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Phosphoinositides regulate ion channels

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Abstract

Phosphoinositides serve as signature motifs for different cellular membranes and often are required for the function of membrane proteins. Here, we summarize clear evidence supporting the concept that many ion channels are regulated by membrane phosphoinositides. We describe tools used to test their dependence on phosphoinositides, especially phosphatidylinositol 4,5-bisphosphate, and consider mechanisms and biological meanings of phosphoinositide regulation of ion channels. This lipid regulation can underlie changes of channel activity and electrical excitability in response to receptors. Since different intracellular membranes have different lipid compositions, the activity of ion channels still in transit towards their final destination membrane may be suppressed until they reach an optimal lipid environment.

Keywords

Phosphatidylinositol 4,5-bisphosphate; Phospholipase C (PLC); G-protein coupled receptor (GPCR); Potassium channel; Calcium channel; Transient receptor potential channels (TRP channels)

1. Introduction

This article considers some key points in the regulation of ion channels by phospholipids. The prescient original concept, from the work of Hilgemann and Ball [1], was that ion channels in the plasma membrane interact with and need local membrane phosphoinositide phospholipids for proper function. We know by now that phosphoinositides do regulate a large number of ion channels. The major themes of our essay are as follows: Definitive evidence and a fairly complete story of ion channel regulation exist for a select few exemplar channels. Partial evidence and a suggestive picture exist for many more, and

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negative evidence or no positive evidence exists for the remainder. Some channels may not be sensitive to phosphoinositides at all. Some ion channels have an obligate requirement for one species of phosphoinositide, whereas others may accept a broader range of phosphoinositides or anionic lipids. Some channels do not function without their preferred lipid and others function differently with and without that lipid. Some channels interact with lipid partners so strongly (high affinity) that the binding site probably remains saturated during most physiological changes of the lipid concentration. Others bind more loosely (low affinity). For the low-affinity channels, variations of lipid levels may act as physiological signals that regulate the computational properties of neurons and the transport properties of secretory cells.

Other articles in this issue review several important properties of phosphoinositide phospholipids that we take here as given. Phosphoinositides derive by phosphorylation from the parent membrane lipid phosphatidylinositol (PI) by combinatorial phosphorylation of the 3, 4, and 5 positions of the inositol head group, so that seven phosphorylated forms exist [2, 3]. The polyphosphoinositides are the most negatively charged of all membrane lipids. They are found only in the cytoplasmic leaflet of the surface membrane and of organellar membranes. All except for PI itself are relatively minority lipids (at most a few mole percent in the membrane) and can serve signaling functions. The different species of phosphoinositides have markedly different abundance in membranes of different organelles of the cell so they act like zip codes or signature motifs of those membranes (Fig. 1; [4]). They are interconverted on a seconds time scale by lipid kinases and lipid phosphatases at the budding and fusing steps of membrane traffic. Finally, phosphoinositides are subject to regulation by membrane receptors. For example, the signature lipid of the plasma membrane, phosphatidylinositol 4,5-bisphosphate ($PI(4,5)P_2$), can be depleted by receptor activation of phospholipase C (PLC), and a small fraction of $PI(4,5)P_2$ can be converted to the potent signal $PI(3,4,5)P_3$ by receptor activation of lipid 3-kinases [5].

2. Two hypotheses

What is regulation by lipids good for? We discuss two hypotheses diagramed in Fig. 1 and Fig. 2. We think both are correct. Ion channels are large integral membrane proteins that become irreversibly imbedded in the lipid bilayer during their synthesis and assembly in the endoplasmic reticulum (ER). They remain membrane imbedded at all times as they traffic through the Golgi and usually continue on to other target membranes via transport vesicles. The vast majority of ion channels that have been studied so far are those targeted to the plasma membrane. During this traffic, integral membrane proteins experience the different signature lipids of each cellular compartment through which they pass. One major hypothesis, regulation in space, has been tested only in very limited ways: It proposes that the phospholipid composition of each compartment controls the activity of channels in transit so that until they arrive at the target membrane, channels may be turned off or altered in function in an adaptive manner [6]. This bold cell biological hypothesis might be broadly relevant to many other integral membrane proteins whether transporters, receptors, enzymes, or ion channels.

A second hypothesis is regulation in time by receptors. While integral membrane proteins like ion channels are residing in the plasma membrane, receptors coupled to PLC or to lipid 3-kinases may become activated and transiently change key membrane lipids sufficiently to modulate channel function. Thus, the electrical and ion-transport properties of the cell may be regulated dynamically by phosphoinositide signaling. As was already pointed out, such regulation in time would apply to channels that do not bind the lipids with very high affinity since the membrane concentration of lipids is likely changed by less than one order of magnitude during receptor activation of enzymes. At times, receptor-induced lipid changes may be graded and modest and their signals and effects on channels may be graded and modest.

3. Tools for study

To study such hypotheses requires tools. Ideally, we should measure the subcellular location and concentrations of the phosphoinositide species, and we should manipulate them up and down. At the same time, we need good assays of the functional changes of the regulated ion channels. Preferably, the measurements, manipulations, and functional assays could be used together in real time on living cells. Fortunately, the techniques and apparatus of patch clamp electrophysiology lend themselves nicely to approaching these ideals. Electrical recording from a cell on a microscope stage is intrinsically a real-time method with millisecond time resolution. Cell photometry or imaging with relevant multicolored fluorescent probes are easily done at the same time on the microscope, and bathing solutions with agonists and inhibitors can be changed by local perfusion, within seconds. Epifluorescence, confocal, and total internal reflection fluorescence (TIRF) microscopy are all possible.

Fluorescently labeled pleckstrin homology domains (PH domains) or other domains with binding specificity for a subset of phosphoinositides are widely used molecular tools for detecting various phosphoinositides [7, 8]. The first was PH-PLCδ1-GFP, which binds selectively to the plasma membrane lipid $PI(4,5)P_2$, showing as a bright fluorescent ring around the edge of a cell in confocal microscopy [9, 10]. It also recognizes inositol 1,4,5-trisphosphate. The probe translocates away from the plasma membrane if $PI(4,5)P_2$ is depleted. Other probes recognize PI(4)P, $PI(3,5)P_2$, etc. Translocation can be followed from the changes of brightness of regions of interest in confocal imaging. Alternatively, translocation of the same fluorescent probes can be studied with Förster resonance energy transfer (FRET) if a suitable FRET-pair of colors is chosen to label the PH domain and a second protein of known location. There will be FRET when they are closely apposed at the same membrane and a loss of FRET when they move apart. A common FRET pair is PH-PLCδ1-YFP and a specific membrane anchor tagged with CFP or even PH-PLCδ1-CFP itself.

A second set of tools are methods to perturb the phosphoinositides of the cell, preferably fast, specific, and compatible with living cells. Several methods manipulate exogenous or endogenous enzymes. Many of these have been outlined by De Camilli and Idevall-Hagren [11] so a short list will suffice. Fig. 2 shows a small subset of phosphoinositide metabolism that includes the enzymes of interest here. The standard biosynthesis of $PI(4,5)P_2$ proceeds

from PI through PI(4)P to PI(4,5)P_2. PI is converted to PI(4)P by a lipid 4-kinase. PI(4)P is converted back to PI by a 4-phosphatase and into PI(4,5)P_2 by a PI(4)P 5-kinase. PI(4,5)P_2 is converted back to PI(4)P by a 5-phosphatase. PI(4,5)P_2 is also cleaved into diacylglycerol and IP_3 by receptor-activated PLC, mobilizing several second messengers and signaling pathways. The conventional approaches of cell biology would be overexpression of the enzyme or of a dominant-negative mutant by transfection, or knockdown by siRNA, or knockout by gene deletion. These methods can change phosphoinositide levels within hours or days.

Other more dynamic enzyme methods can be implemented in the middle of an experiment. These methods, which act in only 1-10 s, include recruitment of transfected enzymes to the membrane using rapamycin-induced dimerization or light-induced optogenetic dimerization. Most typically, the recruited enzyme is a lipid 5-phosphatase that converts PI(4,5)P_2 to PI(4)P after being recruited to the plasma membrane. The optogenetic dimerization is reversible, whereas the rapamycin dimerization is not. Although these enzyme-based methods for PI(4,5)P_2 depletion are effective and easy to use, it should be kept in mind that lipid kinases, lipid synthesis, and cellular regulatory feedbacks also usually continue. With this compensation, depletion cannot be 100%. Further, although a 5-phosphatase depletes PI(4,5)P_2, we should remember that it produces PI(4)P concomitantly.

Additional valuable fast perturbation methods are unique to electrophysiological approaches. These manipulations can be done while monitoring the function of an ion channel. They include (i) electrical activation of a unique voltage-sensing lipid phosphatase (VSP) by depolarizing voltage pulses using whole-cell clamp, (ii) rapid perfusion with enzyme inhibitors, although these drugs are usually not fully selective, and (iii) excising patches of membranes with a patch pipette and applying agents directly to the exposed cytoplasmic surface. After excision, the production of PI(4,5)P_2 in the patch may stop and PI(4,5)P_2 gradually becomes depleted over a minute or two. Channel function declines in parallel, a process called "channel rundown." Applying test phosphoinositide analogs can restore function. Dose-response curves as different concentrations of water-soluble phosphoinositide analogs (e.g. diC_8 analogs) are applied to the cytoplasmic face allow comparison of the efficacy of various analogs and of the relative lipid affinities of different channels. Applying antibodies against specific lipids also blocks lipids and channel function as does screening of the polyanionic lipids with polycations such as Mg^{2+} or poly-lysine. Again lipid analogs can restore function.

The methods we listed have contributed significantly to our growing understanding of lipid dependence of ion channels, but none is definitive and all have reservations. The strongest support of any lipid-regulation hypothesis requires self consistent evidence from several independent techniques. We cannot evaluate methods extensively here, but give a few biases. Since phosphoinositides play major roles in key cellular processes, we favor techniques that perturb the lipid within seconds so that channel effects can be monitored before membrane traffic and the cytoskeleton are disrupted and before compensatory metabolism, new gene expression, or other modulatory processes change the state of the cell. Activation of PLC through receptors provides a quick screen for potential phosphoinositide dependence, but in addition to depleting PI(4,5)P_2, it produces several

other powerful messengers. Thus positive results then need to be followed by confirmations like recruiting a 5-phosphatase to the plasma membrane with rapamycin or activating VSP with depolarizations, two fast methods. Since VSP produces PI(4)P from $PI(4,5)P_2$, with positive results one needs to consider possible actions of both phosphoinositides. With these fast methods, one needs to be sure that the necessary proteins are expressed and, for VSP, that the depolarizing voltage step reaches the membrane under study. One also should ensure with a lipid indicator that the appropriate lipid has been depleted. The three methods use enzymes in intact cells, which we regard as favorable because the baseline and the perturbed levels of endogenous lipid pools are likely to remain within or near a physiologically relevant range.

A different class of experiment is conveniently done with channel expression in oocytes. A patch of membrane is excised in the inside-out configuration and the $PI(4,5)P_2$ is manipulated. It may be allowed to run down in ATP-free solution or to be masked with poly-lysine or with specific antibodies. Exogenous diC_8 lipids are then perfused back on the patch to show recovery. In these procedures, we do not know that the lipid levels are in the physiological range, and the endogenous lipids may become replaced by lipids of much shorter chain length possibly in membrane domains where they are not normally found. The membrane concentration may become excessively high or low. This useful style of experiment may give strong suggestions of lipid dependence, but we feel it is best corroborated by other gentler methods as well.

We finish with a comment on inhibitors and on lipid indicators. The future will probably bring useful inhibitors of steps in lipid synthesis, but for now the drawbacks outweigh the advantages. The ideal would be inhibitors that block formation of specific pools of phosphoinositides. Right now we are uncertain which enzyme subtypes are where in the cell and what pools they serve. Most lack selective inhibitors. Wortmannin is the only agent in significant use. It blocks several kinases at different concentrations. Most relevant for this chapter is the blockage at high concentrations of type III PI 4-kinases, which seem to be required for reformation of $PI(4,5)P_2$ after depletion by PLC.

4. Structural basis of $PI(4,5)P_2$ activation of ion channels

How does the low-abundance plasma membrane lipid $PI(4,5)P_2$ regulate the activity of so many ion channels? All integral membrane proteins sit in intimate contact with a sea of lipids, so it is not surprising that many ion channels have evolved to interact with and recognize the distinctive high negative charge and headgroup geometries of polyphosphoinositides. Two recent papers show crystal structures of $PI(4,5)P_2$ in complex with members of the inwardly rectifying (Kir) potassium channel family: Kir2.2 [12] and G protein-coupled inwardly-rectifying potassium channel GIRK2 (Kir3.2; [13]). Both of these plasma membrane channels require $PI(4,5)P_2$ to be active. The lipid is incorporated right into the channel structure. The structures show specific ion channel-lipid interactions and conformational changes that suggest channel activation when the lipid binds.

Here we focus on the Kir2.2 channel for which phosphoinositide binding is sufficient to activate the wild-type channel in physiological experiments [13, 14]. The x-ray crystal

structure of Kir2.2 in complex with a short-chain analog of $PI(4,5)P_2$, reveals a tetrameric channel composed of a transmembrane domain that forms the potassium-selective pore, and a large cytoplasmic domain that participates in gating (Fig. 3A). One $PI(4,5)P_2$ molecule binds to each of the four channel subunits at the interface between the transmembrane and cytoplasmic domains and induces a translation of the cytoplasmic portion towards the membrane portion (upward in the figure). In effect, the cytoplasmic and transmembrane domains are pulled together by the lipid to favor an active conformation of the channel. The lipid head group sits roughly at the same interfacial level of the membrane as the other head groups of the bulk lipid bilayer with which it can exchange. The $PI(4,5)P_2$ binding site of each Kir2.2 subunit comprises amino acid residues from two distinct regions. First, the glycerol backbone and 1-phosphate of $PI(4,5)P_2$ interact with the inner and outer helices of the channel transmembrane domain (Fig. 3A,B), orienting the inositol head group towards the cytoplasmic domain of the channel. Second, the positioning of the negatively charged 4- and 5-phosphates of the inositol head group of $PI(4,5)P_2$ promotes an electrostatic interaction with positively charged lysine and arginine residues of the cytoplasmic domain and induces the formation of the interfacial "tether helix". These molecular events draw the two domains 6 Å closer to each other and induce a rotation of the inner helices into a conformation that more closely resembles an open channel. In the structure of GIRK2 (Kir3.2) with $PI(4,5)P_2$, there are only modest changes that do not open the channel. In contrast, in the structure of a constitutively-active GIRK channel (GIRK R201A) in complex with $PI(4,5)P_2$ there are striking changes in channel conformation, with rotation of the cytoplasmic domains and a rotation and splaying apart of the inner helices, promoting gate opening [13].

At this writing, only the two Kir channels have been crystallized in complex with $PI(4,5)P_2$. These informative structures revealed a binding interaction that may be generalizable. Given that all Kir channels have some sensitivity to $PI(4,5)P_2$ [14, 15], and the residues forming the $PI(4,5)P_2$ binding pocket are highly conserved within the Kir superfamily [12], we can expect such a mechanism for other Kir's. For example, docking simulations with Kir6.2 predicted a $PI(4,5)P_2$ binding site in good concordance with that of Kir2.2 [16-18]. For other kinds of channels, mutations of specific C-terminal basic residues do change apparent $PI(4,5)P_2$ sensitivity, but the definitive picture awaits further structures. From the incomplete evidence available, we do not anticipate that there will be a recognizable structural element that is common to all $PI(4,5)P_2$-sensitive ion channels. For example, they will not have the fold that defines a classic PH domain. Presumably, each class of channels has its own fold that brings together several basic residues from well-separated regions of the linear sequence to coordinate phosphoinositide phosphates.

In the next four sections we describe highlights of what is known about the phosphoinositide dependence of several classes of ion channels.

5. Regulation of potassium channels by phosphoinositides

Potassium-selective ion channels, typically formed from four homologous subunits as in Fig. 3, are the largest ion channel superfamily with over 80 members. Functionally they maintain the resting membrane potential of cells acting as dampers on excitation as well as

repolarizing the membrane during an action potential. We consider evidence for regulation of voltage-gated potassium (K_V) channels, inwardly-rectifying potassium (Kir) channels, and calcium-activated potassium (K_{Ca}) channels by plasma membrane $PI(4,5)P_2$. There are reports that other phosphoinositides can activate some of these channels to an extent, but $PI(4,5)P_2$ seems to be the principal phosphoinositide regulating potassium channel activity in the plasma membrane [19-21].

5.1. Voltage-gated potassium channels

We begin to summarize the regulation of several potassium channel superfamilies by phosphoinositides. For K_V channels, the clearest examples of regulation by $PI(4,5)P_2$ are the K_V7 channel family. These are slowly activating channels some of which are already open at rest. In particular it is accepted for the common neuronal KCNQ2/KCNQ3 ($K_V7.2/K_V7.3$) heterotetrameric potassium channels, that $PI(4,5)P_2$ is essential for their voltage-gated activity, and that depletion of $PI(4,5)P_2$ renders them inactive so the neuron becomes transiently more excitable until the lipid is resynthesized [22-24]. For many years it was unclear how agonists including acetylcholine could turn off the KCNQ2/3 current of sympathetic neurons (Fig. 4A,B). The answer was that receptor activation couples to PLC, which depletes the plasma membrane $PI(4,5)P_2$. The channels are relatively selective for this one phosphoinositide although they bind $PI(4,5)P_2$ with low enough affinity to be only ~70% activated at the normal resting $PI(4,5)P_2$ level [25]. Many lines of evidence supporting the requirement for $PI(4,5)P_2$ are summarized in Table S1: Activation of PLC through G_q-coupled receptors with subsequent hydrolyses of $PI(4,5)P_2$ causes rapid closure of the channels (Fig. 4A,B). Addition of soluble analogs of $PI(4,5)P_2$ to the cytoplasmic face of excised patches from cells expressing KCNQ2 and KCNQ3 increases the channel activity, and application of $PI(4,5)P_2$-screening substances like poly-L-lysine leads to rapid loss of channel activity [21, 23, 26, 27]. Dephosphorylation of $PI(4,5)P_2$ to PI(4)P by VSP (Fig. 4C,D) or by a chemically- or photo-recruitable lipid-5-phosphatase (Fig. 4E,F) causes loss of KCNQ2/3 channel activity [28-32]. Lastly, after $PI(4,5)P_2$ depletion, restoration of KCNQ2/3 channel activity follows the time course of $PI(4,5)P_2$ resynthesis at the plasma membrane [30, 33-35]. In the experiments with VSP, depolarizing pulses that are only a few hundred milliseconds long suffice to deplete the $PI(4,5)P_2$ and to turn off the channel. Thus the low-affinity channel-lipid complex must be short lived and rapidly turning over. In the structure of Fig. 3, we should imagine that the phosphoinositides are popping in and out of the channel protein, exchanging many times each second with the neighboring membrane phosphoinositides.

Many of these methods have been applied to other K_V channels (Table S1). All members of the K_V7 channel family require $PI(4,5)P_2$. KCNQ1 ($K_V7.1$) channels combine with the β-subunit KCNE1 to form the molecular correlate of the I_{Ks} current in the human heart. Depletion of $PI(4,5)P_2$ by voltage-sensing or rapamycin-recruitable lipid-5-phosphatases strongly inhibits KCNQ1/KCNE1 channel complexes [32], and excising patches from cells expressing KCNQ1/KCNE1 causes current rundown, which is prevented by application of $PI(4,5)P_2$ to the cytoplasmic face of the patch [23]. The presence of KCNE1 enhances the $PI(4,5)P_2$ sensitivity of KCNQ1 by a factor of ~100 [36]. An intriguing mechanistic concept is that $PI(4,5)P_2$ aids the conformational coupling between the channel voltage-sensing

domain and the gate in KCNQ1 [37]. In the absence of $PI(4,5)P_2$, voltage changes can move the voltage sensor, but the channel does not open, and mutations can make the pore open, but the movements of the voltage sensor are not affected. KCNQ4 ($K_V7.4$) channels are sensitive to activation of type 1 muscarinic acetylcholine receptors (M_1R), which activate PLC via G_q [38]. As for KCNQ1/KCNE1 and KCNQ2/3 channel complexes, depletion of $PI(4,5)P_2$ leads to closure of KCNQ4 channels and almost complete loss of channel activity. Again, in excised patches KCNQ4 channel activity can be restored by application of diC_8-$PI(4,5)P_2$ [23, 26]. The remaining channel of this family, KCNQ5 ($K_V7.5$), is similarly sensitive to $PI(4,5)P_2$ [23].

Other than for K_V7 channels, no experiments on other members of the K_V family have suggested that $PI(4,5)P_2$ is essential for their function, although it may have some effects. One member of the K_V1 channel family has clear phosphoinositide sensitivity. The gating properties of $K_V1.2$ channels are changed by lowering $PI(4,5)P_2$. In *Xenopus laevis* oocytes, depletion of $PI(4,5)P_2$ yields a decrease of current amplitude, a left-shift in the voltage dependence of the activation curve, and a marked slowing of channel deactivation [39]. Application of $PI(4,5)P_2$ to an excised patch restores current amplitude and reaccelerates channel deactivation. Comparable results are found in whole-cell recordings from tsA-201 cells following enzymatic depletion of $PI(4,5)P_2$ [40].

For the rest of the K_V family, the evidence for any kind of $PI(4,5)P_2$ effects is either negative or controversial (Table S1). We now mention several controversial examples. For each of them, our own experiments with verified enzymatic depletion of $PI(4,5)P_2$ using VSP or using a recruitable lipid-5-phosphatase showed no effect in intact tsA-201 cells [32]. On the other hand, experiments with membrane patches excised from *Xenopus* oocytes indicated that application of full-length $PI(4,5)P_2$ changed the channel gating. Thus, applying $PI(4,5)P_2$ to the cytoplasmic face of excised patches from oocytes expressing $K_V1.4$ or $K_V3.4$ channels or $K_V1.1/K_V\beta1.1$ or $K_V1.5/K_V\beta1.3$ channel complexes reduces channel inactivation. Subsequent application of poly-L-lysine to screen polyphosphoinositides reverses the effect and restores fast inactivation [41, 42]. Therefore, it still remains to be clarified whether these channels can be regulated by physiological changes in $PI(4,5)P_2$ levels and why these experiments give contrasting results.

Interestingly, for two of these channels, $K_V1.1/K_V\beta1.1$ and $K_V3.4$, inactivation can be removed by activating PLC. This would deplete $PI(4,5)P_2$ but also generate diacylglycerol (DAG) and inositol 1,4,5-trisphosphate (IP_3) and activate protein kinase C (PKC) (Fig. 2). Since direct pharmacological activation of PKC in these cells leads to the same change in channel inactivation, it is plausible that the observed changes in $K_V1.1/K_V\beta1.1$and $K_V3.4$ channel behavior in this experiment are caused by phosphorylation of these channels by PKC rather than by depletion of $PI(4,5)P_2$ [32].

The last controversial channel of this group is $K_V11.1$ (hERG). Dialysis of $PI(4,5)P_2$ into cells via a patch-pipette leads to increased $K_V11.1$-mediated current amplitude, a left-shift in the activation curve, accelerated channel activation, and slowed channel inactivation [43, 44]. In apparent contrast, depletion of $PI(4,5)P_2$ in tsA-201 cells by either a voltage-sensing or a recruitable lipid-5-phosphatase does not influence $K_V11.1$ current amplitude or kinetics

[32]. Again it is unclear at present to what extent alterations in $PI(4,5)P_2$ levels might regulate $K_V11.1$ under physiological conditions.

In short, the evidence that KCNQ (K_V7) channels require $PI(4,5)P_2$ for normal activity is strong. In addition, $K_V1.2$ channel gating can be modified by physiological changes of $PI(4,5)P_2$. However, for the other tested members of the K_V channel family, $PI(4,5)P_2$ is apparently not essential, and whether it has modulatory effects under physiological conditions is controversial in several examples. For reasons not understood, the results seem to depend on choices of expression systems, recording configurations, and the tests tried.

5.2. Inwardly-rectifying potassium channels

Inwardly-rectifying potassium channels conduct best at negative potentials and are most important in regulating the resting potential. Unlike the K_V channel family, every member of the Kir channel family requires $PI(4,5)P_2$ [1, 14, 19, 20, 45-51]. There are many lines of evidence (Table S1), and as we have seen, even crystal structures of $PI(4,5)P_2$ complexes with Kir channels: Measurements of Kir currents in the inside-out patch-clamp configuration show rundown of the currents as soon as membrane patches are excised, and application of diC_8-$PI(4,5)P_2$ restores current amplitudes rapidly and completely [14]. Addition of an anti-$PI(4,5)P_2$ antibody to the cytoplasmic face of excised patches accelerates current rundown, as does the addition of Mg^{2+} that can screen $PI(4,5)P_2$ rendering it less available [49]. In addition, stimulation of the G_q-coupled M_1R receptor or of the EGF receptor reduces the activity of several, although not all, Kir channels [49, 52]. Both receptors activate PLC and initiate hydrolysis and depletion of $PI(4,5)P_2$.

The physiological modulation of Kir channels occurs through at least three different receptor actions, and the various channel subtypes show quite a range of susceptibilities to these signals. Two inhibitory signals are mediated by receptors coupled to G_q, depletion of $PI(4,5)P_2$ and channel phosphorylation via the DAG-PKC pathway. A third signal, excitatory this time, is mediated by βγ subunits of G proteins liberated by receptor activation of $G_{i/o}$. The Gβγ dimer acts directly on GIRK channels (the Kir3 subfamily) and not on the other Kirs.

We focus on the two inhibitory signals. They bear a kind of inverse relation to each other. The different Kir channels bind $PI(4,5)P_2$ with significantly different affinities [49]. Kir channels with high $PI(4,5)P_2$ affinity (Kir1, Kir2.1, Kir4) show only a small inhibition from PKC or from M_1R activation, whereas channels with low $PI(4,5)P_2$ affinity (Kir2.3, Kir3) are more strongly inhibited by PKC and M_1R activation. The Kir channel with the highest measured $PI(4,5)P_2$ affinity, Kir2.1, is insensitive to both PKC and M_1R activation. Site-directed mutagenesis bears out this generalization. While wild-type Kir2.3 channels, which have a relatively low $PI(4,5)P_2$ affinity, are inhibited by about 50% by PKC, increasing their $PI(4,5)P_2$ affinity by introducing a point mutation renders them insensitive to PKC. On the other hand, lowering the $PI(4,5)P_2$ affinity of the normally PKC-insensitive Kir2.1 channel by a point mutation introduces PKC-sensitivity to this mutant channel [49]. Thus, by varying the expression pattern of different Kir channels, a cell could adjust the relative strength of physiological stimuli necessary to cause a decrease in Kir channel conductance (an increase of electrical excitability) by either the PKC or the $PI(4,5)P_2$-depletion pathways.

Kir3 (GIRK) channels show a special kind of regulation as they not only depend on $PI(4,5)P_2$, but also require the binding of Gβγ subunits to be active. $PI(4,5)P_2$ and Gβγ act synergistically to activate these channels [45]. Excising membrane patches from *Xenopus* oocytes expressing GIRK channels leads to rundown of the current due to hydrolysis of $PI(4,5)P_2$, and subsequent application of Gβγ does not restore channel activity under these conditions. Application of $PI(4,5)P_2$ without Gβγ can restore channel activity over several minutes, but applying $PI(4,5)P_2$ together with Gβγ restores GIRK channel activity in only seconds, illustrating an interdependence of Gβγ and $PI(4,5)P_2$ actions.

5.3. Calcium-activated potassium channels

Activated when intracellular calcium rises, calcium-activated potassium channels in neurons gradually slow the rate of firing during trains of action potentials (spike frequency adaptation) and keep cells quiet and hyperpolarized after bursts of firing. There are three families: the small (SK), intermediate (IK) and big conductance (BK) K_{Ca} channels. SK and BK channels are reported to be modulated by phosphoinositides. Thus, the activity of SK channels from apical membranes of rat cortical-collecting duct decreases upon excision of membrane patches and is restored by addition of $PI(4,5)P_2$ to the cytoplasmic face [53]. Exposing the patches to inhibitors of phosphoinositide kinases decreases SK channel activity, and application of $PI(4,5)P_2$ restores it. The BK channels do not run down in excised patches, but nevertheless, channel activity could be increased by application of $PI(4,5)P_2$ to the cytoplasmic face of membrane patches excised from cerebral artery myocytes [54]. In expression systems, potentiation by $PI(4,5)P_2$ was evident when coexpressing BK β1 subunits (those present in the native vascular myocytes) but not β4 subunits (present in skeletal muscle). Mutagenesis of residues in the Ca^{2+}-coordinating RCK1 domain of BK channels suggests a regulatory role of Ca^{2+} in the $PI(4,5)P_2$ sensitivity of BK channels [55]. With low cytoplasmic Ca^{2+} levels, the RCK1 domain limits channel activation by decreasing the $PI(4,5)P_2$ affinity of BK channels, whereas upon binding of Ca^{2+} to the RCK1 domain, this inhibitory influence is removed and $PI(4,5)P_2$ contributes to BK channel activation.

6. Regulation of voltage-gated Ca^{2+} channels by phosphoinositides

Voltage-gated calcium (Ca_V) channels mediate calcium influxes and regulatory calcium transients upon membrane depolarization, transients that initiate all the effector actions of electrically excitable cells [56]. Modulation of Ca_V channels by G protein-coupled receptors (GPCRs) tunes many physiological responses [57, 58]. We focus here on inhibition of Ca_V channel by membrane receptors coupled to G_q where depletion of plasma membrane $PI(4,5)P_2$ is one of the underlying signals.

6.1. Role of membrane $PI(4,5)P_2$ in the activation of Ca_V channels

Early studies showed that $Ca_V2.2$ channel (N-type) currents are partially suppressed by G_q-coupled M_1 muscarinic receptors in sympathetic neurons [59-62]. The signaling pathway was slow (~10 s for inhibition and ~100 s for recovery) and voltage independent. The underlying mechanisms were not clear for many years. Eventually, evidence accumulated that $PI(4,5)P_2$ is required for Ca_V channels to open in response to membrane potential

changes and therefore $PI(4,5)P_2$ depletion might underlie the muscarinic inhibition (Table S2). The first evidence, with membrane patches excised from frog oocytes, was that rundown of $Ca_V2.1$ channel activity was slowed by application of $PI(4,5)P_2$ or Mg-ATP and accelerated by depleting or sequestering $PI(4,5)P_2$ [63]. Subsequently, in sympathetic neurons the G_q-coupled muscarinic inhibition of $Ca_V2.2$ was shown to involve $PI(4,5)P_2$ hydrolysis by PLC, and the recovery of current paralleled $PI(4,5)P_2$ resynthesis [64, 65]. Additional direct evidence for a functional role of membrane $PI(4,5)P_2$ in the activation and modulation of Ca_V channels came from rapid activation of lipid phosphatases [66, 67]. Using both VSP and the rapamycin dimerization system with a 5-phosphatase, it was shown that $PI(4,5)P_2$ depletion alone sufficed to diminish the currents of several high-voltage-activated Ca_V channels but not those of low-voltage activated channels. Further, the recovery from this inhibition needed intracellular ATP-dependent PIP_2 resynthesis. As with receptor activation, the reduction of current was 30-50% and not 100%. And as for modulation of KCNQ currents by VSP, the partial suppression of Ca_V currents required a depolarizing pulse of only 100 ms, implying a rapidly exchanging, low-affinity $PI(4,5)P_2$ interaction. It should be noted that in a slightly different hypothesis, the classical slow receptor-mediated suppression of Ca_V current in sympathetic neurons has also been ascribed to inhibitory actions of arachidonic acid released from $PI(4,5)P_2$ by phospholipase A_2 [68].

6.2. Role of auxiliary β subunits in $PI(4,5)P_2$ regulation of Ca_V channels

As we already saw for some K_V channels, the $PI(4,5)P_2$ regulation of Ca_V channels changes with the coexpression of different Ca_V β subunits [67]. Ca_V β2a subunits bind to the membrane through two palmitoyl side chains in the N-terminus, and the Ca_V channels with this subunit show slow inactivation of current and little $PI(4,5)P_2$ sensitivity [69-71]. Ca_V β3 subunits are not lipidated and do not bind to the membrane until coupled with an α1 channel subunit. Channels coexpressed with β3 subunits inactivate more rapidly and show higher $PI(4,5)P_2$ sensitivity. It was proposed that the two palmitoyl chains of the β2a subunit compete with the interaction of the two fatty acyl chains of $PI(4,5)P_2$ at the channel, reducing the requirement for $PI(4,5)P_2$. When the possibility of lipidation was removed by mutation of the β2a subunit, the channel became $PI(4,5)P_2$-sensitive. In the same vein, when β3 subunits are targeted to the membrane by adding a Lyn_{11} lipidation signal, the chimeric subunit acts like the membrane tethered β2a subunit and diminishes the $PI(4,5)P_2$ sensitivity, suggesting that the subcellular localization of the β subunit governs the phosphoinositide sensitivity of channels [67]. Such experiments raise the interesting concept that $PI(4,5)P_2$ at times may act as a competitor for binding of other lipids to targets and that lipidated proteins may sometimes assume the role of $PI(4,5)P_2$ [72-74]. Even though the Ca_V β subunit is a key determinant of regulation by $PI(4,5)P_2$, the binding sites for $PI(4,5)P_2$ on Ca_V channels seem still to be on the pore-forming α1 subunit. The $PI(4,5)P_2$ sensitivity of Ca_V channels is not lost in channels whose N-terminus has been modified to prevent the inhibitory binding of G protein βγ subunits [66].

6.3. $PI(4,5)P_2$ binds to Ca_V channels as a bidentate ligand

In analogy with the crystal structure of Kir2.2 (Fig. 3), one can speculate that a $PI(4,5)P_2$ molecule acts as a bidentate ligand crosslinking two parts of the Ca_V α1 subunit and favoring active protein conformations [66, 72] (Fig. 5). The hydrophobic recognition site

can be disturbed by the palmitoyl groups of Ca_V β2a [66] or by direct incorporation of fatty acids, such as arachidonic acid or palmitic acid into the interaction sites [72, 73]. The hydrophilic interaction can fail when membrane $PI(4,5)P_2$ is depleted or screened [64, 66, 75].

7. Other plasma membrane channels regulated by phosphoinositides

7.1 TRP channels

Transient receptor potential (TRP) channels form a diverse superfamily. Most of them are non-selective cation channels usually with some Ca^{2+} permeability. They often mediate sensory or homeostatic modalities like stretch, temperature change, pH, vision (in arthropods), or osmotic sense, so their regulation has physiological impact. They are tetrameric complexes with an architecture similar to that of K_V channels, but their opening probability is only mildly increased by depolarization. So far, 20 out of 28 channels in the TRP superfamily have been said to be sensitive to $PI(4,5)P_2$ (summarized in Table S3), and for most of them, $PI(4,5)P_2$ promotes opening [76].

A well-studied example of $PI(4,5)P_2$ dependence is the cold-activated TRPM8 channel. It is expressed primarily in trigeminal and dorsal root ganglion neurons. TRPM8 is activated by low temperatures, voltage, and the "cooling" agents menthol and icilin [77, 78]. It is inhibited by ethanol and desensitized by calcium. TRPM8 channels are not responsive to those stimuli when $PI(4,5)P_2$ is absent. For instance, PLC-mediated hydrolysis of $PI(4,5)P_2$ reduces TRPM8 currents activated by temperature, menthol, or voltage [79-82]. It also reduces inhibition by ethanol [83]. Since by hydrolyzing $PI(4,5)P_2$ PLC also makes IP_3 and DAG, it could be thought that TRPM8 channels are responding to these products of lipid hydrolysis. However, when $PI(4,5)P_2$ levels are reduced without IP_3 or DAG production using the recruitable lipid 5-phosphatases, menthol-induced TRPM8 currents fall concomitantly [81, 84]. TRPM8 channels are also inhibited when $PI(4,5)P_2$ is scavenged by antibodies or screened with poly-L-lysine [79-81, 83]. Mutagenesis of positively charged amino acid residues in the conserved C-terminal TRP domain reduces the apparent affinity for $PI(4,5)P_2$. These residues required for $PI(4,5)P_2$ binding are located in a different part of the protein from that required for the activation by temperature, voltage, or cooling agents [85].

Based on such functional and structural experiments, the current model suggests that temperature, voltage, and $PI(4,5)P_2$ interact allosterically with each other. When $PI(4,5)P_2$ is present, less membrane depolarization or less cooling are needed to activate TRPM8 channels. Molecular simulations docking $PI(4,5)P_2$ on the TRPM8 channel suggest that the aliphatic chains of the lipid bind to a hydrophobic pocket in the transmembrane domain of the channel, modifying the position of the cytoplasmic domain [85]. Removal of $PI(4,5)P_2$ would allow the cytoplasmic domain to move back and render TRPM8 channels less sensitive to any stimulus. Thus, as in Kir channels, $PI(4,5)P_2$ is thought to regulate gating properties of TRPM8 channels by modifying the position of the cytoplasmic domain relative to the transmembrane domain.

Other signals downstream of $PI(4,5)P_2$ hydrolysis also regulate some TRP channels, increasing their activity. This raises the possibility that when $PI(4,5)P_2$ is being hydrolyzed, channel activity can both be stimulated by the products of the hydrolysis and at the same time reduced by $PI(4,5)P_2$ depletion. This potentially complex situation is well exemplified with TRPC channels. TRPC6/7 channels are activated by DAG, a product of $PI(4,5)P_2$ hydrolysis [86, 87]. Nevertheless, $PI(4,5)P_2$ is essential for TRPC6/7 function [88-91]. Therefore, weak or brief activation of PLC stimulates TRPC6/7 currents, and stronger or longer activation of PLC will inactivate them [91]. Evidently it is not necessary to hydrolyze a lot of $PI(4,5)P_2$ before DAG can exert its stimulatory effects on the channel, a situation analogous to the very small cleavage of $PI(4,5)P_2$ needed to get full diacylglycerol action on protein kinase C and full IP_3 action on calcium release [34, 35].

A few TRP channels may have negative regulation by $PI(4,5)P_2$. An interesting case is TRPC4. There the TRPC4α splice variant is negatively regulated when diC_8-$PI(4,5)P_2$ is dialyzed into the cell from the patch pipette [92], whereas the TRPC4β splice variant is not. The effect is attributed to changes in interactions with cytoskeletal elements. In addition, there is a case in which there is not yet agreement on the effect of $PI(4,5)P_2$ despite a lot of work by several groups. TRPV1 channels are sensitive to $PI(4,5)P_2$, and are described as activated or inhibited by $PI(4,5)P_2$--or both--[76]. Here, we summarize some evidence for these not fully reconciled and opposite actions.

Several authors suggest that, like other TRP channels, TRPV1 channels are activated by $PI(4,5)P_2$. For instance, capsaicin-induced TRPV1 currents in a membrane patch can be supported by application to the cytoplasmic face of diC_8 analogs of $PI(4,5)P_2$, $PI(3,4,5)P_3$, or PI(4)P, in order of decreasing potency, whereas currents are reduced by screening $PI(4,5)P_2$ with poly-L-lysine [93-96]. Rapid dephosphorylation of $PI(4,5)P_2$ with VSP or with translocatable 5-phosphatases decreases capsaicin stimulation of TRPV1 currents [95, 97-99]. These phosphatases reduce $PI(4,5)P_2$ levels and simultaneously produce PI(4)P, which is less effective than $PI(4,5)P_2$ at supporting TRPV1 currents. Similarly, $PI(4,5)P_2$ becomes depleted during minute-long applications of capsaicin because Ca^{2+} entering through the open channels activates PLC. In parallel, TRPV1 or TRPV2 channels close, a process called desensitization [94, 97, 100]. Blocking phosphorylation of PI at the 4 position or hydrolyzing $PI(4,5)P_2$ suppresses recovery from desensitization [101].The loss of $PI(4,5)P_2$ is cited as the explanation of this agonist-induced Ca^{2+}-dependent desensitization of channel responses.

Together, the preceding evidence is fully consistent with a $PI(4,5)P_2$ requirement for channel activity. On the other hand, certain experiments suggest inhibitory effects of $PI(4,5)P_2$. For instance, reducing $PI(4,5)P_2$ levels by adding an anti-$PI(4,5)P_2$ antibody increases TRPV1 currents, and reducing the pool of phosphoinositides in general by adding a phosphatidylinositol phospholipase (PI-PLC) increases TRPV1 currents [102, 103].

Possible $PI(4,5)P_2$ binding sites have been sought in the long C-terminus of the TRPV1 channel. Mutagenesis or deletions in regions claimed to be relevant either increase or decrease channel activity [85, 103, 104]. From this evidence, $PI(4,5)P_2$ is variously said to be an inhibitor or stimulator of channel activity. Alternatively, it has been suggested that the

effect of $PI(4,5)P_2$ on TRPV1 channels is mediated not by a domain located on the protein but by a different protein (pirt) that binds both $PI(4,5)P_2$ and TRPV1 channels [105].

One barrier to understanding regulation of TRPV1 in living cells is that the channels are simultaneously sensitive to a large number of different modulatory signaling pathways: phosphorylation, calcium, lipids, other interacting proteins, and voltage. Indeed the same problem applies to most types of channels. TRPV1 channels also seem to be sensitive to several acidic lipids so depletion of just one lipid may not give clear answers. One ideal would be to study the channels in the absence of extraneous signals and proteins. For TRPV1, this has been done by reconstituting purified channel protein in lipid vesicles containing defined lipids [106, 107]. Such channels are inactive in lipid bilayers containing only neutral lipids, but can be made active by including $PI(4,5)P_2$ or other negatively charged lipids. This shows that the purified channel and simple lipids alone suffice to reconstitute function and lipid regulation. When functionally reconstituted with negatively charged lipids, the channel activity decreases secondarily upon addition of high concentrations of $PI(4,5)P_2$. Thus $PI(4,5)P_2$ or some other negatively charged lipids are essential for activity, but high concentrations of $PI(4,5)P_2$ decrease activity. Senning et al. [96] recognized that reconstitution studies might be confounded by the obligatory symmetry of artificial liposome bilayers. They found with membrane patches excised from cells that the addition of $PI(4,5)P_2$ to the cytoplasmic face enhanced channel activity, whereas addition of high concentrations to the extracellular face inhibited it. This observation might resolve some of the apparent contradictions saying that there is an activating site in the inside face of the channel and an inhibiting site on the outside face. Since $PI(4,5)P_2$ and the other polyphosphoinositides are present only in the cytoplasmic leaflet in normal cells, the activating site would be the relevant one in a cellular environment.

7.2 ENaC channels

Epithelial sodium channels (ENaCs) mediate sodium transport in epithelia. Regulation of their activity controls the rate of sodium reabsorption in the kidney, lungs, and colon. They require $PI(4,5)P_2$. Transport through ENaC channels is suppressed by activation of G-protein coupled P2Y purinergic receptors. The suppression does not occur if PLC blockers are present. Activation of P2Y receptors simultaneously depletes $PI(4,5)P_2$ in the plasma membrane, activates PKC, and raises intracellular calcium, but neither the calcium signal nor PKC are necessary for the suppression of sodium transport [108]. $PI(4,5)P_2$ must be added to excised patches to prevent rundown of channel activity. Channel rundown is accelerated by screening $PI(4,5)P_2$ with poly-L-lysine, by adding an anti-$PI(4,5)P_2$ antibody, or by activating PLC. $PI(4,5)P_2$ and $PI(3,4,5)P_3$ promote ENaC activity [109, 110] but at different binding sites. Both bind to β and γ subunits of the channels; however, $PI(4,5)P_2$ binds to the N-terminal whereas $PI(3,4,5)P_3$ binds to the C-terminal [108, 109, 111-113].

7.3 P2X channels

The P2X receptor channels are cation-permeable channels that are opened by ATP and other purinergic ligands binding to the extracellular domain. They are strongly expressed in neurons but also in smooth muscle, platelets, and immune cells. The seven members (P2X1-P2X7) can form homomers or heteromers. All functional homomers are $PI(4,5)P_2$ sensitive

[114, 115]. The principal tests have been to decrease $PI(4,5)P_2$ with inhibitors of phosphatidylinositol 4-kinase and to apply exogenous $PI(4,5)P_2$ to excised patches (Table S3). In excised patches, P2X activity runs down, and application of $PI(4,5)P_2$ reduces rundown, whereas screening of $PI(4,5)P_2$ with polycations accelerates it. In *in vitro* binding assays, $PI(4,5)P_2$, as well as other phosphoinositides, bind the proximal C-terminal [114-116]. A physiological example of $PI(4,5)P_2$ actions is a decrease in P2X1-mediated smooth muscle contraction induced by reduction of $PI(4,5)P_2$ levels [117]. As with other $PI(4,5)P_2$ sensitive channels, P2X channel action is thought to be decreased by a variety of G-protein coupled and tyrosine kinase receptors that activate PLC and deplete $PI(4,5)P_2$. Given the wide distribution of P2X receptor channels in the nervous system, their sensitivity to receptor-mediated $PI(4,5)P_2$ hydrolysis could have relevant consequences in synaptic transmission and neural function.

7.4 CNG channels

Now we come to two families of channels whose activity is depressed by $PI(4,5)P_2$. The first is the cyclic nucleotide gated CNG family, channels that are opened by intracellular ATP or GTP. CNG channels of photoreceptors (cones and rods) and olfactory neurons require more cyclic nucleotide to be activated when levels of $PI(4,5)P_2$ or $PI(3,4,5)P_3$ increase [118-122]. The sensitivity to $PI(4,5)P_2$ varies with the specific channel subtype, being low for olfactory channels, as well as with the splice variant expressed. $PI(3,4,5)P_3$ binds the N-terminal of olfactory CNG channels [120], $PI(4,5)P_2$ and $PI(3,4,5)P_3$ bind both N- and C-termini of cone CNG channels [121]. Interestingly, the N-terminal $PI(3,4,5)P_3$-binding region also interacts with calmodulin [120]. There may be an occlusive functional interaction between the $PI(3,4,5)P_3$ and calmodulin regulatory mechanisms since $PI(3,4,5)P_3$ fails to decrease the activity of the channels while calmodulin is bound. Analogous cross-talk between phosphoinositides and a second regulatory mechanism, is observed for other ion channels such as GIRK channels.

7.5 TMEM16A channels

The recently discovered TMEM16-ANO1 channels are calcium-activated chloride channels that are widely expressed including in smooth muscle, neurons, and epithelial cells [123-125]. They too are apparently inhibited by $PI(4,5)P_2$. Calcium-activated chloride currents recorded in cell lines expressing TMEM16A channels increase when $PI(4,5)P_2$ levels are reduced, either by screening or by activation of PLC, and they are decreased when exogenous $PI(4,5)P_2$ is added. A direct interaction between TMEM16A channels and $PI(4,5)P_2$ is suggested since they co-immunoprecipitate [126]. Negative dependence of TMEM16A on $PI(4,5)P_2$ could explain the depolarization in smooth muscle cells that is induced by PLC-activating factors. Since TMEM16A channels are widely expressed, their regulation by $PI(4,5)P_2$ can have several, yet unexplored, physiological consequences.

8. Regulation of intracellular ion channels by phosphoinositides

Thus far, we have focused on ion channels in the plasma membrane and their dependence on $PI(4,5)P_2$. Now we turn to channels and phosphoinositides in other membranes of the cell. Consider Fig. 1. Ion channels, pumps and transporters, like other membrane proteins, are

synthesized, folded, and assembled within the endoplasmic reticulum (ER) before being transported to the Golgi where they undergo further post-translational modifications. On reaching the trans-Golgi network, membrane proteins are sorted based on specific sequences and earmarked for their final destination, be it other intracellular compartments or the cell surface. Sequential transport along the secretory pathway is mediated by vesicular intermediates that bud from one compartment and fuse with the next, creating almost continuous membrane flow from the ER to the plasma membrane.

As described earlier, ion channels need the coincidence of (*i*) an opening stimulus and (*ii*) the minor phosphoinositide $PI(4,5)P_2$ for full function (Tables S1, S2, S3). In simplified terms, each organelle can be thought of having its own 'signature' phosphoinositide (Fig. 1), although, in reality, organelle membranes probably have a more complex mixture of precursor, signature, and successor phosphoinositides [4, 98, 127-131]. Nevertheless, to discuss the spatial modulation hypothesis [6], we retain the concept that each organelle membrane has a simple phosphoinositide menu as described in Fig. 1. The phosphoinositides are kept in dynamic steady state through the actions of lipid kinases and lipid phosphatases [2]. Although there is evidence for $PI(4,5)P_2$ synthesis on other internal membranes, such as the Golgi [132], its free concentration there is thought to be much less than at the cell surface. Then ion channels and transporters that require $PI(4,5)P_2$ would probably be kept at low activity during trafficking through the secretory pathway. The location-specific control would prevent $PI(4,5)P_2$-requiring transporters and channels from spuriously disturbing the ion content, pH gradients, or electrical potentials across organelle membranes, as they traffic towards the plasma membrane. Conversely, some channels and transporters important for intracellular organelles may be swept forward by membrane flow to the plasma membrane. There, the high $PI(4,5)P_2$ concentration or lack of other appropriate activating phosphoinositides might turn off function. Having specific phosphoinositides to regulate membrane proteins ensures that organelle-specific functions and trafficking can occur on the same platform without deleterious side effects.

How can one study phosphoinositide requirements of intracellular channels? Traditionally, to determine whether a plasma membrane ion channel or transporter is sensitive to phosphoinositides, whole-cell or inside-out patch-clamp recordings have been made from cells overexpressing the membrane protein of interest while one or more of the 7 species of polyphosphoinositides is manipulated. Until recently small size and restricted accessibility have prohibited such patch-clamp analysis of intracellular organelles. One approach is to make the organelles large enough to patch clamp, and another changes their sorting signals to allow them to traffic to the cell surface. For example, the small molecule vacuolin-1 converts endosomes and lysosomes into large vacuoles by fusion, while leaving other cell processes, such as the actin cytoskeleton, receptor mediated endocytosis, and membrane traffic along the secretory pathway, intact [133]. In practice, the heterogeneous endo-lysosomal organelles can be enlarged from ~ 0.5 μm to ~ 5 μm diameter, facilitating patch clamp analysis [134-139].

To date, the electrical activity and phosphoinositide dependence of two classes of endolysosomal cation channels have been described well using vacuolin-enlarged organelles: TRPML of the mucolipin subfamily of TRPs [134, 136, 140], and the two pore-

segment channel 1 TPC1 [138, 139] (Fig. 1). TRPML1-3 and TPC1 and TPC2, are strongly inwardly rectifying channels of intracellular organelles, permeable to Ca^{2+} and Fe^{2+} or to Na^{+}, respectively. Both kinds of channels have been implicated in endosomal and lysosomal trafficking. They are specifically activated in enlarged vesicles by the phosphoinositide of late endosomes and lysosomes, $PI(3,5)P_2$ (Fig. 1) [135, 136, 139]). For TRPML1, a small steady-state pool of channels appears at the plasma membrane that can be activated by "run-up" after patch excision or by adding $PI(3,4)P_2$, and that can be inhibited by $PI(4,5)P_2$, $PI(3,4)P_2$, and $PI(3,4,5)P_3$ [136]. The TRPML1 channel can be mutated to enhance surface expression to make these phenomena more evident. The finding that several phosphoinositides can inhibit the channel and only one can activate it suggests potent suppression of the activity of channels while in transit through "foreign" phosphoinositide/organelle environments until they reach a specific home location.

Studies on this small sample of intracellular channels support the hypothesis of regulation in space. There seems to be a clear dichotomy between channels that function at the plasma membrane and channels that function on intracellular organelles. Extending this concept to $K_V7.2/7.3$ channels (KCNQ2/3) leads us to theorize that they will have higher activity at the plasma membrane than at other places in the cell. This assumes that locations proximal and distal to the plasma membrane do not have sufficient $PI(4,5)P_2$ to active the channel. Perhaps in the future there will be examples of channels that are active across multiple membrane compartments. They would be expected to (i) be activated by multiple phosphoinositides, or (ii) be localized to distinct specific domains with phosphoinositides different from the signature phosphoinositide of an organelle.

9. Concluding challenges

Regulation of ion channels by phosphoinositides is a widespread phenomenon with interesting consequences for cell signaling and during membrane traffic. Certain phenomena are now very clear, but the studies raise important general cell biological questions, points that need to be clarified to understand the extent of the regulation. We speak of signature phosphoinositides in specific membranes, but how different are their levels from those of the other lipids in the same membrane? Is it just two-fold or as much as 100-fold? Further for each organelle and the plasma membrane, do we need to consider a locally sorted mosaic of membrane microdomains that concentrate specific lipids and proteins? Then discussions of traffic would be much more complex, and each membrane has to be imagined as made up of multiple rapidly exchanging "compartments" with different properties. We speak of depletion of certain lipids by receptor-activated lipases. How much are $PI(4,5)P_2$ and other phosphoinositide levels reduced when endogenous receptors are activated under physiological conditions? Many channels have been shown to be phosphoinositide sensitive using the extreme experimental conditions we have listed. How lipid-sensitive does a channel have to be for actual regulation during physiological membrane traffic or by receptor activation? Is localization of the channel with respect to $PI(4,5)P_2$ domains a decisive factor for channel regulation? In many cases we have described, other cellular signals and auxiliary proteins compete with or potentiate lipid signals, either directly or through allosteric interactions. Is this crosstalk the origin of functional differences between

different cell types or in different disease states? Will many other classes of membrane proteins be found to be lipid regulated in the same ways as ion channels clearly are?

Much remains to be done in the future.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

[1]. Hilgemann DW, Ball R. Regulation of cardiac Na^+,Ca^{2+} exchange and K_{ATP} potassium channels by PIP_2. Science. 1996; 273:956–959. [PubMed: 8688080]

[2]. Balla T. Phosphoinositides: tiny lipids with giant impact on cell regulation. Physiol Rev. 2013; 93:1019–1137. [PubMed: 23899561]

[3]. Michell. Phosphoinositides in evolution Biochim Biophys Acta. 2014.

[4]. McCrea HJ, De Camilli P. Mutations in phosphoinositide metabolizing enzymes and human disease. Physiology (Bethesda). 2009; 24:8–16. [PubMed: 19196647]

[5]. Cantley LC. The phosphoinositide 3-kinase pathway. Science. 2002; 296:1655–1657. [PubMed: 12040186]

[6]. Hilgemann DW, Feng S, Nasuhoglu C. The complex and intriguing lives of PIP_2 with ion channels and transporters. Sci STKE. 2001; 2001:re19. [PubMed: 11734659]

[7]. Várnai P, Balla T. Live cell imaging of phosphoinositide dynamics with fluorescent protein domains. Biochim Biophys Acta. 2006; 1761:957–967. [PubMed: 16702024]

[8]. Balla, T. Phosphoinositides binding modules and localization Biochim Biophys Acta. 2014.

[9]. Stauffer TP, Ahn S, Meyer T. Receptor-induced transient reduction in plasma membrane PtdIns(4,5)P_2 concentration monitored in living cells. Curr Biol. 1998; 8:343–346. [PubMed: 9512420]

[10]. Várnai P, Balla T. Visualization of phosphoinositides that bind pleckstrin homology domains: calcium- and agonist-induced dynamic changes and relationship to myo-[^{3}H]inositol-labeled phosphoinositide pools. J Cell Biol. 1998; 143:501–510. [PubMed: 9786958]

[11]. De Camilli, P.; Idevall-Hagren, O. Manipulating phosphoinositide levels Biochim Biophys Acta. 2014.

[12]. Hansen SB, Tao X, MacKinnon R. Structural basis of PIP_2 activation of the classical inward rectifier K^+ channel Kir2.2. Nature. 2011; 477:495–498. [PubMed: 21874019]

[13]. Whorton MR, MacKinnon R. Crystal structure of the mammalian GIRK2 K^+ channel and gating regulation by G proteins, PIP_2, and sodium. Cell. 2011; 147:199–208. [PubMed: 21962516]

[14]. Rohács T, Lopes CM, Jin T, Ramdya PP, Molnar Z, Logothetis DE. Specificity of activation by phosphoinositides determines lipid regulation of Kir channels. Proc Natl Acad Sci U S A. 2003; 100:745–750. [PubMed: 12525701]

[15]. Zhang H, He C, Yan X, Mirshahi T, Logothetis DE. Activation of inwardly rectifying K^+ channels by distinct PtdIns(4,5)P_2 interactions. Nat Cell Biol. 1999; 1:183–188. [PubMed: 10559906]

[16]. Stansfeld PJ, Hopkinson R, Ashcroft FM, Sansom MS. PIP_2-binding site in Kir channels: definition by multiscale biomolecular simulations. Biochemistry. 2009; 48:10926–10933. [PubMed: 19839652]

[17]. Haider S, Tarasov AI, Craig TJ, Sansom MS, Ashcroft FM. Identification of the PIP_2-binding site on Kir6.2 by molecular modelling and functional analysis. EMBO J. 2007; 26:3749–3759. [PubMed: 17673911]

[18]. Schmidt MR, Stansfeld PJ, Tucker SJ, Sansom MS. Simulation-based prediction of phosphatidylinositol 4,5-bisphosphate binding to an ion channel. Biochemistry. 2013; 52:279–281. [PubMed: 23270460]

[19]. Fan Z, Makielski JC. Anionic phospholipids activate ATP-sensitive potassium channels. J Biol Chem. 1997; 272:5388–5395. [PubMed: 9038137]

[20]. Rohács T, Chen J, Prestwich GD, Logothetis DE. Distinct specificities of inwardly rectifying K^+ channels for phosphoinositides. J Biol Chem. 1999; 274:36065–36072. [PubMed: 10593888]

[21]. Telezhkin V, Reilly JM, Thomas AM, Tinker A, Brown DA. Structural requirements of membrane phospholipids for M-type potassium channel activation and binding. J Biol Chem. 2012; 287:10001–10012. [PubMed: 22303005]

[22]. Suh BC, Hille B. Recovery from muscarinic modulation of M current channels requires phosphatidylinositol 4,5-bisphosphate synthesis. Neuron. 2002; 35:507–520. [PubMed: 12165472]

[23]. Zhang H, Craciun LC, Mirshahi T, Rohács T, Lopes CM, Jin T, Logothetis DE. PIP_2 activates KCNQ channels, and its hydrolysis underlies receptor-mediated inhibition of M currents. Neuron. 2003; 37:963–975. [PubMed: 12670425]

[24]. Hughes S, Marsh SJ, Tinker A, Brown DA. PIP_2-dependent inhibition of M-type (Kv7.2/7.3) potassium channels: direct on-line assessment of PIP_2 depletion by G_q-coupled receptors in single living neurons. Pflugers Arch. 2007; 455:115–124. [PubMed: 17447081]

[25]. Suh BC, Horowitz LF, Hirdes W, Mackie K, Hille B. Regulation of KCNQ2/KCNQ3 current by G protein cycling: the kinetics of receptor-mediated signaling by G_q. J Gen Physiol. 2004; 123:663–683. [PubMed: 15173220]

[26]. Li Y, Gamper N, Hilgemann DW, Shapiro MS. Regulation of Kv7 (KCNQ) K^+ channel open probability by phosphatidylinositol 4,5-bisphosphate. J Neurosci. 2005; 25:9825–9835. [PubMed: 16251430]

[27]. Hernandez CC, Zaika O, Shapiro MS. A carboxy-terminal inter-helix linker as the site of phosphatidylinositol 4,5-bisphosphate action on K_V7 (M-type) K^+ channels. J Gen Physiol. 2008; 132:361–381. [PubMed: 18725531]

[28]. Suh BC, Inoue T, Meyer T, Hille B. Rapid chemically induced changes of PtdIns(4,5)P_2 gate KCNQ ion channels. Science. 2006; 314:1454–1457. [PubMed: 16990515]

[29]. Murata Y, Okamura Y. Depolarization activates the phosphoinositide phosphatase Ci-VSP, as detected in Xenopus oocytes coexpressing sensors of PIP_2. J Physiol. 2007; 583:875–889. [PubMed: 17615106]

[30]. Falkenburger BH, Jensen JB, Hille B. Kinetics of PIP_2 metabolism and KCNQ2/3 channel regulation studied with a voltage-sensitive phosphatase in living cells. J Gen Physiol. 2010; 135:99–114. [PubMed: 20100891]

[31]. Idevall-Hagren O, Dickson EJ, Hille B, Toomre DK, De Camilli P. Optogenetic control of phosphoinositide metabolism. Proc Natl Acad Sci U S A. 2012; 109:E2316–2323. [PubMed: 22847441]

[32]. Kruse M, Hammond GR, Hille B. Regulation of voltage-gated potassium channels by PI(4,5)P_2. J Gen Physiol. 2012; 140:189–205. [PubMed: 22851677]

[33]. Falkenburger BH, Jensen JB, Hille B. Kinetics of M_1 muscarinic receptor and G protein signaling to phospholipase C in living cells. J Gen Physiol. 2010; 135:81–97. [PubMed: 20100890]

[34]. Dickson EJ, Falkenburger BH, Hille B. Quantitative properties and receptor reserve of the IP_3 and calcium branch of G_q-coupled receptor signaling. J Gen Physiol. 2013; 141:521–535. [PubMed: 23630337]

[35]. Falkenburger BH, Dickson EJ, Hille B. Quantitative properties and receptor reserve of the DAG and PKC branch of G_q-coupled receptor signaling. J Gen Physiol. 2013; 141:537–555. [PubMed: 23630338]

[36]. Li Y, Zaydman MA, Wu D, Shi J, Guan M, Virgin-Downey B, Cui J. KCNE1 enhances phosphatidylinositol 4,5-bisphosphate (PIP_2) sensitivity of I_{Ks} to modulate channel activity. Proc Natl Acad Sci USA. 2011; 108:9095–9100. [PubMed: 21576493]

[37]. Zaydman MA, Silva JR, Delaloye K, Li Y, Liang H, Larsson HP, Shi J, Cui J. Kv7.1 ion channels require a lipid to couple voltage sensing to pore opening. Proc Natl Acad Sci U S A. 2013; 110:13180–13185. [PubMed: 23861489]

[38]. Hernandez CC, Falkenburger B, Shapiro MS. Affinity for phosphatidylinositol 4,5-bisphosphate determines muscarinic agonist sensitivity of K_V7 K^+ channels. J Gen Physiol. 2009; 134:437–448. [PubMed: 19858360]

[39]. Rodriguez-Menchaca AA, Adney SK, Tang QY, Meng XY, Rosenhouse-Dantsker A, Cui M, Logothetis DE. PIP_2 controls voltage-sensor movement and pore opening of Kv channels through the S4-S5 linker. Proc Natl Acad Sci U S A. 2012; 109:E2399–2408. [PubMed: 22891352]

[40]. Kruse M, Hille B. The phosphoinositide sensitivity of the KV channel family. Channels (Austin, Tex.). 2013; 7:530–536.

[41]. Oliver D, Lien C-C, Soom M, Baukrowitz T, Jonas P, Fakler B. Functional conversion between A-type and delayed rectifier K^+ channels by membrane lipids. Science. 2004; 304:265–270. [PubMed: 15031437]

[42]. Decher N, Gonzalez T, Streit AK, Sachse FB, Renigunta V, Soom M, Heinemann SH, Daut J, Sanguinetti MC. Structural determinants of Kvβ1.3-induced channel inactivation: a hairpin modulated by PIP_2. EMBO J. 2008; 27:3164–3174. [PubMed: 18987637]

[43]. Bian J, Cui J, McDonald TV. hERG K^+ channel activity is regulated by changes in phosphatidylinositol 4,5-bisphosphate. Circ. Res. 2001; 89:1168–1176. [PubMed: 11739282]

[44]. Bian JS, Kagan A, McDonald TV. Molecular analysis of PIP_2 regulation of HERG and IKr. Am J Physiol Heart Circ Physiol. 2004; 287:H2154–2163. [PubMed: 15231497]

[45]. Huang CL, Feng S, Hilgemann DW. Direct activation of inward rectifier potassium channels by PIP_2 and its stabilization by Gβγ. Nature. 1998; 391:803–806. [PubMed: 9486652]

[46]. Ho IH, Murrell-Lagnado RD. Molecular mechanism for sodium-dependent activation of G protein-gated K^+ channels. The Journal of Physiology. 1999; 520:645–651. Pt 3. [PubMed: 10545132]

[47]. Dong K, Tang L, MacGregor GG, Hebert SC. Localization of the ATP/phosphatidylinositol 4,5 diphosphate-binding site to a 39-amino acid region of the carboxyl terminus of the ATP-regulated K^+ channel Kir1.1. J Biol Chem. 2002; 277:49366–49373. [PubMed: 12381730]

[48]. Zeng WZ, Liou HH, Krishna UM, Falck JR, Huang CL. 2002; 282:F826–834.

[49]. Du X, Zhang H, Lopes C, Mirshahi T, Rohács T, Logothetis DE. Characteristic interactions with phosphatidylinositol 4,5-bisphosphate determine regulation of Kir channels by diverse modulators. J Biol Chem. 2004; 279:37271–37281. [PubMed: 15155739]

[50]. Ryan DP, da Silva MRD, Soong TW, Fontaine B, Donaldson MR, Kung AWC, Jongjaroenprasert W, Liang MC, Khoo DHC, Cheah JS, Ho SC, Bernstein HS, Maciel RMB, Brown RH, Ptácek LJ. Mutations in potassium channel Kir2.6 cause susceptibility to thyrotoxic hypokalemic periodic paralysis. Cell. 2010; 140:88–98. [PubMed: 20074522]

[51]. Logothetis DE, Petrou VI, Adney SK, Mahajan R. Channelopathies linked to plasma membrane phosphoinositides. Pflugers Arch. 2010; 460:321–341. [PubMed: 20396900]

[52]. Lopes CM, Zhang H, Rohacs T, Jin T, Yang J, Logothetis DE. Alterations in conserved Kir channel-PIP_2 interactions underlie channelopathies. Neuron. 2002; 34:933–944. [PubMed: 12086641]

[53]. Lu M, Hebert SC, Giebisch G. Hydrolyzable ATP and PIP_2 modulate the small-conductance K^+ channel in apical membranes of rat cortical-collecting duct (CCD). J Gen Physiol. 2002; 120:603–615. [PubMed: 12407074]

[54]. Vaithianathan T, Bukiya A, Liu J, Liu P, Asuncion-Chin M, Fan Z, Dopico A. Direct regulation of BK channels by phosphatidylinositol 4,5-bisphosphate as a novel signaling pathway. J Gen Physiol. 2008; 132:13–28. [PubMed: 18562499]

[55]. Tang QY, Zhang Z, Meng XY, Cui M, Logothetis DE. Structural determinants of PIP_2 regulation of BK channel activity through the RCK1 Ca^{2+} coordination site. J Biol Chem. 2014

[56]. Hille, B. Ion Channels of Excitable Membranes. 3rd. Sinauer Associates, Inc.; Sunderland, MA.: 2001.

[57]. Catterall WA. Structure and regulation of voltage-gated Ca^{2+} channels. Annual review of cell and developmental biology. 2000; 16:521–555.

[58]. Zamponi GW, Currie KP. Regulation of Ca_V2 calcium channels by G protein coupled receptors. Biochim Biophys Acta. 2013; 1828:1629–1643. [PubMed: 23063655]

[59]. Bernheim L, Beech DJ, Hille B. A diffusible second messenger mediates one of the pathways coupling receptors to calcium channels in rat sympathetic neurons. Neuron. 1991; 6:859–867. [PubMed: 1647174]

[60]. Hille B. Modulation of ion-channel function by G-protein-coupled receptors. Trends Neurosci. 1994; 17:531–536. [PubMed: 7532338]

[61]. Haley JE, Delmas P, Offermanns S, Abogadie FC, Simon MI, Buckley NJ, Brown DA. Muscarinic inhibition of calcium current and M current in $G\alpha_q$-deficient mice. J Neurosci. 2000; 20:3973–3979. [PubMed: 10818132]

[62]. Kammermeier PJ, Ruiz-Velasco V, Ikeda SR. A voltage-independent calcium current inhibitory pathway activated by muscarinic agonists in rat sympathetic neurons requires both $G_{\alpha q/11}$ and $G_{\beta\gamma}$. J Neurosci. 2000; 20:5623–5629. [PubMed: 10908599]

[63]. Wu L, Bauer CS, Zhen XG, Xie C, Yang J. Dual regulation of voltage-gated calcium channels by PtdIns(4,5)P_2. Nature. 2002; 419:947–952. [PubMed: 12410316]

[64]. Gamper N, Reznikov V, Yamada Y, Yang J, Shapiro MS. Phosphatidylinositol 4,5-bisphosphate signals underlie receptor-specific $G_{q/11}$-mediated modulation of N-type Ca^{2+} channels. J Neurosci. 2004; 24:10980–10992. [PubMed: 15574748]

[65]. Vivas O, Castro H, Arenas I, Elias-Vinas D, Garcia DE. PIP_2 hydrolysis is responsible for voltage independent inhibition of $Ca_V2.2$ channels in sympathetic neurons. Biochemical and biophysical research communications. 2013; 432:275–280. [PubMed: 23396054]

[66]. Suh BC, Leal K, Hille B. Modulation of high-voltage activated Ca^{2+} channels by membrane phosphatidylinositol 4,5-bisphosphate. Neuron. 2010; 67:224–238. [PubMed: 20670831]

[67]. Suh BC, Kim DI, Falkenburger BH, Hille B. Membrane-localized β-subunits alter the PIP_2 regulation of high-voltage activated Ca^{2+} channels. Proc Natl Acad Sci U S A. 2012; 109:3161–3166. [PubMed: 22308488]

[68]. Liu L, Roberts ML, Rittenhouse AR. Phospholipid metabolism is required for M_1 muscarinic inhibition of N-type calcium current in sympathetic neurons. Eur Biophys J. 2004; 33:255–264. [PubMed: 15004729]

[69]. Chien AJ, Zhao X, Shirokov RE, Puri TS, Chang CF, Sun D, Rios E, Hosey MM. Roles of a membrane-localized β subunit in the formation and targeting of functional L-type Ca^{2+} channels. J Biol Chem. 1995; 270:30036–30044. [PubMed: 8530407]

[70]. Hurley JH, Cahill AL, Currie KP, Fox AP. The role of dynamic palmitoylation in Ca^{2+} channel inactivation. Proc Natl Acad Sci U S A. 2000; 97:9293–9298. [PubMed: 10900273]

[71]. Feng ZP, Arnot MI, Doering CJ, Zamponi GW. Calcium channel β subunits differentially regulate the inhibition of N-type channels by individual Gβ isoforms. J Biol Chem. 2001; 276:45051–45058. [PubMed: 11560937]

[72]. Roberts-Crowley ML, Rittenhouse AR. Arachidonic acid inhibition of L-type calcium ($Ca_V1.3b$) channels varies with accessory $Ca_V\beta$ subunits. J Gen Physiol. 2009; 133:387–403. [PubMed: 19332620]

[73]. Heneghan JF, Mitra-Ganguli T, Stanish LF, Liu L, Zhao R, Rittenhouse AR. The Ca^{2+} channel β subunit determines whether stimulation of G_q-coupled receptors enhances or inhibits N current. J Gen Physiol. 2009; 134:369–384. [PubMed: 19858357]

[74]. Mitra-Ganguli T, Vitko I, Perez-Reyes E, Rittenhouse AR. Orientation of palmitoylated $Ca_V\beta2a$ relative to $Ca_V2.2$ is critical for slow pathway modulation of N-type Ca^{2+} current by tachykinin receptor activation. J Gen Physiol. 2009; 134:385–396. [PubMed: 19858358]

[75]. Horowitz LF, Hirdes W, Suh BC, Hilgemann DW, Mackie K, Hille B. Phospholipase C in living cells: activation, inhibition, Ca^{2+} requirement, and regulation of M current. J Gen Physiol. 2005; 126:243–262. [PubMed: 16129772]

[76]. Rohács T. Phosphoinositide Regulation of TRP Channels. Handbook of experimental pharmacology. 2014; 223:1143–1176. [PubMed: 24961984]

[77]. McKemy DD, Neuhausser WM, Julius D. Identification of a cold receptor reveals a general role for TRP channels in thermosensation. Nature. 2002; 416:52–58. [PubMed: 11882888]

[78]. Peier AM, Moqrich A, Hergarden AC, Reeve AJ, Andersson DA, Story GM, Earley TJ, Dragoni I, McIntyre P, Bevan S, Patapoutian A. A TRP channel that senses cold stimuli and menthol. Cell. 2002; 108:705–715. [PubMed: 11893340]

[79]. Liu B, Qin F. Functional control of cold- and menthol-sensitive TRPM8 ion channels by phosphatidylinositol 4,5-bisphosphate. J Neurosci. 2005; 25:1674–1681. [PubMed: 15716403]

[80]. Rohács T, Lopes CM, Michailidis I, Logothetis DE. PI(4,5)P_2 regulates the activation and desensitization of TRPM8 channels through the TRP domain. Nat Neurosci. 2005; 8:626–634. [PubMed: 15852009]

[81]. Daniels RL, Takashima Y, McKemy DD. Activity of the neuronal cold sensor TRPM8 is regulated by phospholipase C via the phospholipid phosphoinositol 4,5-bisphosphate. J Biol Chem. 2009; 284:1570–1582. [PubMed: 19019830]

[82]. Fujita F, Uchida K, Takaishi M, Sokabe T, Tominaga M. Ambient temperature affects the temperature threshold for TRPM8 activation through interaction of phosphatidylinositol 4,5-bisphosphate. J Neurosci. 2013; 33:6154–6159. [PubMed: 23554496]

[83]. Benedikt J, Teisinger J, Vyklicky L, Vlachova V. Ethanol inhibits cold-menthol receptor TRPM8 by modulating its interaction with membrane phosphatidylinositol 4,5-bisphosphate. J Neurochem. 2007; 100:211–224. [PubMed: 17074062]

[84]. Várnai P, Thyagarajan B, Rohács T, Balla T. Rapidly inducible changes in phosphatidylinositol 4,5-bisphosphate levels influence multiple regulatory functions of the lipid in intact living cells. J Cell Biol. 2006; 175:377–382. [PubMed: 17088424]

[85]. Brauchi S, Orta G, Mascayano C, Salazar M, Raddatz N, Urbina H, Rosenmann E, Gonzalez-Nilo F, Latorre R. Dissection of the components for PIP_2 activation and thermosensation in TRP channels. Proc Natl Acad Sci U S A. 2007; 104:10246–10251. [PubMed: 17548815]

[86]. Hofmann T, Obukhov AG, Schaefer M, Harteneck C, Gudermann T, Schultz G. Direct activation of human TRPC6 and TRPC3 channels by diacylglycerol. Nature. 1999; 397:259–263. [PubMed: 9930701]

[87]. Okada T, Inoue R, Yamazaki K, Maeda A, Kurosaki T, Yamakuni T, Tanaka I, Shimizu S, Ikenaka K, Imoto K, Mori Y. Molecular and functional characterization of a novel mouse transient receptor potential protein homologue TRP7. Ca^{2+}-permeable cation channel that is constitutively activated and enhanced by stimulation of G protein-coupled receptor. J Biol Chem. 1999; 274:27359–27370. [PubMed: 10488066]

[88]. Lemonnier L, Trebak M, Putney JW Jr. Complex regulation of the TRPC3, 6 and 7 channel subfamily by diacylglycerol and phosphatidylinositol-4,5-bisphosphate. Cell Calcium. 2008; 43:506–514. [PubMed: 17942152]

[89]. Jardin I, Redondo PC, Salido GM, Rosado JA. Phosphatidylinositol 4,5-bisphosphate enhances store-operated calcium entry through hTRPC6 channel in human platelets. Biochim Biophys Acta. 2008; 1783:84–97. [PubMed: 17719101]

[90]. Imai Y, Itsuki K, Okamura Y, Inoue R, Mori MX. A self-limiting regulation of vasoconstrictor-activated TRPC3/C6/C7 channels coupled to PI(4,5)P_2-diacylglycerol signalling. J Physiol. 2012; 590:1101–1119. [PubMed: 22183723]

[91]. Itsuki K, Imai Y, Hase H, Okamura Y, Inoue R, Mori MX. PLC-mediated PI(4,5)P_2 hydrolysis regulates activation and inactivation of TRPC6/7 channels. J Gen Physiol. 2014; 143:183–201. [PubMed: 24470487]

[92]. Otsuguro K, Tang J, Tang Y, Xiao R, Freichel M, Tsvilovskyy V, Ito S, Flockerzi V, Zhu MX, Zholos AV. Isoform-specific inhibition of TRPC4 channel by phosphatidylinositol 4,5-bisphosphate. J Biol Chem. 2008; 283:10026–10036. [PubMed: 18230622]

[93]. Stein AT, Ufret-Vincenty CA, Hua L, Santana LF, Gordon SE. Phosphoinositide 3-kinase binds to TRPV1 and mediates NGF-stimulated TRPV1 trafficking to the plasma membrane. J Gen Physiol. 2006; 128:509–522. [PubMed: 17074976]

[94]. Lukacs V, Thyagarajan B, Varnai P, Balla A, Balla T, Rohács T. Dual regulation of TRPV1 by phosphoinositides. J Neurosci. 2007; 27:7070–7080. [PubMed: 17596456]

[95]. Klein RM, Ufret-Vincenty CA, Hua L, Gordon SE. Determinants of molecular specificity in phosphoinositide regulation. Phosphatidylinositol (4,5)-bisphosphate ($PI(4,5)P_2$) is the endogenous lipid regulating TRPV1. J Biol Chem. 2008; 283:26208–26216. [PubMed: 18574245]

[96]. Senning EN, Collins MD, Stratiievska A, Ufret-Vincenty CA, Gordon SE. Regulation of TRPV1 ion channel by phosphoinositide (4,5)-bisphosphate: the role of membrane asymmetry. J Biol Chem. 2014; 289:10999–11006. [PubMed: 24599956]

[97]. Yao J, Qin F. Interaction with phosphoinositides confers adaptation onto the TRPV1 pain receptor. PLoS biology. 2009; 7:e46. [PubMed: 19243225]

[98]. Hammond GR, Fischer MJ, Anderson KE, Holdich J, Koteci A, Balla T, Irvine RF. PI4P and $PI(4,5)P_2$ are essential but independent lipid determinants of membrane identity. Science. 2012; 337:727–730. [PubMed: 22722250]

[99]. Lukacs V, Yudin Y, Hammond GR, Sharma E, Fukami K, Rohács T. Distinctive changes in plasma membrane phosphoinositides underlie differential regulation of TRPV1 in nociceptive neurons. Journal of Neuroscience. 2013; 33:11451–11463. [PubMed: 23843517]

[100]. Mercado J, Gordon-Shaag A, Zagotta WN, Gordon SE. Ca^{2+}-dependent desensitization of TRPV2 channels is mediated by hydrolysis of phosphatidylinositol 4,5-bisphosphate. J Neurosci. 2010; 30:13338–13347. [PubMed: 20926660]

[101]. Liu B, Zhang C, Qin F. Functional recovery from desensitization of vanilloid receptor TRPV1 requires resynthesis of phosphatidylinositol 4,5-bisphosphate. J Neurosci. 2005; 25:4835–4843. [PubMed: 15888659]

[102]. Chuang HH, Prescott ED, Kong H, Shields S, Jordt SE, Basbaum AI, Chao MV, Julius D. Bradykinin and nerve growth factor release the capsaicin receptor from $PtdIns(4,5)P_2$-mediated inhibition. Nature. 2001; 411:957–962. [PubMed: 11418861]

[103]. Prescott ED, Julius D. A modular PIP_2 binding site as a determinant of capsaicin receptor sensitivity. Science. 2003; 300:1284–1288. [PubMed: 12764195]

[104]. Ufret-Vincenty CA, Klein RM, Hua L, Angueyra J, Gordon SE. Localization of the PIP_2 sensor of TRPV1 ion channels. J Biol Chem. 2011; 286:9688–9698. [PubMed: 21224382]

[105]. Kim AY, Tang Z, Liu Q, Patel KN, Maag D, Geng Y, Dong X. Pirt, a phosphoinositide-binding protein, functions as a regulatory subunit of TRPV1. Cell. 2008; 133:475–485. [PubMed: 18455988]

[106]. Cao E, Cordero-Morales JF, Liu B, Qin F, Julius D. TRPV1 channels are intrinsically heat sensitive and negatively regulated by phosphoinositide lipids. Neuron. 2013; 77:667–679. [PubMed: 23439120]

[107]. Lukacs V, Rives JM, Sun X, Zakharian E, Rohács T. Promiscuous activation of transient receptor potential vanilloid 1 (TRPV1) channels by negatively charged intracellular lipids: the key role of endogenous phosphoinositides in maintaining channel activity. J Biol Chem. 2013; 288:35003–35013. [PubMed: 24158445]

[108]. Kunzelmann K, Bachhuber T, Regeer R, Markovich D, Sun J, Schreiber R. Purinergic inhibition of the epithelial Na^+ transport via hydrolysis of PIP_2. FASEB J. 2005; 19:142–143. [PubMed: 15504951]

[109]. Ma HP, Saxena S, Warnock DG. Anionic phospholipids regulate native and expressed epithelial sodium channel (ENaC). J Biol Chem. 2002; 277:7641–7644. [PubMed: 11809744]

[110]. Tong Q, Gamper N, Medina JL, Shapiro MS, Stockand JD. Direct activation of the epithelial Na^+ channel by phosphatidylinositol 3,4,5-trisphosphate and phosphatidylinositol 3,4-bisphosphate produced by phosphoinositide 3-OH kinase. J Biol Chem. 2004; 279:22654–22663. [PubMed: 15028718]

[111]. Yue G, Malik B, Yue G, Eaton DC. Phosphatidylinositol 4,5-bisphosphate (PIP_2) stimulates epithelial sodium channel activity in A6 cells. J Biol Chem. 2002; 277:11965–11969. [PubMed: 11812779]

[112]. Zaika O, Mamenko M, O'Neil RG, Pochynyuk O. Bradykinin acutely inhibits activity of the epithelial Na^+ channel in mammalian aldosterone-sensitive distal nephron. Am J Physiol Renal Physiol. 2011; 300:F1105–1115. [PubMed: 21325499]

[113]. Pochynyuk O, Tong Q, Medina J, Vandewalle A, Staruschenko A, Bugaj V, Stockand JD. Molecular determinants of PI(4,5)P_2 and PI(3,4,5)P_3 regulation of the epithelial Na^+ channel. J Gen Physiol. 2007; 130:399–413. [PubMed: 17893193]

[114]. Zhao Q, Yang M, Ting AT, Logothetis DE. PIP_2 regulates the ionic current of P2X receptors and $P2X_7$ receptor-mediated cell death. Channels (Austin). 2007; 1:46–55. [PubMed: 19151591]

[115]. Bernier LP, Ase AR, Chevallier S, Blais D, Zhao Q, Boue-Grabot E, Logothetis D, Seguela P. Phosphoinositides regulate P2X4 ATP-gated channels through direct interactions. J Neurosci. 2008; 28:12938–12945. [PubMed: 19036987]

[116]. Fujiwara Y, Kubo Y. Regulation of the desensitization and ion selectivity of ATP-gated $P2X_2$ channels by phosphoinositides. J Physiol. 2006; 576:135–149. [PubMed: 16857707]

[117]. Bernier LP, Ase AR, Tong X, Hamel E, Blais D, Zhao Q, Logothetis DE, Seguela P. Direct modulation of P2X1 receptor-channels by the lipid phosphatidylinositol 4,5-bisphosphate. Mol Pharmacol. 2008; 74:785–792. [PubMed: 18523136]

[118]. Womack KB, Gordon SE, He F, Wensel TG, Lu CC, Hilgemann DW. Do phosphatidylinositides modulate vertebrate phototransduction? J Neurosci. 2000; 20:2792–2799. [PubMed: 10751430]

[119]. Zhainazarov AB, Spehr M, Wetzel CH, Hatt H, Ache BW. Modulation of the olfactory CNG channel by Ptdlns(3,4,5)P_3. J Membr Biol. 2004; 201:51–57. [PubMed: 15635812]

[120]. Brady JD, Rich ED, Martens JR, Karpen JW, Varnum MD, Brown RL. Interplay between PIP_3 and calmodulin regulation of olfactory cyclic nucleotide-gated channels. Proc Natl Acad Sci U S A. 2006; 103:15635–15640. [PubMed: 17032767]

[121]. Dai G, Peng C, Liu C, Varnum MD. Two structural components in CNGA3 support regulation of cone CNG channels by phosphoinositides. J Gen Physiol. 2013; 141:413–430. [PubMed: 23530136]

[122]. Dai G, Sherpa T, Varnum MD. Alternative splicing governs cone cyclic nucleotide-gated (CNG) channel sensitivity to regulation by phosphoinositides. J Biol Chem. 2014; 289:13680–13690. [PubMed: 24675082]

[123]. Caputo A, Caci E, Ferrera L, Pedemonte N, Barsanti C, Sondo E, Pfeffer U, Ravazzolo R, Zegarra-Moran O, Galietta LJ. TMEM16A, a membrane protein associated with calcium-dependent chloride channel activity. Science. 2008; 322:590–594. [PubMed: 18772398]

[124]. Schroeder BC, Cheng T, Jan YN, Jan LY. Expression cloning of TMEM16A as a calcium-activated chloride channel subunit. Cell. 2008; 134:1019–1029. [PubMed: 18805094]

[125]. Yang YD, Cho H, Koo JY, Tak MH, Cho Y, Shim WS, Park SP, Lee J, Lee B, Kim BM, Raouf R, Shin YK, Oh U. TMEM16A confers receptor-activated calcium-dependent chloride conductance. Nature. 2008; 455:1210–1215. [PubMed: 18724360]

[126]. Pritchard HA, LeBlanc N, Albert AP, Greenwood IA. Inhibitory role of phosphatidylinositol 4,5 bisphosphate on TMEM16A encoded calcium-activated chloride channels in rat pulmonary artery. Br J Pharmacol. 2014

[127]. Di Paolo G, De Camilli P. Phosphoinositides in cell regulation and membrane dynamics. Nature. 2006; 443:651–657. [PubMed: 17035995]

[128]. De Matteis MA, Godi A. PI-loting membrane traffic. Nat Cell Biol. 2004; 6:487–492. [PubMed: 15170460]

[129]. Bojjireddy N, Botyanszki J, Hammond G, Creech D, Peterson R, Kemp DC, Snead M, Brown R, Morrison A, Wilson S, Harrison S, Moore C, Balla T. Pharmacological and genetic targeting of the PI4KA enzyme reveals its important role in maintaining plasma membrane phosphatidylinositol 4-phosphate and phosphatidylinositol 4,5-bisphosphate levels. J Biol Chem. 2014; 289:6120–6132. [PubMed: 24415756]

[130]. Hammond GR, Machner MP, Balla T. A novel probe for phosphatidylinositol 4-phosphate reveals multiple pools beyond the Golgi. J Cell Biol. 2014; 205:113–126. [PubMed: 24711504]

[131]. Dickson EJ, Jensen JB, Hille B. Golgi and plasma membrane pools of PI(4)P contribute to plasma membrane PI(4,5)P_2 and maintenance of KCNQ2/3 ion channel current. Proc Natl Acad Sci U S A. 2014; 111:E2281–2290. [PubMed: 24843134]

[132]. De Matteis M, Godi A, Corda D. Phosphoinositides and the Golgi complex. Curr Opin Cell Biol. 2002; 14:434–447. [PubMed: 12383794]

[133]. Cerny J, Feng Y, Yu A, Miyake K, Borgonovo B, Klumperman J, Meldolesi J, McNeil PL, Kirchhausen T. The small chemical vacuolin-1 inhibits Ca^{2+}-dependent lysosomal exocytosis but not cell resealing. EMBO Rep. 2004; 5:883–888. [PubMed: 15332114]

[134]. Dong XP, Cheng X, Mills E, Delling M, Wang F, Kurz T, Xu H. The type IV mucolipidosis-associated protein TRPML1 is an endolysosomal iron release channel. Nature. 2008; 455:992–996. [PubMed: 18794901]

[135]. Dong XP, Shen D, Wang X, Dawson T, Li X, Zhang Q, Cheng X, Zhang Y, Weisman LS, Delling M, Xu H. PI(3,5)P_2 controls membrane trafficking by direct activation of mucolipin Ca^{2+} release channels in the endolysosome. Nat Commun. 2010; 1:38. [PubMed: 20802798]

[136]. Zhang X, Li X, Xu H. Phosphoinositide isoforms determine compartment-specific ion channel activity. Proc Natl Acad Sci U S A. 2012; 109:11384–11389. [PubMed: 22733759]

[137]. Shen D, Wang X, Li X, Zhang X, Yao Z, Dibble S, Dong XP, Yu T, Lieberman AP, Showalter HD, Xu H. Lipid storage disorders block lysosomal trafficking by inhibiting a TRP channel and lysosomal calcium release. Nat Commun. 2012; 3:731. [PubMed: 22415822]

[138]. Wang X, Zhang X, Dong XP, Samie M, Li X, Cheng X, Goschka A, Shen D, Zhou Y, Harlow J, Zhu MX, Clapham DE, Ren D, Xu H. TPC proteins are phosphoinositide-activated sodium-selective ion channels in endosomes and lysosomes. Cell. 2012; 151:372–383. [PubMed: 23063126]

[139]. Jha A, Ahuja M, Patel S, Brailoiu E, Muallem S. Convergent regulation of the lysosomal two-pore channel-2 by Mg^{2+}, NAADP, PI(3,5)P_2 and multiple protein kinases. EMBO J. 2014; 33:501–511. [PubMed: 24502975]

[140]. Cheng X, Shen D, Samie M, Xu H. Mucolipins: Intracellular TRPML1-3 channels. FEBS Lett. 2010; 584:2013–2021. [PubMed: 20074572]

Highlights

- Phosphoinositides regulate functional characteristics of many ion channels.
- For study, need defined perturbations of phosphoinositides levels in living cells.
- Phosphoinositides may silence ion channel during trafficking.

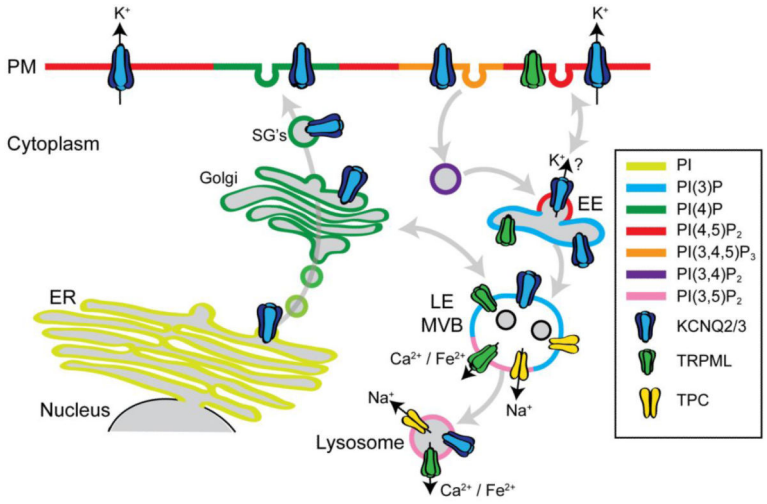

Fig. 1.

Organelle phosphoinositide signature helps regulate ion channel function. Schematic representation of the predominant subcellular localization of phosphoinositide species in each organelle. PI(4,5)P_2 and PI(3,4,5)P_3 are concentrated at the plasma membrane (PM). PI(3,4)P_2 is found mostly in early endocytic pathways distal to the plasma membrane. PI(4)P is principally concentrated in the Golgi complex, but can also be found at the plasma membrane and secretory pathways (SGs). PI(3)P is located in early endosomes (EE) and PI(3,5)P_2 on late endosomal (LE), multi-vesicular body (MVB), and lysosomal compartments. PI is found in the endoplasmic reticulum (ER). Grey arrows represent the continuous flow of membrane between organelles. Note that heterotetrameric KCNQ2/3 ion channels are closed (no arrow) during trafficking and retrieval from the plasma membrane, but are open (with arrow) at the plasma membrane due to the presence of appropriate activating phosphoinositide (PI(4,5)P_2) and membrane voltage.

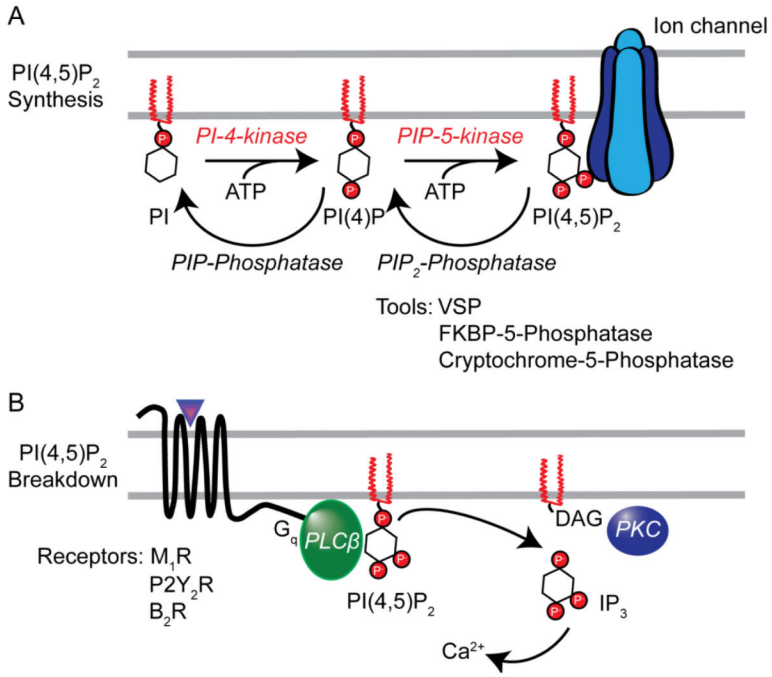

Fig. 2.

Metabolic synthesis and cleavage of $PI(4,5)P_2$. A) Production of $PI(4,5)P_2$ from PI in two steps of phosphorylation. Lipid kinases transfer phosphates from ATP to the inositol 4- and 5-hydroxyls, and lipid phosphatases remove the phosphates in continuous steady-state cycles. B) G_q-couple receptors activate PLCβ, which cleaves $PI(4,5)P_2$ into soluble inositol 1,4,5-trisphosphate (IP_3) and membrane-bound diacylglycerol. IP_3 releases calcium from intracellular stores and diacylglycerol recruits and activates PKC.

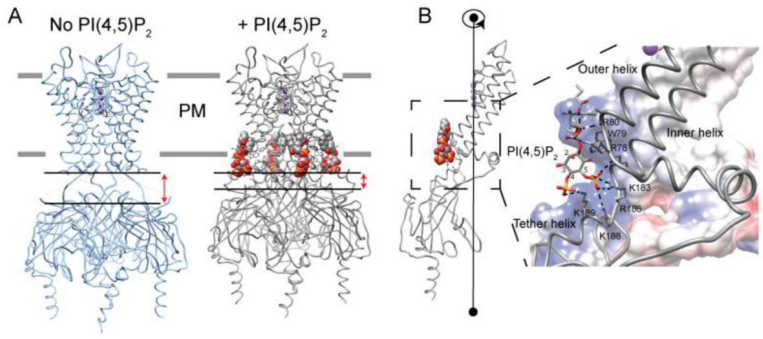

Fig. 3.

Structural basis of $PI(4,5)P_2$ activation of Kir2.2. A) X-Ray crystal structures of the Kir2.2 homotetrameric channel in the absence (left, blue α-carbon chain, Protein Data Base coordinates, PDB: 3JYC) and presence of $PI(4,5)P_2$ (right, gray, PDB: 3SPI) [12]. The Kir2.2 tetramer is viewed from the membrane side with the extracellular face up. The approximate position of the plasma membrane (PM) lipid bilayer is represented as grey bars. Purple spheres represent potassium ions on the axis of the pore. Each channel subunit is in complex with a single $PI(4,5)P_2$ molecule represented as spheres and colored according to atom type: carbon, grey; phosphorous, orange; and oxygen, red. Binding of $PI(4,5)P_2$ induces a translation of the cytoplasmic domains towards the transmembrane domains and overall 6 Å compression of the channel (compare red arrows) and an opening of the inner helix 'activation' gate. B) A single Kir2.2 subunit in complex with a single $PI(4,5)P_2$ molecule. The vertical line is the pore axis with four K^+ ions visible. Inset: detailed view of the $PI(4,5)P_2$ binding site. Helices (shown as ribbon) are colored grey. All side chains are shown as sticks. Di-C_8-$PI(4,5)P_2$ is shown as sticks and colored according to atom type: carbon, grey; phosphorous, orange; oxygen, red; and nitrogen blue. N-O hydrogen bonds are dashed lines. Upon $PI(4,5)P_2$ binding, a flexible linker contracts to form a compact helical structure, the cytoplasmic domain translates and becomes tethered to the transmembrane domain, this causes the inner gate to open. Note the electrostatic map of the same subunit behind the Kir2.2 structure (blue is positive; red negative; white neutral). The $PI(4,5)P_2$ binding site comprises numerous basic residues (blue) that interact electrostatically with the negatively charged phosphates of $PI(4,5)P_2$.

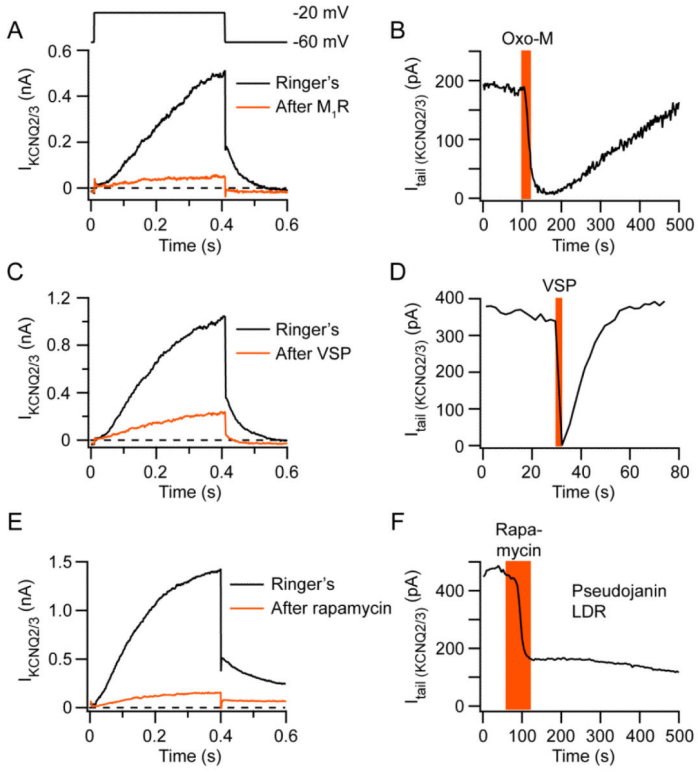

Fig. 4.

Depletion of plasma membrane PI(4,5)P_2 by different approaches closes KCNQ2/3 channels. A) Current traces of KCNQ2/3 channels activated by a depolarizing pulse from −60 mV to −20 mV in the whole-cell configuration before (black) and after (red) activation of muscarinic acetylcholine receptor type I (M_1R) by 10 μM oxotremorine methiodide (Oxo-M) in tsA-201 cells. Test pulse protocol is shown above the current traces. B) KCNQ2/3-mediated tail current amplitude against time in the same experiment as A. Orange bar indicates Oxo-M application. C-F) Same type of experiment as in A and B, but PI(4,5)P_2 was depleted either by a voltage-pulse to +100 mV to activate a voltage-sensitive lipid-5-phosphatase (VSP, panels C and D), or by recruitment of a lipid-5-phosphatase (pseudojanin) to an anchor protein at the plasma membrane (LDR) by addition of 5 μM rapamycin (panels E and F).

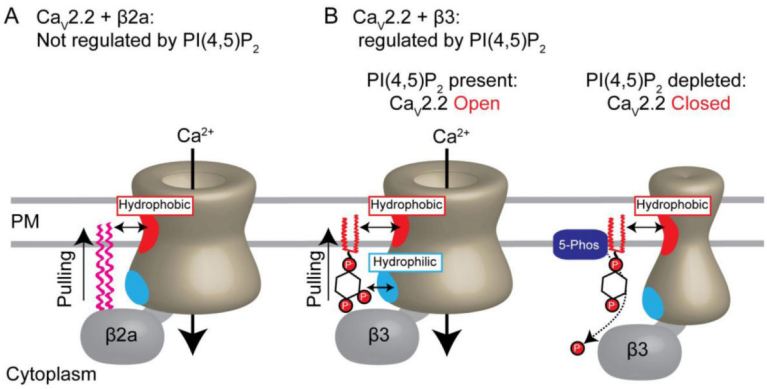

Fig. 5.

Bidentate model of $Ca_V2.2$ channel regulation by $PI(4,5)P_2$ with different coexpressed Ca_V β subunits. A) Interactions between $Ca_V2.2$ and β2a subunit. The two palmitoyl fatty acyl chains of Ca_V β2a compete with the those of $PI(4,5)P_2$ at the channel, reducing the need for $PI(4,5)P_2$. See text. B). Interactions between $Ca_V2.2$, $PI(4,5)P_2$, and β3 subunit. Non lipidated Ca_V β3 subunits do not compete with $PI(4,5)P_2$ binding. The electrostatic interaction of $PI(4,5)P_2$ with the channel is disturbed after dephosphorylation by a lipid $PI(4,5)P_2$ 5-phosphatase. See text.