Emerging complexities of lipid regulation of potassium channels

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Inwardly rectifying potassium (Kir) channels are important regulators of cellular excitability, enabling cells to alter electrical activity in response to diverse autonomic and metabolic signals (Hibino et al., 2010). Fairly or not, the Kir channel family is sometimes derided as the least complex or simplest of the eukaryotic potassium channels because they lack the widely studied transmembrane architecture of their voltage-gated cousins. However, there are many instances where discoveries about Kir channel regulation have presaged important changes in our understanding of electrical signaling mechanisms. For example, the demonstration that GIRK (G protein–coupled inwardly rectifying K⁺) channels are regulated by Gβγ subunits was among the first evidence that Gβγ can directly target effectors in a GPCR (G protein–coupled receptor)-initiated signaling cascade (Logothetis et al., 1987). Kir channels also led the way toward our current understanding of ion channel regulation by lipid environments and the notion that PIP₂ (phosphatidyl-inositol-4,5-bisphosphate) is an essential ion channel modulator. Although PIP₂ is now recognized as an important regulator of a large number of ion channel types, its effects on electrical signaling were first recognized in K₄, which is a well-defined interface (Tao et al., 2009; Hansen et al., 2011). The structural consequences of PIP₂ binding in a series of Kir3.2 structures are less pronounced than in Kir2.2, but these conformational changes around the channel gate highlight the importance of a cluster of positively charged residues that bind PIP₂ (Whorton and MacKinnon, 2011, 2013). From a functional perspective, it is important to note that, in most Kir channels, there is a strong preference for PI(4,5)P₂. Other combinations of inositol head group phosphorylation are often insufficient for function (Rohács et al., 2003), and other anionic phospholipids are ineffective substitutes for PI(4,5)P₂ in this canonical binding site.

Despite the fairly strict requirement for PIP₂, it has become increasingly clear that PIP₂ is not the only determinant of Kir channel activity and that lipid membrane composition can modulate PIP₂ effects. In a series of articles investigating the functional consequences of defined lipid environments on Kir2.x channels, a secondary lipid requirement for Kir2 channels was revealed, which is distinct from the canonical PIP₂ binding site described in crystal structures to date (Cheng et al., 2011; D’Avanzo et al., 2013; Lee et al., 2013). The initial observation leading to this hypothesis was that reconstituted Kir2.x channels required significantly different PIP₂ levels for activity, depending on whether the channel was reconstituted in a neutral lipid (e.g., with phosphatidylethanolamine [PE] or phosphatidylcholine [PC] head groups) versus supplementation with an anionic lipid (e.g., with a phosphatidylglycerol [PG] head

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The presence of 10–20% anionic lipid (abbreviated PL−) sensitized reconstituted Kir2.1 and Kir2.2 channels to PIP2 by roughly two orders of magnitude, shifting the PIP2 concentration–response curve to lower concentrations and markedly increasing channel activity in saturating PIP2 levels. The requirement for this secondary anionic phospholipid was shown to lack the strong specificity previously observed in the PIP2 binding site, as phosphatidylserine (PS), PG, PI, phosphatidic acid (PA), and cardiolipin lipids were all sufficient to sensitize channels to PIP2 (Cheng et al., 2011). Subsequent computational and mutagenic studies highlighted the importance of two residues on the intracellular side of the channel, distinct from the canonical PIP2 binding site, but lying near the interface between the slide helix and the CTD (Fig. 1; D'Avanzo et al., 2013; Lee et al., 2013). Lys62 and Lys219 were proposed to form an essential site in Kir2.2 where the channel interacts with anionic lipids, leading to the hypothesis that these residues are involved in maintaining a close association of the slide helix and CTD to the inner leaflet of the plasma membrane. This conformational constraint was predicted to influence the canonical PIP2 binding site and increase the apparent channel affinity for PIP2, generating positive allosteric coupling between the secondary nonselective PL− site and the recognized PIP2 binding site (Lee et al., 2013).

This suggestion of slide helix–mediated “tuning” of the PIP2 binding site has received direct support from acyl “tethering” experiments: cysteine mutation of Lys62 on Kir2.2 causes loss of function, but these silent channels can be rescued by modification with long-chain acyl-MTS (methanethiosulfonate) reagents. These decyl-MTS–modified Lys62Cys channels are intrinsically sensitized to PIP2 and exhibit no requirement for anionic phospholipids to generate PIP2 sensitivity that is comparable with WT Kir2.2 (Lee et al., 2013). The interpretation of these findings was that the acyl “tail” adduct on the Kir2.2 slide helix mimics the interaction of Lys62 with a phospholipid, helping to tether the slide helix to the membrane and thereby facilitating PIP2 binding. Importantly, acyl-MTS modification was only effective at the Lys62 position; acylation of Lys219Cys channels was not sufficient to sensitize channels to PIP2. The ability to rescue channel function by MTS tethering to the membrane was reminiscent of similar experiments on KirBac channels, in which certain cysteine mutations in the slide helix caused loss of function but could be rescued by long chain acyl-MTS modification (Enkvetchakul et al., 2007). The KirBac channels have notably different regulation by PIP2 (they are inhibited by PIP2; Enkvetchakul et al., 2005), but this likely highlights a common structural theme in which close orientation of the slide helix with the plasma membrane is required for Kir channel function.

In the present study, Lee et al. (2016) present an analysis of Kir2.2 carrying a targeted mutation at position Lys62, deliberately designed to bypass the secondary lipid requirement in these channels (Lee et al., 2016). Their findings nicely confirm a hypothetical basis for

Figure 1. Kir2.2 forms a defined slide helix–CTD interface in the presence of PIP2. (A and B) Previously published structures (PDB accession no. 3JYC [Tao et al., 2009] for A and 3SPI [Hansen et al., 2011] for B) of the Kir2.2 channel in the apo (A)- or PIP2-bound (B) state illustrate the location of the proposed auxiliary lipid binding residues (yellow, Lys62 and Lys219) along with other potentially stabilizing interactions (green, Asp81 and Arg218) that may form when the slide helix–CTD interface is engaged. PIP2 alone appears sufficient to drive formation of the interface, but the presence of an auxiliary lipid is suggested to alter the arrangement of the slide helix and PIP2 binding site.
the complex lipid dependence of Kir2 channels. As mentioned above, in the absence of PIP₂, the Kir2.2 channel structures exhibited a disengaged CTD, lacked a defined interface between the TMD and the CTD, and exhibited altered geometry of PIP₂-binding residues in the canonical PIP₂ site (Hansen et al., 2011). The Kir2.2[Lys62Trp] mutant was motivated by the idea that substitution with a Trp side chain might promote membrane association of the slide helix (Landolt-Marticorena et al., 1993; van der Wel et al., 2007) and mimic the decyl-MTS modification experiments in Kir2.2[Lys62Cys] channels. Indeed, in functional experiments using reconstituted liposomes, Lee et al. (2016) show that Kir2.2[Lys62Trp] channels exhibit strong PIP₂ sensitivity in the absence of PL⁻, which is comparable with WT Kir2.2 channel activity in 10% PG. This suggests that the mutation is working as predicted, bypassing the requirement for lipid binding in the secondary site.

Complementing this functional data are interesting structures of the Kir2.2[Lys62Trp] channel in apo- and PIP₂-bound forms. Unlike the previously reported “disengaged” apo-Kir2.2 structure, the Kir2.2[Lys62Trp] mutant exhibits a close assembly of the CTD and slide helix and is largely indistinguishable from the PIP₂-bound Kir2.2[Lys62Trp] channel structure. The authors interpret these findings as a demonstration that the Lys62Trp mutation mimics occupancy of the auxiliary binding site, bringing the slide helix into close proximity with the plasma membrane and tuning the PIP₂ binding site without the requirement for auxiliary lipid binding. In the structure, a maltoside detergent molecule seems to act as a surrogate for membrane lipids and forms a close association with the substituted Trp; this very likely contributes to the alternative slide helix conformation observed. It is also noteworthy that, although the PIP₂-bound Kir2.2[Lys62Trp] structure resembles the previously published PIP₂-bound WT Kir2.2, the CTD and TMD are arranged even more closely in the Lys62Trp mutant, and several small differences are apparent in the modeled arrangement of PIP₂ bound to its canonical site. These findings are consistent with the proposal that the conformation of the slide helix acts to shape the PIP₂ binding site and alter subtle details of PIP₂ binding.

Within the greater context of PIP₂ regulation of Kir channels, this study highlights the importance of peripheral regulators in tuning PIP₂ sensitivity of channels. Importantly, the conserved residues that underlie the auxiliary lipid binding site are absent in most Kir subtypes. Only some members of the Kir2 family (Kir2.1, 2.2, 2.3), along with Kir4.1, possess a lysine in an equivalent position to Lys62 in Kir2.2. However, there is considerable variability within this channel region (making confident alignment somewhat challenging), and many channels have one or more positively charged residues in this initial region of the slide helix. At the 219 position, a lysine residue is more common (found in Kir1.1, 1.2, and 1.3; Kir2.1, 2.2, and 2.3; Kir4.1; Kir6.1 and 6.2; and Kir7.1), suggesting a more general role for the Lys219 equivalent residue. A theme that emerges from this inspection of sequences is that the auxiliary lipid binding site is generally apparent in channels that are not subject to other mechanisms of regulation. For example, the essential residues are not present in the Gβγ-sensitive Kir3 family channels, nor the Kir6 channels that assemble constitutively with sulfonlyurea receptors to form K_{ATP}. Thus, one possibility is that as these other regulatory mechanisms developed, the requirement for an auxiliary lipid binding site may have been lost. Assembly with auxiliary subunits may enable formation of a stable interface between the CTD and slide helix/TMD. Also, one might imagine that dynamically regulated channels like K_{ATP} or GIRKs require specifically timed, ligand-dependent activation to fulfill their physiological role, and so they may have evolved to respond to extrinsic signals independently of the proposed auxiliary lipid binding site.

It is interesting to consider why the lipid tethering approach can effectively rescue the Lys62Cys mutation but not Lys219Cys. An explanation that seems consistent with the data presented is that Lys219 may not be an essential mediator of lipid binding, but rather acts downstream of lipids binding to Lys62 to transduce conformational effects to the CTD and PIP₂ binding site. It is striking that Lys219 is directly adjacent to Arg218, which, along with Asp81, forms a very highly conserved residue pair (Li et al., 2013). Inspection of channel structures reveals that these two residues lie very close to one another, sandwiched between the top of the CTD and the slide helix, although it is not always apparent that they form a close ionic interaction (Fig. 1). One might envisage that nonspecific auxiliary lipid binding to Lys62 contributes to membrane association of the slide helix, whereas the Asp81-Arg218 pair, along with Lys219, may help to stabilize the interface between the CTD and slide helix.

An important implication of this study is that many existing crystal structures may not reflect the influence of functionally important lipids on channel structure. In this study, the presence of a maltoside near the Lys62Trp side chain is suggested to have an important influence on the position of the slide helix (and PIP₂ binding site), in a manner that is consistent with the functional effects of the mutation. However, the facilitating Lys62Trp mutation is required to sample these alternative, and possibly important, configurations of the slide helix and domain interface. The inference is that an adequate lipid binding partner for the native Lys62 was not present in previously reported Kir2.2 structures, potentially influencing important structural details of the channel. A growing amount of structural work has described lipids or detergent molecules associated with ion channels (Long et al., 2007),
but it is unclear whether these reproduce functionally important lipid interactions similar to those identified for Kir2.1 channels. Ongoing detailed functional characterization of lipid effects on channel function (often requiring challenging purification and reconstitution of channels into bilayers of defined composition) may help to inform future experimental conditions for structural determination. Recent advances in cryo-EM techniques are compatible with structure determination of channel proteins embedded in nanodiscs of defined lipid composition. Engineering nanodiscs to reflect the native lipid environment of an ion channel may be of great benefit for recapitulating functionally important states that have not yet been described by structural studies and might have the added benefit of bypassing the need to use channel mutants to mimic certain lipid interactions.

Moving forward, this series of papers highlights many important questions that will help us navigate the complexities of ion channel regulation by their neighboring lipids. Historically, ion channel genes and sequences have been fairly easy to manipulate, whereas we have been at the behest of experimental expression systems that recapitulate functionally important states that have not been at the behest of experimental expression systems to determine ambient lipid environments. This experimental shortcoming is highlighted by a growing number of studies systematically investigating functional relationships between ion channels and their various potential lipid partners (Cheng et al., 2011; Hite et al., 2014). Thus, we are now in a position to ask the daunting questions of what lipid interactions might influence channel gating or the stability of certain channel states, whether lipid composition has generally important effects on other channel types, and what physiological roles these regulatory interactions may have.

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