholesterol sensitivity between the two channels residing in the N terminus, in the C-linker (the connection between the transmembrane domains and the C terminus), in the CD-loop, and in the G-loop gate, forming a cytosolic belt that surrounds the pore of the channel close to its interface with the transmembrane domain. Interestingly, modeling studies showed that cholesterol docked at sites that did not involve directly any of the identified residues, suggesting that the effect of cholesterol is transmitted to these sites allosterically. Finally, motions of the cytosolic belt residues were shown to correlate or anticorrelate with residues located in the most flexible region of the G-loop, suggesting that these residues are critical for channel gating. It is noteworthy that the cytosolic belt residues reside in the same regions in which $PI(4,5)P_2$-sensitive residues are found and that most of them have been directly or indirectly implicated in the $PI(4,5)P_2$ sensitivity of Kir2.1 (54). Thus, although cholesterol seems to act independently of $PI(4,5)P_2$, it converges to allosterically control residues similar to those that $PI(4,5)P_2$ controls.

Modulation of Channel-$PI(4,5)P_2$ Interaction by Protein Phosphorylation

One well-studied case of phosphorylation affecting channel-$PI(4,5)P_2$ interactions involves the Kir1.1 channel, which is responsible for renal K^+ secretion. Liou and colleagues (70) used the well-established activation of Kir1.1 by PKA to show that PKA influences $PI(4,5)P_2$-mediated activation of Kir1.1, presumably by enhancing the channel's affinity for $PI(4,5)P_2$. PKA stimulation reduced the ability of $PI(4,5)P_2$ antibody to inhibit channel activity and sensitized the channel to

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$PI(4,5)P_2$. The C terminus of Kir1.1 contains juxtamembrane positive residues as well as two PKA phosphorylation sites at S219 and S313. These sites are found next to basic residues that are sensitive to $PI(4,5)P_2$ (54). Mutation of these phosphorylation sites to alanine destabilized channel-$PI(4,5)P_2$ interactions (70). PKA also activates G protein–gated Kir3 channels. Similarly to Kir1.1, PKA-mediated phosphorylation of heteromeric Kir3.1/3.4 in excised patches results in increased $PI(4,5)P_2$ affinity (71).

In contrast to PKA activation, PKC activation by phorbol esters inhibits Kir1.1 and Kir3.1/3.4 activity. PKC appears to reduce the $PI(4,5)P_2$ content in excised membrane patches of Kir1.1, rather than reducing channel affinity for $PI(4,5)P_2$. Consistent with this finding, purified PKC had no effect on wild-type or mutant Kir1.1 with reduced $PI(4,5)P_2$ affinity, and the PKC activators PMA (phorbol myristate acetate) and OAG (oleoyl acetyl glycerol) inhibited mutant Kir1.1 (72). Investigating Kir3.1/3.4 channels, Keselman and colleagues (73) found that purified PKCδ dose-dependently inhibited wild-type channels in excised patches while also reducing the apparent affinity for $PI(4,5)P_2$. Furthermore, PKC activation by phorbol esters did not alter $PI(4,5)P_2$ levels. Given these differing results, it is unclear whether PKC inhibits Kir channel activity by affecting channel-$PI(4,5)P_2$ interactions directly (in the case of Kir3.1/3.4) or by reducing the available $PI(4,5)P_2$ for channel activation (in the case of Kir1.1). Clearly, divergent mechanisms of modulation by protein kinase phosphorylation converge on $PI(4,5)P_2$, which is a central effector of channel activation.

The Ca^{2+}-activated small-conductance K^+ (SK) channels were recently shown to be $PI(4,5)P_2$ sensitive (74). SK channels are activated exclusively by Ca^{2+}-bound calmodulin (CaM) (75) and are negatively regulated by phosphorylation of CaM in response to neurotransmitters (76–78). The putative $PI(4,5)P_2$-binding site in the SK channel subtype 2 (SK2)/CaM complex involves the central linker of CaM and an SK2 channel fragment that connects the transmembrane S6 domain to the CaM-binding domain, a region previously implicated in coupling Ca^{2+} binding of CaM and the opening of SK channels (**Figure 4*a***) (79). A threonine residue (T79) in the CaM central linker phosphorylated by casein kinase 2 lies near the putative $PI(4,5)P_2$-binding site of the SK2/CaM complex (**Figure 4*b***). Introduction of negative charge at this site (mimicked by T79D mutation) can effectively reduce the apparent affinity of $PI(4,5)P_2$ for the channel complex (**Figure 4*c***). Indeed, the phosphomimetic T79D mutant CaM reduces the $PI(4,5)P_2$ apparent affinity of SK2 channels ~11-fold and confers much more prominent inhibition by $PI(4,5)P_2$ hydrolysis stimulated by Gq protein–coupled muscarinic receptors (74). These results provide an example of how physiological stimuli, such as protein phosphorylation, can decrease the affinity of $PI(4,5)P_2$ for transmembrane proteins, such as ion channels, and make them more susceptible to modulation by $PI(4,5)P_2$.

Another study explored the relationship between Ca^{2+} sensitivity of the Slo1 subunit of big-conductance K^+ (BK) channels and $PI(4,5)P_2$ regulation (80). BK channels show little to no rundown in inside-out patch recordings. Yet, rundown can be observed under conditions of low $[Ca^{2+}]_i$. The $PI(4,5)P_2$ sensitivity of these channels (81) was tightly coupled to the RCK1 Ca^{2+} coordination site (80). In this case, increases in $[Ca^{2+}]_i$ allosterically enhance channel-$PI(4,5)P_2$ interactions and thus channel activity.

Other Mechanisms of Modulating Channel-$PI(4,5)P_2$ Interactions

A complex interplay between intracellular Na^+, Gβγ subunits, and $PI(4,5)P_2$ occurs to open Kir3.1/3.4 channels. Whereas exogenous $PI(4,5)P_2$ can fully activate excised patches containing Kir3.1/3.4 channels, albeit with a slow time course, concomitant application of purified Gβγ sensitizes the channel for rapid activation by $PI(4,5)P_2$ (82, 83). Recent structures of Kir3.1 or

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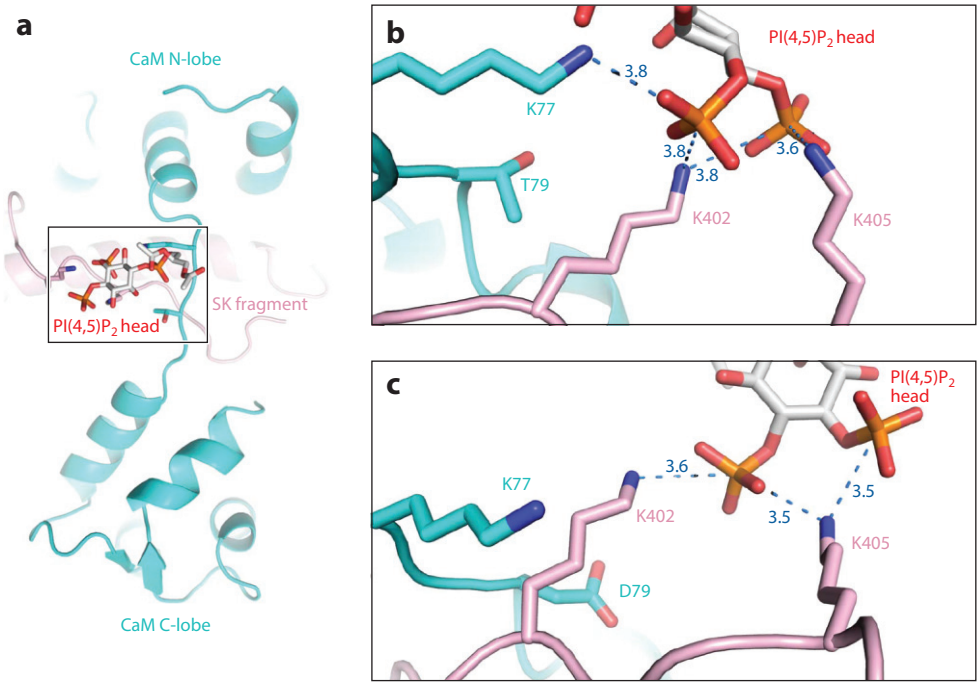

Figure 4

Regulation of $PI(4,5)P_2$ interactions with the SK2/CaM complex by phosphorylation. (*a*) The putative $PI(4,5)P_2$-binding site includes the CaM linker and the SK2 channel fragment before the CaM-binding domain. (*b*) Molecular dynamics simulations showing the interactions of the $PI(4,5)P_2$ phosphates with the positively charged K77 (CaM) and K402 and K405 (SK2). (*c*) Phosphomimetic T79D mutation of CaM decreases the interaction between the $PI(4,5)P_2$-binding site and the $PI(4,5)P_2$ head group (atomic distances between interacting residues are shown in angstroms). Abbreviation: CaM, calmodulin; $PI(4,5)P_2$, phosphatidylinositol 4,5-bisphosphate; SK2, small-conductance K^+ channel subtype 2.

Kir3.2 in complex with Gβγ (52, 84) have revealed how the interactions of the two proteins may stabilize the channel in a conformation that strengthens channel-$PI(4,5)P_2$ interactions.

Interaction with intracellular Na^+ in Kir3.2 and Kir3.4 depends on an aspartate residue located in the CD-loop, near a conserved arginine residue that is critical for $PI(4,5)P_2$ sensitivity. Molecular modeling suggested that Na^+ coordinates with the negatively charged aspartate, freeing a nearby positive residue to exert its influence on $PI(4,5)P_2$ binding. This residue is an asparagine in the Na^+-insensitive Kir3.1 or Kir2.1, and the D223N mutation in Kir3.4 renders the channel insensitive to Na^+, effectively increasing $PI(4,5)P_2$ sensitivity (85). Evidence from a crystal structure has confirmed these results (51).

Multiple Kir channels, including Kir1.1 and Kir1.2, Kir2.3, and Kir4.1/5.1 heteromers, also sense changes in intracellular pH. The ability to monitor intracellular pH makes these channels important in controlling K^+ secretion and acid/base balance in the kidney, as well as in sensing CO_2 levels in the brain. Hypercapnia and acidosis inhibit Kir1.1, Kir1.2, Kir2.3, and Kir4.1/5.1 channels. This inhibition depends on a critical lysine (K80 in Kir1.1, K61 in Kir1.2, and K67 in Kir4.1) near the HBC. Channels with this lysine mutant in the presence of $PI(4,5)P_2$ show almost no sensitivity to changes in pH. In the presence of $PI(4,5)P_2$, homomeric Kir4.1 has relatively low pH sensitivity, but heteromeric Kir4.1/5.1 has enhanced CO_2 and pH sensing. Whether the

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pH sensor controls the channel gate, or whether protonation directly alters channel-$PI(4,5)P_2$ interactions, is unclear. Application of $PI(4,5)P_2$ in excised patches left-shifts the pH sensitivity of Kir4.1/5.1, making the channel less responsive to intracellular proton–mediated inhibition. $PI(4,5)P_2$ in the cellular environment probably reduces the pH-mediated inhibition inherent in the channel (86).

Rapedius and colleagues (87) suggest that $PI(4,5)P_2$ and pH may converge upon similar gating mechanisms, pinpointing a critical hydrogen bond at the HBC between the M1 and M2 transmembrane helices as responsible for stabilizing the closed state. Mutation that abrogates this hydrogen bond results in faster $PI(4,5)P_2$-mediated activation, indicating a faster closed-to-open transition. Although the kinetics of inhibition onset are unchanged by mutating the hydrogen bonding residues, the recovery from inhibition reflecting the closed-to-open transition is slowed in the presence of residues supporting a hydrogen bond. The authors conclude that $PI(4,5)P_2$ activation and recovery from pH inhibition converge on a similar gating mechanism at this hydrogen bond and that hydrogen bonding further stabilizes a pH-inhibited closed state (87). More examples of intracellular signaling modulating channel-$PI(4,5)P_2$ interactions will undoubtedly become apparent as familiar pathways of channel modulation are reexamined for $PI(4,5)P_2$ dependence.

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Voltage-Dependent Channel Modulation by $PI(4,5)P_2$

Although many studies investigating channel-$PI(4,5)P_2$ interactions rely on nonphysiological conditions, using cell-free patches or exogenous $PI(4,5)P_2$, there are multiple examples of physiological pathways coupled to altered $PI(4,5)P_2$ levels. The KCNQ2/3 heteromeric current termed the M current in sympathetic neurons is subject to modulation from PLC-coupled stimuli like acetylcholine and bradykinin. Hydrolysis of $PI(4,5)P_2$ results in a decrease in the slow voltage-activated current carried by KCNQ2/3, ultimately increasing synaptic excitability and firing rate (88). Similar mechanisms exert a dual effect on P/Q-type and N-type voltage-gated calcium channels in neurons (89, 90). Rodríguez-Menchaca et al. (91, 92) and Abderemane-Ali et al. (93) observed a similar dual effect for Kv1.2 and Shaker-like channels: $PI(4,5)P_2$ depletion left-shifted the voltage dependence of activation, increasing current while at the same time decreasing the open probability. These events decreased the current level. A dual effect was also seen in the sea urchin HCN channel, but in the reverse order (91). Kruse et al. (94) tested a number of Kv channels in intact cells and found most of them to be insensitive to $PI(4,5)P_2$; this list includes Kv1.1/Kvβ1.1, Kv1.3, Kv1.4, Kv1.5/Kvβ1.3, Kv2.1, Kv3.4, Kv4.2, and Kv4.3 (with different Kv channel–interacting proteins). Kruse & Hille (95) replicated the PIP_2 dependence of Kv1.2. Using genetic mutation approaches in *Drosophila*, Krause and colleagues (96) demonstrated that $PI(4,5)P_2$ exerts a voltage-dependent inhibition on a delayed rectifier K^+ channel (Shab). Light stimulation activated PLC in rods, resulting in $PI(4,5)P_2$ hydrolysis that subsequently activated the Shab repolarizing current. Why, under intact cell conditions, some Kv channels show clear $PI(4,5)P_2$ dependence, whereas others do not, remains to be further investigated.

PHOSPHOINOSITIDE EFFECTS ON TRAFFICKING OF ION CHANNELS AND TRANSPORTERS

We discuss above that regulation of ion channel activity by PIPs feeds directly into the gating mechanisms of ion channels and that other regulatory mechanisms that change the gating behavior of ion channels often proceed through or complement the effect of PIP binding on the channel. However, there seems to be yet another side to PIP regulation of ion channels. PIPs are intricately involved in both endocytic and exocytic processes of the cell, as discussed above. Thus, it should

come as no surprise that PIPs are capable of altering ion channel and transporter trafficking, ultimately affecting the population of molecules that are active (or are available for activation) on the cell surface.

The Na^+-Ca^{2+} exchanger (NCX1) is one of the first ion transport proteins shown to be regulated by $PI(4,5)P_2$ (34). Direct application of $PI(4,5)P_2$ on excised membrane patches containing NCX1 induces potentiation of the activity of the transporter (34, 97). Yet, in whole-cell patches, perfusion of $PI(4,5)P_2$ leads to an initial increase in the outward exchange current, followed by a decrease in the current and a concomitant decrease in cell capacitance (i.e., membrane area) over a period of 3 min (97). Utilizing an approach to permanently modify the surface-exposed exchangers through addition of a polyethylene glycol moiety, Shen et al. (98) showed that expression of PIP5KIβ (and other manipulations that increase the PIP content of baby hamster kidney cells) decreases the surface fraction of the exchanger. The underlying form of endocytosis likely represents massive endocytosis, a clathrin-independent endocytic process that is thought to be activated by Ca^{2+}. Importantly, the presence of $PI(4,5)P_2$ negates the requirement for ATP in the induction of this process (99).

A similar case involves the epithelial Na^+ channel (ENaC). ENaC is activated by $PI(4,5)P_2$ and PIP_3, likely through direct interactions (e.g., Reference 100). Yet, coexpression of PIP5KIα decreases ENaC current, and this decrease is attributed to decreased surface expression of the channel due to epsin-mediated endocytosis (101).

At least two TRP channels exhibit some paradoxical regulation by PIPs. As was the case with NCX1, TRPC5 is activated by $PI(4,5)P_2$ in inside-out patches, but $PI(4,5)P_2$ perfusion in whole-cell patches inhibits current (102). Moreover, EGFR stimulation enhances TRPC5 currents. From a gating standpoint, this effect would be contradictory because EGFR activation leads to decreased $PI(4,5)P_2$ concentration in the membrane and should decrease channel current. However, EGFR activation initiates membrane insertion of TRPC5 channels, a process that depends on PI3K, Rac1, and PIP5Kα and that ultimately increases TRPC5 currents (103). Another TRP channel, TRPV1, presents a similar case. TRPV1 is activated by $PI(4,5)P_2$ (and other PIPs) in inside-out macropatches (104), but growth factor receptor activation leads to potentiation of TRPV1 activity. Similarly to TRPC5, the potentiating effect of growth factor receptor stimulation relies on PI3K signaling and involves the insertion of channels into the plasma membrane after phosphorylation of a single tyrosine residue on the channel by Src kinase (105, 106).

Glutamate receptors also seem to exhibit some form of regulation by PIPs related to trafficking events. Activation of PLC-coupled receptors decreases NMDA receptor (NMDAR) currents by disrupting interactions between $PI(4,5)P_2$ and the cytoskeleton-associated protein α-actinin, which is an adaptor between the lipid and the channel (107). NMDAR regulation by $PI(4,5)P_2$ seems to be dependent largely on trafficking, as disruption of dynamin binding to amphiphysin and conditions that alter actin depolymerization negate the regulation (108). Moreover, the amplitude of excitatory postsynaptic currents is increased in synaptojanin 1 knockout neurons because of an increase in surface-exposed AMPA receptors (AMPARs) (109). Synaptojanin 1 is a lipid phosphatase that is important in clathrin-mediated endocytosis because it enables shedding of endocytic factors from the membrane by dephosphorylating $PI(4,5)P_2$. Thus, defects in endocytosis increase AMPAR whole-cell current due to an enlarged pool of receptors that can be activated upon stimulation. Finally, PIP_3 is involved in the clustering and stabilizing of AMPARs in the synaptic compartment, a process that involves the recruitment of the scaffolding molecule PSD-95 and that is necessary for the induction and maintenance of long-term potentiation (110).

The mechanistic basis of how PIPs regulate trafficking processes that target ion channels is still lacking and will require further study. Yet, given the fact that most ion channels are activated by PIPs, it is perhaps reassuring to think that, in the native environment of a cell, PIPs represent

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something more than a general ON switch. Thus, cases of paradoxical regulation likely represent an integration of processes controlling direct gating mechanisms and trafficking. Such integration could be seen as a manifestation of physiological checks and balances that are in place to provide feedback (positive or negative) to the system. From all available evidence, a picture of PIPs acting as signaling integrators is emerging.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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Phosphoinositide Control of Membrane Protein Function: A Frontier Led by Studies on Ion Channels
Logothetis et al.

Supplemental Table 1. List of non-selective cation channels that are sensitive to PIPs.
The specificity towards PIPs is presented where available, and so are the regulatory effects for each channel. Whenever a single PIP species is listed, the effect of other PIP species on the channel has not been tested in most cases.

Channel	PIP species	Regulatory effect	References
Nucleotide-gated channels			
Hyperpolarization-activated channels (HCN)			
HCN1	$PI(4,5)P_2$	Activation, shift G-V	(1, 2)
HCN2	$PI(4,5)P_2$, PIP, PIP_3	Activation, shift G-V	(1–3)
HCN4	$PI(4,5)P_2$	Activation, shift G-V	(2)
CNG (cGMP-gated)			
Native rod CNG channel	$PI(4,5)P_2$, PS, PA	Inhibition, right-shift cGMP dose response	(4)
Native olfactory CNG channel	$PI(3,4,5)P_3 > PI(4,5)P_2$	Inhibition, right-shift cAMP dose response	(5)
CNGA1	$PI(4,5)P_2$	Inhibition, right-shift cGMP dose response	(4)
CNGA1/B1	$PI(4,5)P_2$	Inhibition, right-shift cGMP dose response	(4)
CNGA2	$PI(3,4,5)P_3$	Inhibition, right-shift cAMP dose response	(5, 6)
CNGA2/CNGA4	$PI(3,4,5)P_3$	Inhibition, right-shift cAMP dose response	(5)
CNGA3/CNGB3	$PI(3,4,5)P_3$	Inhibition, right-shift cAMP dose response	(7)
TRP channels			
TRPV1	$PI(4,5)P_2$, $PI(3,4)P_2$, $PI(3,5)P_2$, PI(4)P, PI(5)P, $PI(3,4,5)P_3$	Activation or inhibition dependent on stimulus strength, membrane insertion	(8–15)
TRPV1t (taste receptor variant)	$PI(4,5)P_2$	Inhibition	(16)
TRPV5	$PI(4,5)P_2$	Activation	(17)
TRPV6	$PI(4,5)P_2 > PI(3,4)P_2 \approx PI(3,4,5)P_3$	Activation	(18, 19)
TRPM4	$PI(4,5)P_2$	Activation, shift G-V	(20, 21)
TRPM5	$PI(4,5)P_2$	Activation	(22)
TRPM7	$PI(4,5)P_2$	Activation	(23)
TRPM8	$PI(4,5)P_2 >> PI(3,4)P_2$, PI(4)P, $PI(3,4,5)P_3$	Activation, shift G-V	(12, 24–27)
TRPA1	$PI(4,5)P_2$	Inhibition	(28–30)
TRPC1	$PI(4,5)P_2$	Activation	(31)
TRPC3	$PI(4,5)P_2$	Activation	(32)
TRPC4	$PI(4,5)P_2$	Inhibition	(33)

TRPC5	$PI(4,5)P_2$>PI(4)P	Activation or inhibition, membrane insertion	(34, 35)
TRPC6	$PI(4,5)P_2$, $PI(3,4,5)P_3$	Activation	(32, 36–38)
TRPC7	$PI(4,5)P_2$	Activation	(32)
TRPC1/C5/C6 (coronary artery myocytes)	$PI(4,5)P_2$, $PI(3,4,5)P_3$	Activation	(39)
Ligand-gated channels			
P2X Receptors (ATP-gated)			
P2X1	$PI(4,5)P_2$	Activation	(40, 41)
P2X2	$PI(4,5)P_2$, $PI(3,5)P_2$, PI	Activation	(40, 42, 43)
P2X3	$PI(4,5)P_2$	Activation	(40, 43)
P2X2/3	$PI(4,5)P_2$,$PI(3,4,5)P_3$	Activation	(43)
P2X4	$PI(4,5)P_2$>$PI(3,4,5)P_3$	Activation	(40, 44)
P2X5	$PI(4,5)P_2$	Activation	(40)
P2X7	$PI(4,5)P_2$	Activation	(40)
Ionotropic Glutamate Receptors			
Native NMDA receptors (cortical pyramidal neurons)	$PI(4,5)P_2$	Activation, retention at cell surface	(45)
NR1/NR2A	$PI(4,5)P_2$	Activation	(46)
NR1/NR2B	$PI(4,5)P_2$	Activation	(46)
NR1/NR2C	$PI(4,5)P_2$	Activation	(46)
Native AMPA receptors (CA1 neurons)	$PI(3,4,5)P_3$	Activation, clustering	(47)

Supplemental Table 2. List of additional channels and some transporters that are PIP sensitive.
In some cases the specificity of the ion transport protein towards PIPs is listed. The relevant regulatory effect is also listed for each channel/transporter. Whenever a single PIP species is listed, the effect of other PIP species on the channel has not been tested in most cases. For the Na^+/Ca^+ exchanger (NCX1) early experiments by Vemuri and Philipson have shown that the exchanger is also sensitive to phosphatidyl serine and phosphatidic acid in reconstituted membranes (79).

Channel	PIP species	Regulatory effect	References
Voltage-gated Ca^{2+} channels			
$Ca_v1.2$ (L-type)	$PI(4,5)P_2$	Slowed rundown	(48, 49)
$Ca_v2.1$ (P/Q-type)	$PI(4,5)P_2$	Activation, slowed rundown, shift G-V	(48, 50, 51)
$Ca_v2.2$ (N-type)	$PI(4,5)P_2$	Shift G-V, slowed rundown, recovery from inhibition	(50, 52–54)
Na+ channels			
ENaC (α/β/γ)	$PI(4,5)P_2$, $PI(3,4)P_2$, $PI(3,4,5)P_3$, PS, PA	Activation, decrease of channel number on cell surface	(55–65)
Cl^- channels			
CFTR	$PI(4,5)P_2 >> PI(4)P \approx PI$	Activation	(66)
Volume-regulated anion channel (VRAC)	$PI(3,4,5)P_3$	Activation	(67)
Ca^{2+}-release channels			
$Ins(1,4,5)P_3$ receptor	IP_3, $PI(4,5)P_2$	Inhibition	(68–70)
Ryanodine receptor (RyR1)	$PI(4,5)P_2$	Activation	(71, 72)
Transporters and Exchangers			
Plasmalemmal Ca^{2+} pump (PMCA)	$PI(4,5)P_2$, PS, PA, PI	Activation, increase of V_{max}, decrease of K_m for Ca^{2+}	(73–75)
Na^+/Ca^+ Exchanger (NCX1)	$PI(4,5)P_2$	Activation, decrease of channel number on cell surface	(76–83)
Na^+/H^+ Exchanger (NHE1)	$PI(4,5)P_2$	Activation	(84)
Na^+/HCO_3 Cotransporter (NBCe1-A)	$PI(4,5)P_2$	Activation	(85)
Other channels			
Connexin Cx43	$PI(4,5)P_2$	Activation	(86)
Hair cell mechanotransduction channels	$PI(4,5)P_2$	Regulates the rates of fast and slow adaptation	(87)

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