Review

Lipid agonism: The PIP$_2$ paradigm of ligand-gated ion channels

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ABSTRACT

The past decade, membrane signaling lipids emerged as major regulators of ion channel function. However, the molecular nature of lipid binding to ion channels remained poorly described due to a lack of structural information and assays to quantify and measure lipid binding in a membrane. How does a lipid–ligand bind to a membrane protein in the plasma membrane, and what does it mean for a lipid to activate or regulate an ion channel? How does lipid binding compare to activation by soluble neurotransmitter? And how does the cell control lipid agonism? This review focuses on lipids and their interactions with membrane proteins, in particular, ion channels. I discuss the intersection of membrane lipid biology and ion channel biophysics. A picture emerges of membrane lipids as bona fide agonists of ligand-gated ion channels. These freely diffusing signals reside in the plasma membrane, bind to the transmembrane domain of protein, and cause a conformational change that allosterically gates an ion channel. The system employs a catalog of diverse signaling lipids ultimately controlled by lipid enzymes and raft localization. I draw upon pharmacology, recent protein structure, and electrophysiological data to understand lipid regulation and define inward rectifying potassium channels (K$_{ir}$) as a new class of PIP$_2$ lipid-gated ion channels.

1. Introduction

Signaling lipids are important regulators of ion channels and exert a central role in tissue function including functional heartbeat, neuronal signaling, kidney dialysis, sight, smell, pain, and touch [1–5]. In the past, most biochemist and ion channel experts viewed lipids as unwieldy, hydrophobic molecules physically supporting ion channels in a cell membrane or liposomes but not as ligands. Recent past models of lipid signaling to ion channels suggested that the formation of anionic lipids caused a change in the plasma membrane surface charge. Little was known about how lipids engaged and disengaged the channel or how the contact of a lipid with protein might affect the conformation of ion channels in the membrane. A lack of binding constants for lipids and ion channels challenged our ability to think about lipids as ligands. Aspects of this problem remain an important hurdle.

In 1998, Hilgemann and colleagues [6] eloquently showed that a signaling lipid could directly activate an ion channel. The lipid, phosphatidylinositol 4,5-bisphosphate (PIP$_2$), a minor constituent of the plasma membrane, was required and sufficient for the activation of a potassium channel [6]. Despite more than a decade of experimentation, the nature of PIP$_2$ binding remained clouded by an inability to accurately measure its concentration in the membrane and directly detect binding to protein. Simple terminology such as lipid concentration and affinity are difficult to define for insoluble molecules in an aqueous environment [7]. Absent a well-characterized ligand protein interaction, the initial non-specific theories of surface charge and membrane curvature dominated [8,9] but struggled to account for the specificity of signaling lipids in many systems. Recently, a more accurate model emerges that includes structural and pharmacological evidence that lipids bind to and activate ion channels analogous to classic ligand-like agonist properties [10,11].

Herein, a model of lipid agonism is built on PIP$_2$ and inward rectifying potassium (K$_{ir}$) channels. Aspects of many other classes of channels and signaling lipids appear to function in a similar way; select examples are included throughout this review. The intent of this review is to

facilitate an understanding at the interface of ion channel activation and membrane lipid biology, although neither field is reviewed in a comprehensive way.

2. The signaling lipid PIP2 is an agonist that gates ion channels

PIP2, arguably the best-studied signaling lipid, is comprised of an inositol head group (the named feature), a phosphoglycerol backbone, and two acyl chains (Fig. 1A). PIP2 bears four negative charges and is a permanent and minor component (<1%) of the Eukaryotic plasma membrane inner leaflet [9,12].

2.1. PIP2 ion channel physiology

PIP2 signaling dictates the activatable state of a plethora of ion channels [2,13,14] (Fig. 1) with broad reaching cellular function. The first indication that a channel is PIP2 dependent usually arises when a channel, excised from the plasma membrane (e.g., inside out patch), steadily decreases in conductance until the channel inactivates. This is known as "rundown" [2,15]. The excised patch lacks the cytosolic factors to maintain sufficient PIP2 levels in the membrane to support ion channel function; hence, the channels in the patch close. Adding ATP and Mg was shown to delay rundown [15]. Presumably, PIP2 synthesizing enzymes are excised in the patch with the channels and that these enzyme utilize the ATP to replenish PIP2 [2,15]. Adding back a soluble PIP2 analog dioctanoyl PIP2 (C8PIP2) rescues activity [2,14] of many ion channel types [16–19]. In a second method, PIP2 scavengers (e.g., polyamines or PIP2 antibodies) are used to deplete or mask PIP2 availability [20–22]. Polyamines are positively charged polymers that bind via avidity to the multiple negative charges of PIP2. More complete descriptions of PIP2-dependent ion channels and PIP2 cellular function are reviewed by Suh and Hille [2,11], Xie [5], and McLaughlin [9]. Recently, a voltage-sensitive phosphatase (Ci-VSP) was shown to provide direct control over PIP2 signaling in the membrane [23–25]. When Ci-VSP is co transfected with K_{ir} [23–25], $K_v7.1$ [26], Ca_v2 [27,28], and TRP [29,30], channels are voltage-dependent consistent with Ci-VSP regulation of

PIP2. This method provides better control of PIP2; however, indirect effects of PIP2 remain a possibility.

In order to directly show PIP2 modulation, an ion channel can be purified and reconstituted (reinserted) into lipid vesicles with a known lipid composition. A lack of purified ion channels limited this technique, but recent advancements in membrane protein expression and purification [31,32] has overcome this problem for select channel types [33–37]. The nAChR was among the first channels to show direct dependence on a lipid for activation, phosphatidic acid (PA) [38]. Recently, PIP2-dependent channels were reconstituted into lipid vesicles and shown to respond directly to PIP2 modulation. This includes GIRK [39,40], TRPV1 [41], TRPM8 [42], and $K_{ir}2.1$-2 [43] channels.

2.2. PIP2 ion channel structure

Despite robust channel modulation by indirect methods, absent a crystal structure, an understanding of the molecular action of PIP2 and the precise binding site remained speculative. In 2011, an X-ray crystal structure complex of $K_{ir}2.2$ with PIP2 revealed a PIP2 binding site in the channel's transmembrane domain [10] (Fig. 2). The glycerol backbone and 1′ phosphate of PIP2 capped the first transmembrane spanning helix (TM1) of K_{ir}. An intimate coordination of the 5′ inositol phosphate in the distal end of the second transmembrane spanning helix (TM2) accounted for PIP2 specificity. Moreover, a conformational change appeared to initiate or open the ion conduction pathway. Basic residues on a linker between the transmembrane domain and cytoplasmic domain directly contacted PIP2, but distal basic residues proposed in the CTD [44] did not; rather, they were buried and stabilized proper folding of the cytoplasmic domain structure [10]. Prior to the $K_{ir}2.2$/PIP2 complex, structures of PIP2/protein complexes were limited to soluble membrane localization domains, which lack a transmembrane domain and share few if any functional similarities with ion channels. A lack of appropriate structural examples and an understanding of how lipids and proteins interact in the plasma membrane hindered a complete mechanistic interpretation of PIP2 data. Furthermore, early studies on the C-terminus of K_{ir} included residues that turned out to be in the TMD of K_{ir} and key to binding the 5′ inositol phosphate [6] (Fig. 2). Only with recent structural
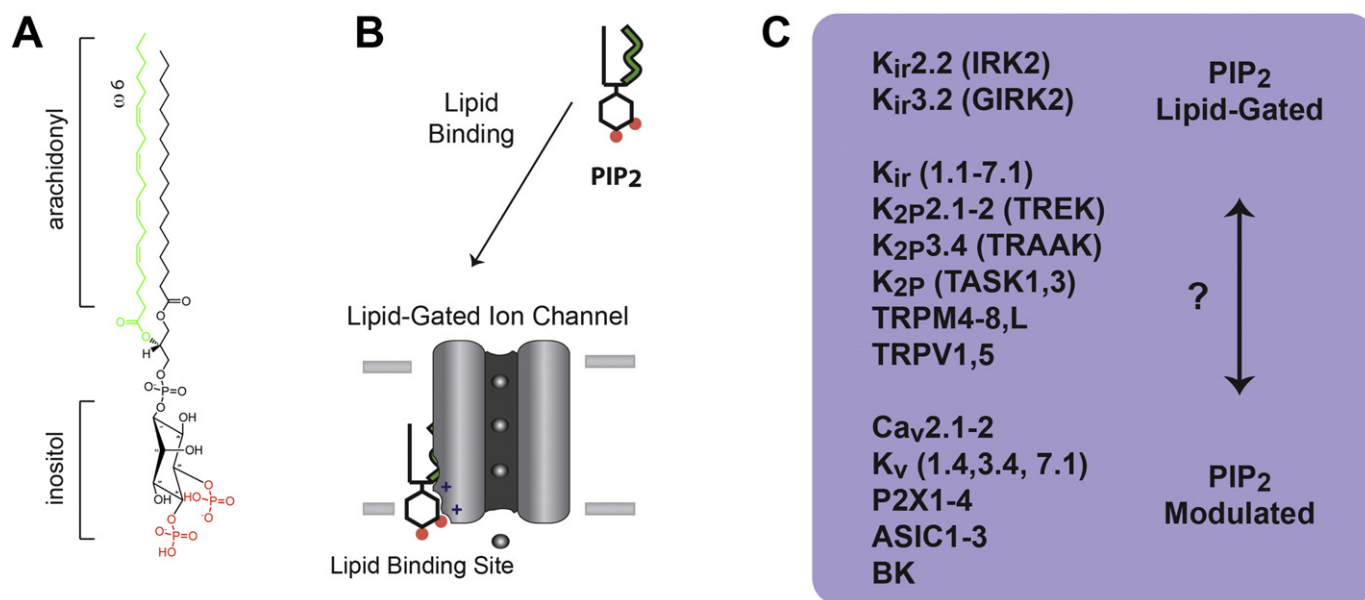
Fig. 1. PIP2 lipid regulation of ion channels. (A) The chemical structure of plasma membrane PIP2 is shown with an arachidonyl acyl chain (green) and inositol phosphates at the 4′ and 5′ position (red). (B) A cartoon representation of a PIP2 lipid-gated ion channel. PIP2 is shown bound to a lipid-binding site in the transmembrane domain of an ion channel. (C) List of ion channels with lipid gating properties. $K_{ir}2.2$ and 3.2 are the most clearly "lipid gated." A second group appears to be dual regulated, or "PIP2 modulated." PIP2 modulates channel gating, but gating also requires either voltage or a second ligand. A third group of channels behave similar to K_{ir} but await definitive proof of lipid gating vs. PIP2 modulation (?). The list of channels is exemplary and not comprehensive.
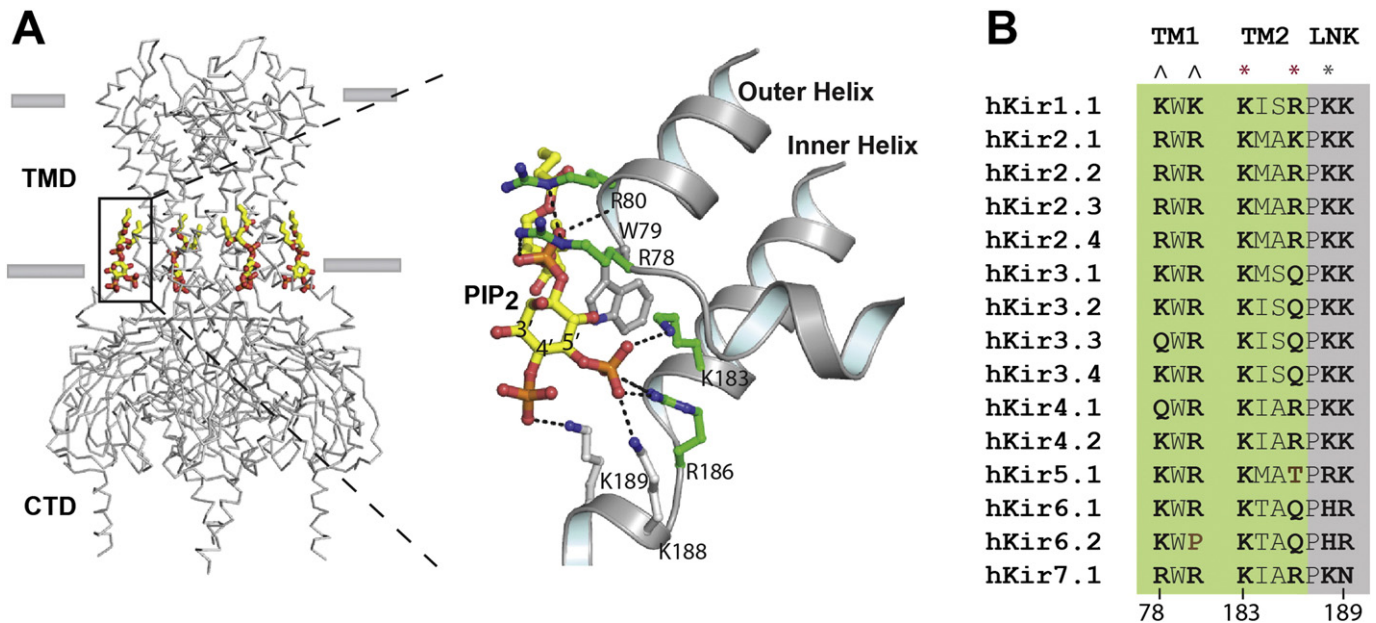
Fig. 2. Conserved PIP$_2$ binding site in K$_{ir}$2.2. PIP$_2$ binds the transmembrane domain (TMD) of K$_{ir}$ and causes a conformational change that allosterically gates the channel. (A) The PIP$_2$ binding site is specific for inositol 5′ phosphate. (B) A sequence alignment of all K$_{ir}$ family members reveals a highly structured PIP$_2$ binding site comprised of basic residues. Amino acid residues that directly contact PIP$_2$ are shown in bold type. Only two residues (brown type) at the conserved site lack a positive charge. Residues originating from the TMD and a linker (LNK) are shaded green and gray, respectively. ^ indicates residues that strongly coordinate the lipid backbone phosphate, and * indicates the residues that strongly (red) and weakly (gray) bind the PIP$_2$ 5′ phosphate. PIP$_2$ atoms are colored yellow for carbon, orange for phosphate, and red for oxygen. Amino acid side chains with carbons colored green are located on transmembrane outer helix 1 (TM1) or inner helix 2 (TM2). Lysines colored gray are located on the start of a linker helix (LNK) or "tether helix" connecting the transmembrane domain (TMD) and the cytoplasmic domain (CTD). Residue numbering is according to K$_{ir}$2.2.

data has a model emerged where lipids bind to specific sites in the transmembrane domain of ion channels [10,45–48].

2.3. Lipid-gating theory

Taken together, these finding suggest a ligand-gating theory of PIP$_2$ activation. In biochemistry, the term ligand refers to the reversible, specific, and dose-dependent binding of a substance to a protein to form a complex. Ligands include small molecule drugs, hormones, peptides, and metabolites. Normally, ligands stabilize at least two states, one bound and one unbound [49,50].

The binding of PIP$_2$ to K$_{ir}$ has many features of a ligand. First, PIP$_2$ is in low abundance [9,12]. This requires that PIP$_2$ bind with high affinity to its targets to exert an effect. Second, PIP$_2$ binds reversibly to ion channels in a dose-dependent manner [19,22]. Third, PIP$_2$ binds with specificity; for example, PI(4,5)P$_2$ activates K$_{ir}$2.1 and PI(3,4)P$_2$ inhibits the same channel [51]. This specificity is striking since the two lipids are chemical isomers and only differ in the position of the 5′ phosphate. Another anionic lipid, oleoyl-CoA, competitively and reversibly inhibits all K$_{ir}$'s [51] except K$_{atp}$, which is specifically activated by oleoyl-CoA [52,53]. Fourth, like neurotransmitter, PIP$_2$ is a dynamically regulated molecule [54,55]; a signaling cascade can rapidly change the concentration of PIP$_2$ to cause the channels to open or close [56–58]. And lastly, PIP$_2$ channel affinity determines channel function [59]. Mutations that allosterically decrease the affinity of PIP$_2$ cause disease (e.g., the Andersen–Tawil syndrome) [44,60].

The ligand-like characteristics of PIP$_2$ binding to the entire family of inward rectifiers warrant classification of these channels as ligand gated. The unique properties of lipids logically give rise to a lipid subclass suggested here "lipid-gated" ion channels.

3. The evolving view of PIP$_2$

3.1. Membrane surface charge theory

PIP$_2$ was first speculated to induce ion channel activation by non-specific avidity of negatively charged phospholipid binding to clusters of basic amino acids in the C-terminus of channels [2,5,8]. Anionic lipids were thought to accumulate on the inner leaflet and non-specifically attract positively charged residues on the surface of K$_{ir}$'s cytoplasmic domain (CTD). The rational for the theory is sound and was based on data from K$_{atp}$ (K$_{ir}$7.x) [20,61–63] and proteins like MARCKS [2,8]. However, in light of the PIP$_2$/K$_{ir}$ complexes, the previous role of electrostatic theory appears inadequate for K$_{ir}$. The glycerol backbone of PIP$_2$ bound tightly to the transmembrane domain (TMD), and the inositol phosphates interacted with residues in or proximal to the TMD, not the CTD. The original influential lack of K$_{atp}$'s specificity is an anomaly among K$_{ir}$'s and appears to be an adaptation that allowed regulation by oleoyl-CoA [19] and not a mechanistic requirement as speculated. If non-specific anionic interactions regulate K$_{ir}$, the site of anion lipid binding are likely distal to the canonical PIP$_2$ site [64] or act synergistically with PIP$_2$ [43,65] by binding to one of the 4 canonical sites. The notion that the cytoplasmic domain is the binding site for PIP$_2$ and that PIP$_2$ localizes the CTD similar to a PH domain appears to be incorrect. The K$_{ir}$2.2 CTD did move toward the membrane and may reflect an evolutionary origin; but the primary mechanism appears to be an allosteric conformational change, not non-specific electrostatic attractions of the CTD to the membrane surface. The key PIP$_2$ binding interactions were confirmed in a complex of PIP$_2$ with GIRK2 [47], suggesting a common mechanism in related K$_{ir}$ channels (Fig. 2B).

Voltage activated ion channels better exemplify non-specific electrostatic interaction. A well-studied domain called the "voltage sensor domain" (VSD) senses and responds to changes in surface charge [32, 46,66,67]. Conserved basic residues in the VSD electrostatically move towards the charge causing a conformational change that gates the channel (Fig. 3). The charge is non-specific and can be applied by external current or by changing the charge of lipids in the plasma membrane. The latter was shown in recent bilayers studies where K$_v$ responded symmetrically and non-specifically to anionic lipids [68]. The same study showed a distinct phosphatidic acid site in the cytoplasmic leaflet that specifically and dramatically affected K$_v$ gating [68]. This suggests both ligand and electrostatic modes can operate in the same channel, however the structural determinants of the two are likely distinct. A

similar arrangement exists in Ca_v2, which has a voltage sensor and a putative PIP_2 specific binding site [11,69].

Few other channels currently have sufficient molecular description to definitively discriminate the mechanism of action seen in K_{ir} and K_v. Many tetrameric channels exhibit a C-terminal charged cluster and varying degrees of specificity reminiscent of K_{ir}, including TRP [18,41,70–74], and P2X4 [75,76] (see Table 1). Typically, these charges immediately follow or are located in the last transmembrane domain. Many other channels respond to PIP_2 in ways that parallel K_{ir} responses, including Ca_v [69], NMDA [77], K_v [26], P2X1-3 [78] channels (see also Fig. 1C), but it is unknown if the interactions are direct with the TMD or indirect through membrane charge or other proteins. Since numerous soluble domains use polybasic clusters to target to the plasma membrane [79], some yet undefined cytoplasmic domains could utilize a membrane surface charge as previously speculated [2,8]. Future structural studies will continue to reveal the details and breadth of electrostatic theory.

3.2. Cofactor theory

Lipids are sometimes viewed as cofactors. Before discussing PIP_2 as a cofactor I must first define a cofactor and distinguish it from a ligand. The term cofactor stems from enzymology and generally refers to a permanent organic compound or metal that is required for the enzyme to function. A cofactor normally derives its function by remaining bound to a protein. In contrast, a ligand derives its function by binding and dissociating from its partner protein. Lipids have always existed in cells and it is reasonable to assume that some lipids may bind as cofactors. A crystal structure of K_v in a lipid like environment revealed phospholipid-binding sites near the voltage sensor and some of these appear to be lipid cofactors [46]. In other words, they facilitate the proper organization of the channel, but at present they do not appear to initiate a change in the channel state by dynamic regulation of the lipid.

In a speculative role, PIP_2 was proposed to act as a 'coincidence detector' in order to facilitate transport of an inactive channel [2,14,72,80]. A nascent channel in the endoplasmic reticule (ER), where PIP_2 is scarce, remains inactive until it arrives at the plasma membrane where an abundance of PIP_2 constitutively activates the ion channel. This fits well a definition of cofactor in the resting state. Directly demonstrating the physiological contribution remains a challenge since PIP_2 is dynamically regulated [2]. For example, the PLC hydrolysis of PIP_2 in the plasma membrane inhibits K_{ir} [57,58], a function also consistent with ligand-like properties.

In another speculative role, PIP_2 might function as a cofactor in sensing protons. The pK_a's of inositol phosphates are around 6.5 and 6.9, an optimal range for sensing physiological changes in proton concentration [81]. The lipid could remain bound and simply supply the metal phosphate as a proton sensing cofactor. Ions interacting with lipids were recently shown to regulate a receptor [82]. Acid-sensing ion channels (ASIC) are likely candidates for such a mechanism since they bind PIP_2 and sense protons. Alternatively, PIP_2 may serve as a proton-sensitive ligand. An atomic structure is known for ASIC [37], but the role of PIP_2 in channel activation requires further investigation.

Perhaps one reason for a slow adaptation of a "lipid-gating" model for PIP_2 is the fact that the prototypical PIP_2-gated channel K_{ir} is active during the resting state of excitable cells. These channels are often considered "constitutively active" leak channels. While it is true they allow potassium out of the cell during the resting state, the acetylcholine stimulation of M1 muscarinic receptor inactivates K_{ir} [57,58]. An early study on high-affinity $K_{ir}2.1$ in oocytes showed resistance to ACh inactivation [59], but later studies in mammalian cells demonstrated robust and complete inhibition of $K_{ir}2.1$ through activation of M1 receptor [58]. Thus, the neurotransmitter-induced closure of K_{ir} potassium channels is presumably synergistic with the opening of calcium, sodium, and voltage-gated channels and should result in a stronger action potential or sustained excitability.

4. Cellular regulation of PIP_2 agonism

The agonist properties of lipids broaden the cell-signaling role of PIP_2 regulation. Similar to neurotransmitter, the release, degradation, and localization of PIP_2 must govern ion channel function.

4.1. Lipid-mediated localization of PIP_2 in the plasma membrane

Phosphoinositides distributes heterogeneously in the plasma membrane [83–85]. Hydrophobicity causes lipids to partition (see Fig. 5). Saturated lipid chains partition into cholesterol-rich lipid rafts, often referred to as detergent-resistant membranes (DRMs). Lipids with unsaturation partition into the liquid-disordered phase (L_d). Mass spec of resting cells indicate that PIP_2 is comprised of a polyunsaturated fatty acyl chain [86–88] and localizes in the L_d region of the membrane [85]. Quantitative studies of PIP_2 suggest close to 85% of PIP_2 is polyunsaturated and 70% comprised of an arachidonyl acyl chain [88]. In contrast, PIP_3 is primarily comprised of saturated or monounsaturated lipid acyl chains [87]. Strikingly, arachidonyl PIP_3 was not detected in quiescent cells [87]. Based on standard lipid partitioning, the saturated PIP_3 is likely located in cholesterol rafts. In agreement with this arrangement, PI3 kinase (the enzyme that generates PIP_3 from PIP_2) localizes to lipid rafts [89]. Taken together, these data indicate an acyl chain-based localization of PIPs in the plasma membrane. Fig. 4 shows a hypothetical layout of the quiescent cell based on available, but limited, mass spec, super resolution imaging, and localization studies [85–88].

4.2. GPCR signaling through lipases

Famously, Gq-coupled GPCRs (guanine nucleotide-coupled receptors) hydrolyze PIP_2 through phospholipase C (PLC) activation. G-protein-mediated PIP_2 hydrolysis was known more than 30 years ago
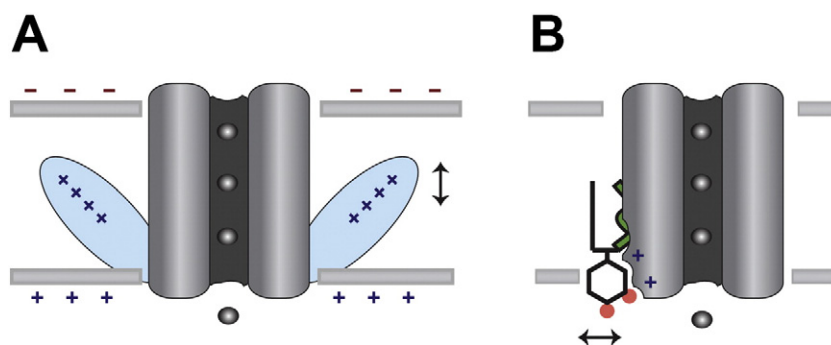
Fig. 3. Mechanistic comparison of surface charge gating vs. direct lipid gating. (A) Non-specific surface charge gates an ion channel through a charge sensor domain (blue). The vertical arrow indicates charge driven movement. (B) A lipid-gated channel reversibly binds the signaling lipid PIP_2 to allosterically gate the channel. A horizontal arrow indicates PIP_2 dissociation from the channel.

Table 1
Inositol phosphate ion channel specificity.

Channel	PIP$_2$ effect	Selectivity over	Comments	Ref
TRPM8	Activation*	PI(3,4)P$_2$ and PIP$_3$	5′ activates, 3′ inhibits	[18,42]
TRPV1	Mixed	PI(4)P and PIP$_3$	Likely acyl chain dependence	[41,70]
TRPM4	Activation	PI(4)P and PI(5)P	Modest selectivity over PIP$_3$	[74]
P2X4	Activation	PIP$_3$	Modest selectivity over PIP$_3$	[76]
TRPML	Inhibition	PI(3,5)P$_2$ (activation)	Direct competition of (3,5) with PI(4,5)P$_2$	[71,72]
Kir2.1,1.1	Activation*	PI(3,4)P$_2$ (inhibition)	Direct competition of (3,4) with PI(4,5)P$_2$	[19,51]
Kir3 (Girk2/4)	Activation*	PI(4)P	Gbg increases PI(4,5)P$_2$ binding	[19]

* PIP$_2$ is known to be necessary and sufficient for channel activation.

[90]. However, most cell biologist viewed (and many still do) PIP$_2$ as little more than a substrate for second messenger signaling [91]. This view is inadequate for K$_{ir}$ channels; PIP$_2$ must also be viewed as an ion channel activator [3,6] or agonist. Hence, the hydrolysis of PIP$_2$ by M1 muscarinic receptors should be viewed as a direct regulatory mechanism to deplete agonist. PIP$_2$ hydrolysis inactivates both high and low affinity K$_{ir}$ channels [57,58]. The downstream modulation of K$_{ir}$ by phosphatases and kinases appear secondary to this direct PIP$_2$ regulation [6, 92], a rational also supported by the central and highly conserved role of PIP$_2$ in channel activation as described above (2.5). The PLC regulation of Ca$_v$ [69], K$_{ir}$ [57], HCN [93], K$_v$7 [26], K$_{2P}$ [94], and TRP [95,96] channels (among others) is well documented.

In addition to PLC, GPCR signaling activates phospholipase D [97] (PLD). PLD produces PA and free choline. PA has emerged as an important signaling lipid [98]. PA and PIP$_2$ appear to synergistically activate K$_{ir}$ [43] and K$_{2P}$ [99] channels; in contrast, the nAChR [38] and some K$_v$ [68] respond specifically to PA and not PIP$_2$. A third important class of lipases phospholipase A2 (PLA$_2$) also exhibits GPCR regulation [100]. PLA$_2$ hydrolyzes arachidonyl-lipids creating lysophospholipids and arachidonic acid. Downstream and second messenger signaling are well studied for PLA$_2$ and PLC and include the arachidonic cascade and IP$_3$ second messenger signaling respectively. In comparison, the upstream role of the intact bioactive arachidonyl-phospholipids and PIP$_2$ is much less understood. Nonetheless, the added role of PIP$_2$ in directly gating ion channels solidifies a direct route for GPCR regulation of ion channels independent of downstream kinases and calcium signaling [6,92].

Several ion channels bind G-proteins directly, this role is widely accepted for the G-protein regulated inward rectifiers (GIRK/K$_{ir}$3.x) and N-type calcium channels (Ca$_v$2) [101]. A trimeric complex of GIRK with Gβγ (a G-protein) and PIP$_2$ revealed the GIRK/Gβγ interface [39]. And biochemical studies suggest that Gβγ is important for increasing binding of PIP$_2$ to GIRK [40]. The precise mechanism by which Gβγ enhances PIP$_2$ activation needs further clarification.

4.3. Protein-mediated localization of lipid modifying enzymes

Lipases localize with ion channels to increase the speed and specificity of PIP$_2$ channel gating [95,102]. For example, rhodopsin-activated PLC hydrolyzes PIP$_2$ opening TRPL channels. The colocalization of PLC with TRPL [95] allows for a fast 20 ms response time [103]. In addition, TRPM7 directly binds PLC to locally affect channel activation [92]. PLC functionally colocalized with NMDA receptors [77], and the IP3 receptor co-localizes with PLC to regulate calcium release [104]. PLD lipases directly localize to ion channels, including TRPM8 [105] and TREK-1 [106]. There are many subtypes of lipases; their diverse regulation and specific localization satisfies cells with the needed diversity for signaling.
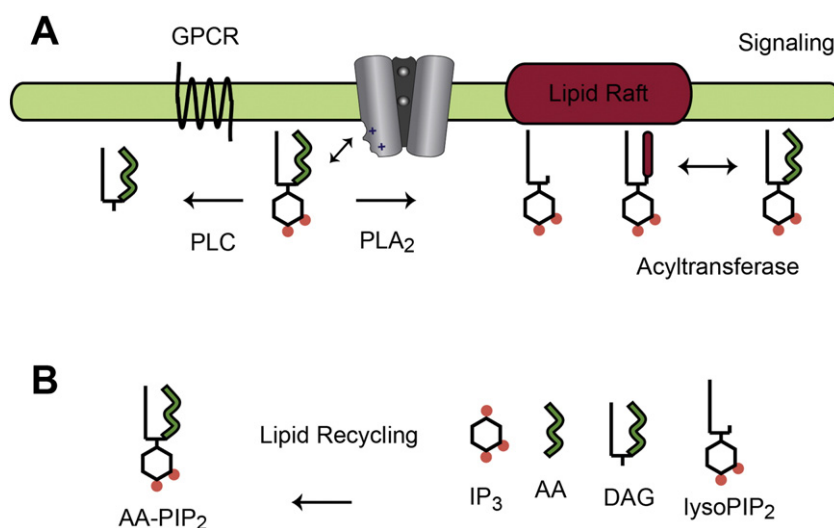
4.4. Transient PIP$_2$ signaling

The partitioning of PIPs and their modifying enzymes appears primed to deliver dynamic cell signaling. During a signaling event, G-proteins control PIP kinases, lipases, and phosphatases to degrade PIP$_2$ signaling. This signaling generates lipid degradation products (Fig. 5B). For example, it was shown PLC activation generates arachidonyl-diacyl-glycerol [88]. In addition, PLA$_2$ activation removes the arachidonyl sn2 acyl chain generating lysoPIP$_2$ [107]. In order to return to a resting state, degradation products need to be removed from the membrane and PIP$_2$ resynthesized.

Endocytosis recycles lipid micro domains and lipid rafts after signaling [108]. The late endosome and ER feed back into PIP$_2$ signaling. This postsynaptic lipid reuptake would then reset the membrane for another signaling event analogous to presynaptic neurotransmitter reuptake. Further studies are needed to understand the temporal and spatial regulation of PIP$_2$ in vivo in particular during a signaling event. However, signaling lipids are known to control the ion channel desensitization
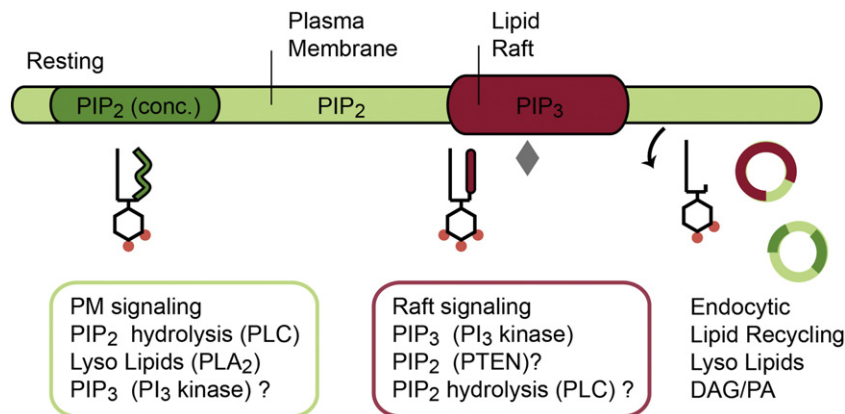
Fig. 4. Phosphoinositide (PI) partitioning in the plasma membrane. In the absence of a stimulus, arachidonyl-PIP$_2$ (green) localizes in the disordered region of the plasma membrane and sometimes in concentrated lipid micro domains (dark green) apart from cholesterol-rich lipid rafts (red). Inositol lipids are distributed according to their acyl chains; hence, saturated PIP$_2$ enters lipid rafts where PI3 kinase generates PIP$_3$. A saturated lysoPIP$_2$ may also associates with raft like domains. Key signaling enzymes (see colored boxes) appear localized in lipid micro domains where they are optimally positioned to remodel the PIP acyl chains and head groups during signaling. Lipid degradation products are found in endocytic vesicles, which suggest a lipid-recycling event analogous to recycling of some soluble neurotransmitters. Gray diamond represents PI3 kinase.

Fig. 5. PIP$_2$ transient signaling. (A) In the proposed model, PIP$_2$ dissociates from K$_{ir}$ and diffuses laterally in the plasma membrane. G-proteins activate lipid-hydrolyzing enzymes that deplete PIP$_2$ from the plasma membrane or laterally redistribute PIP$_2$ into distinct lipid micro domains (e.g., lipid rafts). Dynamic PIP$_2$ signaling gives rise to a transient inactivation of K$_{ir}$ that contributes to an action potential. (B) PIP$_2$ degradation products are taken up by endocytosis and PIP$_2$ resynthesis returns the cell to a resting state.

[18], voltage dependence [74], and recovery from inactivation [92], and these events correlate with ion channel rundown. Lipid regulated desensitization may prove to be a central function of many channel types. Much more data are needed to build a complete picture.

4.5. Other mechanistic considerations

Lipids localize topically by leaflets generating a lipid signal. For example, phosphatidylserine (PS) is found on the inner leaflet of the plasma membrane. Enzymes known as flippases and floppases move lipids between leaflets [109]. PS signals by flipping outside the cell [110]. PS is negatively charged and movement outside the cell has the ability to change the membrane surface charge from negative inside to negative outside. Recently, asymmetric changes to the charge of lipids in a bilayer dramatically shifted the voltage midpoint potential of a K$_v$ channel [68]. Hence, lipids may "flip" as a rapid mechanism to impose a lipid induced change on the cell membrane potential, a mechanism that would have likely preceded a synapse.

In a separate mechanism, lipid acyl transferases (LAT) could signal to ion channels by changing the unsaturation of a lipid acyl chain. LAT enzymes add acyl chains to lipids or move acyl chains between existing lipids [111]. If a LAT enzyme swaps an arachidonyl acyl chain with a saturated one, the signaling lipid would most likely translocate to a lipid raft (Fig. 5). This may simply sequester the signal away from the ion channel by moving the lipid into or out of a lipid micro domain. Alternatively, the translocation could make the lipid available to other modifying enzymes that would then deplete the signal from the membrane.

Alternatively, lipid acyl chains may directly contribute to gating of an ion channel. The acyl chains contain chemical diversity and putative specificity could determine the affinity of the lipid for the channel or cause a specific conformational change that gates the channel. Hydrophobic sites for lipid acyl chains affect PIP$_2$ activation of Ca$_v$2.2 [11].

The four identical binding sites in K$_{ir}$ are positioned for PIP$_2$ cooperatively and allosteric competition. Tetrameric channels engineered to have only one binding pocket indicated that one PIP$_2$ molecule is sufficient to activate the channel [65]. In wild-type channels with four binding sites, PIP$_2$ in combination with PA, PG, or PS dramatically increased channel conductance. However, absent PIP$_2$, these lipids failed to activate K$_{ir}$ [43]. A structure of K$_{ir}$ with PA bound showed PA binding to the canonical PIP$_2$ site [10], a site also compatible with PG and PS. In biochemical studies, oleoyl-CoA, an endogenous inhibitor, also competes directly with PIP$_2$ [51]. Taken together, these studies suggest that in K$_{ir}$ the lipid-binding site is always occupied, and K$_{ir}$ integrates the sum

total of the lipid environment in a cooperative way. At least one site must be occupied by PIP$_2$; the remaining three canonical sites appear to be available to exert cooperative activation or inhibition through a rigid conformational change [10] in the CTD. Thus, additional PIP$_2$ binding events are poised to activate K$_{ir}$ with increasing affinity consistent with electrophysiology recordings [65].

Lastly, the relative abundance of diet-derived fatty acids may affect the levels of PIP$_2$ signaling in the plasma membrane. Cells appear to incorporate the relative amounts of saturated and unsaturated fats into their cell membranes (phospholipids) [112]. It is tempting to speculate that diets with excess saturated fat would lead to saturated PIP$_2$ signaling, which most likely favors PIP$_3$ signaling. Diets with large amounts of polyunsaturated fats (PUFAs) would lead to more arachidonyl-PIP$_2$ and more PIP$_2$ signaling. This may account for the positive affect of dietary PUFAs on heart arrhythmias and insulin resistance since PIP$_2$ channels (including K$_{ir}$) are central to both these diseases. Consistent, with this model, loss of PIP$_2$ channel activation is associated with the disease states [60]. Similar speculation could be made of chronic pain and perhaps some cancers. Understanding PIP$_2$ acylation may shed light on these important medical problems.

5. The future of lipid ion channel interactions

5.1. Pharmacology of lipids

Better methods are needed for assaying lipid interactions with ion channels. Most studies rely on crude pharmacological shifts of PIP$_2$ concentrations in biological membranes; this is inadequate. Varying the concentration of lipids in a liposome is a good step in the right direction. Normally, one describes a ligand in terms of an on and off rate. Certainly, lipids have an affinity for ion channels, but we lack the methodology for effectively measuring lipid channel interactions. Better quantitative lipid-binding assays are needed. New mass spec techniques will likely allow for quantitative measurements of lipids in vivo and in vitro. And there is no doubt lipidomics will continue to find ways to improve the quantitative, temporal, and spatial identification of lipids in a membrane.

5.2. Implications on the plasma membrane

The plasma membrane holds thousands of lipids with functions that remain largely a mystery [113]. A catalog of lipid signals appears poised to exert exquisite regulation on membrane proteins perhaps rivaled

only by protein phosphorylation. Certainly, the phosphodiester bonds in lipids are equally suited for rapid signaling. And lipid acyl chains may be as diverse in function as they are in chemistry. Recognizing low abundant phospholipid signaling molecules as potential ligands for membrane proteins reveals a vast pool of putative effector ligands for cellular signaling.

6. Concluding remarks

The added role of PIP$_2$ activation presented here takes shape from the recent crystallographic K$_{ir}$ structures. The non-specific model of PIP$_2$ activating K$_{ir}$ is gradually making room for a PIP$_2$ site with specificity and ligand-like properties. How does a lipid–ligand influence its target molecule? It does so just like any other molecule; it binds in a concentration-dependent manner to a binding site and elicits a conformational change in the protein. While in hindsight this seems an obvious possibility, the plasma membrane has always been a little mysterious [114] and the understanding of membrane proteins slow in coming. No doubt lipid modulation of proteins is diverse with more surprises yet to come.

Conflict of interest

The author declares no conflict of interest.

Transparency Document

The Transparency document associated with this article can be found, in the online version.

Acknowledgments

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