PIP_2 PIP_2 Hooray for Maxi K^+

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Voltage-gated K^+ (K_V) channels contain a voltage-sensing transmembrane segment that moves in response to changes in membrane potential. A small "gating" current arises from the translocation of positively charged residues within the channel's voltage sensor. These gating currents occur at the microsecond timescale (Gilly and Armstrong, 1980), indicating a highly ordered translocation (or reorganization) of the voltage sensors within the membrane. These initial events trigger additional, complex conformational changes that open, close, or inactivate the channel, thereby providing distinct kinetics to different types of K_V channels (Benzanilla, 2008). Initial x-ray crystallography data of a mammalian K_V chimeric channel provided the first glimpse of the protein organization of a K_V channel when associated with phospholipid in a mixed phospholipid/detergent environment (Long et al., 2007). However, in vivo, K_V channels are surrounded by a complex sea of lipids. How do these lipids interact with channels, where are critical sites of interaction, and how do they influence voltage sensing and gating? Answers to these important questions are beginning to be provided by x-ray crystal structures of pore regions, voltage sensors, and cytoplasmic domains of several K^+ channels, in combination with biophysical, pharmacological and mutagenesis studies (Jiang et al., 2002; Kuo et al., 2003; Long et al., 2005; 2007; Logothetis et al., 2007; Lundbæk, 2008; Tucker and Baukrowitz, 2008).

A consensus is emerging that the anionic phospholipid, phosphatidylinositol-4,5-bisphosphate (PIP_2) has physiologically important interactions with many K_V channels and other transmembrane proteins, including ion transporters and ligand-gated K^+ channels. In an explosion of interest for this idea, a rapid succession of studies identified a number of K^+ channels that are positively regulated by PIP_2, including all members of the Kir family of inward rectifiers (Sui et al., 1998; Liou et al., 1999; Zhang et al., 1999; Enkvetchakul et al., 2007; Logothetis et al., 2007), as well as voltage-gated KCNQ channels (Suh and Hille, 2002; Loussouarn et al., 2003; Zhang et al., 2003) and delayed rectifier K^+ channels (Oliver et al., 2004). Among the reasons for the interest in PIP_2 regulation of channels is that a number of G_q-coupled receptors that stimulate PIP_2's breakdown also modulate K^+ channel activity, most likely by decreasing the PIP_2 available to associate with channels (Xie et al., 1999; Cho et al., 2001; Suh and Hille, 2002).

Hilgemann and Ball (1996) made the initial observation that PIP_2 positively regulates the cardiac Na^+/Ca^{2+} exchanger. Addition of ATP to the cytoplasmic side of inside-out (I/O) giant patches of cardiac myocyte membrane increased the Na^+/Ca^{2+} exchange current; in patches pretreated with a bacterial PLC that selectively metabolizes phosphatidylinositol (PI), ATP no longer was able to enhance the current. ATP's stimulatory action recovered if PI was added to the bath before ATP; PI by itself had no effect, whereas PIP_2 alone stimulated current in patches pretreated with PLC. These seminal findings suggested that phosphorylation of PI produced PIP_2, which then directly interacted with the exchanger to stimulate its activity. To test their model, Hilgemann and Ball (1996) exposed ATP-stimulated patches to a PIP_2-selective PLC, and found that current returned to pre-ATP levels, a result consistent with PIP_2's actions underlying increased exchanger activity. Using similar approaches, they found an analogous stimulation of K_{ATP} channel activity with conditions that increased endogenous PIP_2 and decreased activity under conditions that decreased PIP_2 levels. Lastly they detected a possible increase in K^+ inward rectifier (Kir) current. Soon after, Fan and Makielski (1997) reported for the first time the potentiation of Kir current by PIP_2, a finding confirmed by Hilgemann's group (Huang et al., 1998). These early studies suggested that the requirement for PIP_2 to elicit channel opening might be a broad principle for a number of K^+ channels.

The same approach that implicated PIP_2 as a regulator of Na^+/Ca^{2+} exchangers and K_{ATP} channels led to negative outcomes in studies on the large conductance, Ca^{2+}-activated K^+ channel (also known as slo1, maxi-K^+, or BK channel), in skeletal muscle and cardiac myocytes (Hilgemann et al., 2001; Hilgemann, D.W., personal communication). Throughout the body, maxi-K^+ channels integrate intracellular Ca^{2+} signals with changes in membrane potential, often providing an important negative feedback mechanism that limits depolarization-driven Ca^{2+} influx. Previous findings demonstrated that metabolic products of PIP_2 and downstream targets

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Abbreviations used in this paper: I/O, inside-out; PIP_2, phosphatidylinositol-4,5-bisphosphate.

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such as PLC, ryanodine receptors, kinases, and free fatty acids modulate BK channel activity (Dopico et al., 1994; Kirber et al., 1992; Salkoff et al., 2006). From beautifully executed experiments, Alex Dopico and his colleagues (see article by Vaithianathan et al., p. 13) now provide evidence that PIP_2 can indeed interact with BK channels, adding another type of K^+ channel to the ever-growing list of PIP_2-sensitive channels. However, in order to observe enhanced activity, BK channels must co-express with a specific accessory β subunit giving rise to tissue-specific PIP_2 effects.

BK channels arise from a single mammalian gene (*KCNMA1*) with multiple splice sites providing for diverse expression patterns and differing biophysical properties (Salkoff et al., 2006). Not surprisingly, BK channels differ in topological organization from the conventional K_V channels, which are comprised of six transmembrane segments (S1–S6) with both N and C termini located intracellularly. Distinct regions within S1–S4 (S3b and S4 in particular) appear responsible for voltage sensing while a P-loop spans segments S5 and S6 to contribute to the channel's pore (Long et al., 2005; 2007). Four of these subunits form a functional channel that has a central pore and four voltage sensors. BK channel structure deviates in several ways from this organization. First, the BK channel has an additional S0 segment preceding S1–S6 that results in the N terminus ending extracellularly. S0 may interface with voltage-sensing areas, introducing additional regulation of voltage sensor movements (Liu et al., 2008). Second, each S0 interacts with an accessory β subunit that contains two transmembrane segments. Encoded by distinct genes, expression of different β subunits (β1–β4) in different tissues adds complexity to BK gating, kinetics, and pharmacology. Third, as the name indicates, this Ca^{2+}-sensitive maxi-K^+ channel opens in response to rises in intracellular Ca^{2+} levels, membrane depolarization, or a combination of the two. BK's long C terminus tail contains a variety of functional domains involved in divalent sensing, such as the Ca^{2+} bowl, and two RCK (regulators of conductance of K^+) domains, conferring Ca^{2+} sensitivity to the channel, yet the molecular underpinnings are not fully understood (Salkoff et al., 2006).

To test whether PIP_2 may interact directly with BK channels, Dopico and colleagues performed several experiments in the presence of basal levels of Ca^{2+} (300 nM), where exogenous PIP_2 robustly and reproducibly increased steady-state activity (NP_o) of native BK channels in I/O patches from freshly isolated cerebral vascular myocytes, with no changes in the unitary current amplitude. In contrast, little change in NP_o occurred following extracellular PIP_2 application to cell-attached or outside-out patches, indicating that the putative site of interaction is most accessible from the inner leaflet, as would be expected because that is where endogenous PIP_2 is found (Laux et al., 2000). The authors then used a dizzying array of pharmacological conditions to examine the effect of endogenous PIP_2 on BK current in I/O patch and in perforated-patch recordings where the myocytes remained intact. Every experiment that favored increased endogenous PIP_2 levels yielded increased channel activity. Dopico and colleagues then took the investigation a further step. They pressurized endothelium-free cerebral arteries that develop myogenic tone in order to determine whether changes in PIP_2 levels could alter vessel diameter. Under conditions that minimized PIP_2 metabolism, arterial diameter significantly increased, indicating that elevated levels of PIP_2 increase BK channel activity. The resulting increase in BK-driven, outward current then hyperpolarizes myocyte membrane, promoting muscle relaxation and vessel dilation. They confirmed this hypothesis by showing that when BK activity was blocked with paxilline, arteries no longer relaxed under conditions that increased PIP_2 levels. This in vivo demonstration is the first report documenting the consequences of a PIP_2 interaction with an ion channel on organ function.

Having demonstrated physiological significance for PIP_2's actions on BK channel activity, the authors examined the structural requirements for increased channel activity. They found that more water soluble forms of PIP_2 (diC4 and diC8) also increased NP_o but to a lesser degree than the longer-chain form, and reversed with washout more easily than PIP_2. This result suggested that the longer fatty acid tails on the one hand facilitate increased partitioning of PIP_2 into the lipid environment and hence access to channels and, on the other, slow the off rate from the bilayer to the aqueous phase. Additionally, the authors found that channel activation positively correlated with the number of anionic head group charges (−1 to −4). Moreover, when they included PIP_2 antibodies or the anion scavenger poly-L-lysine along with PIP_2, the normally robust increase in NP_o was significantly reduced. Lastly, the authors tested whether 1,2-dipalmtoyl-*sn*-glycero-3-phospho-L-serine (PS) versus 1,2-dipalmtoyl-*sn*-glycero-3-phosphoinositol (PI) are equally effective in increasing BK activity. Both phospholipids were matched for charge (−1) and "flavor" of fatty acid tails. PI increased NP_o 2.5 times greater than PS. This finding is especially important since it indicates that not only is charge critical, but so too is the structure of the moiety in the *sn*-3 position of the phospholipid. Taken together, the structural specifications for BK activation, which include the importance of long-chain fatty acid tails, the phospho-moiety in the *sn*-3 position, and the number of negative charges are consistent with the negatively charged phosphoinositol head group directly interacting with a specific binding site on the channel.

From these findings with native BK channels in cerebral vascular myocytes, Dopico and colleagues turned to the oocyte heterologous expression system to search

for a potential PIP_2 binding site in recombinant BK channels, with surprising results. Following expression of cvb1 (encoded by *KCNMA1)*, a BK splice variant prevalent in cerebral artery myocytes (Liu, J., P. Liu, M. Asuncion-Chin, and A. Dopico. 2005. *Soc. Neurosci. Abstr. Online*. 960.913), PIP_2 robustly increased the current recorded from I/O patches, establishing that cbv1, together with its immediate lipid environment, was sufficient for PIP_2 to enhance current. They then identified a three–amino acid sequence (RKK) in the S6–S7 cytosolic linker of cbv1 that, when mutated to AAA, blunted increases in NP_o following PIP_2 application, indicating that this sequence may serve as a site of interaction with PIP_2. In contrast, mutation of a positively charged amino acid (K239A), located in the S4–S5 cytosolic loop, had no effect on cvb1 sensitivity to PIP_2. Taken together, these studies demonstrate that loss of PIP_2's actions does not depend on all positive residues contained within cytoplasmic loops, but rather, on specific positive residues in the S6–S7 segment. Moreover, current enhancement by PIP_3 occurred in wt and K239A mutant but not in the RKK to AAA substitution, indicating that PIP_2 and PIP_3 act at a common site of action.

Cvb1's RKK sequence is most likely a PIP_2 interaction site because it shares certain characteristics with PIP_2 binding sites in other K^+ channels, which normally contain at least two (though often more than five) positively charged residues (arginine and lysine). At least one of the residues must be arginine. As with cvb1, critical sequences for PIP_2 binding have been located to the proximal portion of the C-terminal tail of bacterial and mammalian Kir channels (Huang et al., 1998; Shyng and Nichols, 1998; Logothetis et al., 2007), KCNQ1, the subunit that confers PIP_2 sensitivity to KCNQ1/KCNE1 channels (Loussouarn et al., 2003), and KCNQ2, the subunit that when coexpressed with KCNQ3 gives rise to M-current (Zhang et al., 2003). For certain channels, additional positively charged residues in the proximal N terminus also confer channel sensitivity to PIP_2. Interestingly, the RKK mutation in cbv1 decreased but did not ablate BK channel sensitivity to PIP_2, raising the possibility that additional nearby residues participate in PIP_2 binding and/or additional sites of interaction mediate further enhancement; these sites await future discovery.

Analysis of macroscopic currents showed that PIP_2 causes a parallel leftward shift in voltage sensitivity of activation without altering the effective valence of BK channels. Thus the negative charge of the PIP_2 head group does not appear to alter critical aspects of voltage gating. Further analysis of unitary gating kinetics by the authors revealed increases in both open and closed dwell time distributions caused by PIP_2 amplification of Ca^{2+}_i-driven gating. Indeed, when channels were opened with voltage in the absence of Ca^{2+}, PIP_2 had little effect on BK NP_o, whereas in the presence of 300 nM or greater, PIP_2 robustly enhanced NP_o. Another observation highlights the importance of PIP_2 control of Ca^{2+}-driven gating. The authors found that increases in cbv1 channel NP_o following PIP_2 was markedly less than that observed in I/O patches from cerebral artery myocytes. One notable difference between the two sets of experiments was the absence of β1 subunits in the heterologous expression system, whereas cvb1 tightly associates with β1 subunits in cerebral vascular myocytes (Liu et al., 2005; Salkoff et al., 2006). Therefore, the authors tested whether β1 might potentiate PIP_2's actions when coexpressed with cvb1. As suspected, PIP_2's actions now recapitulated the remarkably large increase in NP_o of vascular myocyte BK channels. In contrast, coexpression of β4 had no potentiating effect. Native BK channels in skeletal myocytes, cells known to barely express β1, responded to PIP_2 similarly to homomeric slo1 consistent with β1, but not β4, facilitating PIP_2's effects. These findings add a new wrinkle to PIP_2 actions on channels where the accessory β1 subunit, known to robustly increase the apparent Ca^{2+} sensitivity of the BK channel complex, confers an additional amplification mechanism that controls the final effect of PIP_2.

Though increasing evidence indicates that PIP_2 regulates channel availability and P_o of other K^+ channels, how this occurs remains ill defined. Nonspecific deformation in lipid packing can alter channel gating (Lundbæk, 2008). However, the first x-ray crystal structures of the initial shell of lipid surrounding a chimeric voltage-sensing paddle yielded evidence that lipids may alter K^+ channel conformational changes due to specific electrostatic interactions (Schmidt et al., 2006; Long et al., 2007). In particular, the finding of a phospholipid "wedged" between the voltage sensor and the S4–S5 linker, a domain of the channel that couples voltage sensor movement to S5–S6 channel gating (Long et al., 2007), suggests specific molecular interactions between individual amino acid residues and phospholipid molecules. The specific phospholipids interacting with the channels in mammalian cell membranes remain unidentified. However, molecular modeling of PIP_2 with Kir 3.4 (Logothetis et al., 2007) or Kir 6.2 (Haider et al., 2007) find PIP_2 localized to a location comparable to the phospholipid "wedge" between the S4–S5 linker in the crystal structures (Long et al., 2007). Similarly, PIP_2's binding to the proximal region of the BK channel's cytoplasmic tail is consistent with an analogous orientation of the phosphoinositol head, embedded in BK's large tail region like an anchor; PIP_2's long hydrophobic fatty acid tails serve as tethers by extending into transmembrane regions of the channel to influence gating. Whether additional molecular interactions of the maxi-K^+ channel (perhaps via β_1 subunit and Ca^{2+}) reorient PIP_2 within the bilayer and increase coupling between voltage sensors and gating regions within S5–S6 remains to be tested.

In summary, the results of Dopico and colleagues (Vaithianathan et al., 2008) provide an important addition to our understanding of the role of PIP_2 in regulating transmembrane proteins. The similar changes occurring across multiple preparations make it unlikely that PIP_2's interaction with the proteolipid environment around the BK channel complex indirectly mediates changes in channel gating. More likely the cbv1 + β1 complex is sufficient to support channel activation by PIP_2. The fact that a specific β subunit appears to confer endogenous PIP_2 sensitivity to a specific BK channel complex, which exhibits tissue-specific coexpression, strongly suggests that modulation by PIP_2 is physiologically significant. In particular, the novel action of PIP_2 in increasing channel sensitivity to Ca^{2+}-dependent regulation of gating is reminiscent of PIP_2's ability to increase sensitivity to G-protein subunits and Na^+ binding observed with other K^+ channels (Logothetis et al., 2007). The experimental findings in this report advance the importance of PIP_2's interaction with the cytoplasmic region following S6 in a number of K^+ channels.

REFERENCES

Benzanilla, F. 2008. How membrane proteins sense voltage. *Nat. Rev. Mol. Cell Biol.* 9:323–332.

Cho, H., G.B. Nam, S.H. Lee, Y.E. Earm, and W.K. Ho. 2001. Phosphatidylinositol 4,5-bisphosphate is acting as a signal molecule in α1-adrenergic pathway via the modulation of acetylcholine-activated K^+ channels in mouse atrial myocytes. *J. Biol. Chem.* 276(1):159–164.

Dopico, A.M., M.T. Kirber, J.J. Singer, and J.V. Walsh Jr. 1994. Membrane stretch directly activates large conductance Ca^{2+}-activated K^+ channels in mesenteric artery smooth muscle cells. *Am. J. Hypertens.* 7:82–89.

Enkvetchakul, D., I. Jeliazkova, J. Bhattacharyya, and C.G. Nichols. 2007. Control of inward rectifier K channel activity by lipid tethering of cytoplasmic domains. *J. Gen. Physiol.* 130:329–334.

Fan, Z., and J.C. Makielski. 1997. Anionic phospholipids activate ATP-sensitive potassium channels. *J. Biol. Chem.* 272:5388–5395.

Gilly, W.F., and C.M. Armstrong. 1980. Gating current and potassium channels in the giant axon of the squid. *Biophys. J.* 29:485–492.

Haider, S., A.I. Tarasov, T.J. Craig, S.P. Sansom, and F.M. Ashcroft. 2007. Identification of the PIP2-binding site on Kir6.2 by molecular modelling and functional analysis. *EMBO J.* 26:3749–3759.

Hilgemann, D.W., and R. Ball. 1996. Regulation of cardiac Na^+,Ca^{2+} exchange and KATP potassium channels by PIP_2. *Science.* 273:956–959.

Hilgemann, D.W., S. Feng, and C. Nasuhoglu. 2001. The complex and intriguing lives of PIP2 with ion channels and transporters. *Sci. STKE.* 111:RE19.

Huang, C., S. Feng, and D. Hilgemann. 1998. Direct activation of inward rectifier potassium channels by PIP_2 and its stabilization by Gβγ. *Nature.* 391:803–806.

Jiang, Y., A. Lee, J. Chen, V. Cadene, B.T. Chait, and R. MacKinnon. 2002. Crystal structure and mechanism of a calcium-gated potassium channel. *Nature.* 417:515–522.

Kirber, M.T., R.W. Ordway, L.H. Clapp, S.M. Sims, J.V. Walsh Jr., and J.J. Singer. 1992. Both membrane stretch and fatty acids directly activate large conductance Ca^{2+}-activated K^+ channels in vascular smooth muscle cells. *FEBS Lett.* 297:24–28.

Kuo, A., J.M. Gulbis, J.F. Antcliff, T. Rahman, E.D. Lowe, J. Zimmer, J. Cuthbertson, F.M. Ashcroft, T. Ezaki, and D.A. Doyle. 2003. Crystal structure of the potassium channel KirBac1.1 in the closed state. *Science.* 300:1922–1926.

Laux, T., K. Fukami, M. Thelen, T. Golub, D. Frey, and P. Caroni. 2000. GAP43, MARCKS, and CAP23 modulate PI(4,5)P(2) at plasmalemmal rafts, and regulate cell cortex actin dynamics through a common mechanism. *J. Cell Biol.* 149:1455–1472.

Liou, H.H., S.S. Zhou, and C.L. Huang. 1999. Regulation of ROMK1 channel by protein kinase A via a phosphatidylinositol 4,5-bisphosphate-dependent mechanism. *Proc. Natl. Acad. Sci. USA.* 96:5820–5825.

Liu, G., S.I. Zakharov, L. Yang, S.X. Deng, D.W. Landry, A. Karlin, and S.O. Marx. 2008. Position and role of the BK channel α subunit S0 helix inferred from disulfide crosslinking. *J. Gen. Physiol.* 131:537–548.

Logothetis, D.E., T. Jin, D. Lupyan, and A. Rosenhouse-Dantsker. 2007. Phosphoinositide-mediated gating of inwardly rectifying K^+ channels. *Pflugers Arch.* 455:83–95.

Long, S.B., E.B. Campbell, and R. Mackinnon. 2005. Crystal structure of a mammalian voltage-dependent *Shaker* family K^+ channel. *Science.* 309:897–903.

Long, S.B., X. Tao, E.B. Campbell, and R. MacKinnon. 2007. Atomic structure of a voltage-dependent K^+ channel in a lipid membrane-like environment. *Nature.* 450:376–382.

Loussouarn, G., K. Park, C. Bellocq, I. Baro, F. Charpentier, and D. Escande. 2003. Phosphatidylinositol-4,5-bisphosphate, PIP2, controls KCNQ1/KCNE1 voltage-gated potassium channels: a functional homology between voltage-gated and inward rectifier K^+ channels. *EMBO J.* 22:5412–5421.

Lundbæk, J.A. 2008. Lipid bilayer–mediated regulation of ion channel function by amphiphilic drugs. *J. Gen. Physiol.* 131:421–429.

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

Salkoff, L., A. Butler, G. Ferreira, C. Santi, and A. Wei. 2006. High-conductance potassium channels of the SLO family. *Nat. Rev. Neurosci.* 7:921–931.

Schmidt, D., Q.X. Jiang, and R. MacKinnon. 2006. Phospholipids and the origin of cationic gating charges in voltage sensors. *Nature.* 444:775–779.

Shyng, S.L., and C.G. Nichols. 1998. Membrane phospholipid control of nucleotide sensitivity of KATP channels. *Science.* 282:1138–1141.

Suh, B.C., and B. Hille. 2002. Recovery from muscarinic modulation of M current channels requires phosphatidylinositol 4,5-bisphosphate synthesis. *Neuron.* 35:507–520.

Sui, J.L., J. Petit-Jacques, and D.E. Logothetis. 1998. Activation of the atrial KACh channel by the βγ subunits of G proteins or intracellular Na^+ ions depends on the presence of phosphatidylinositol phosphates. *Proc. Natl. Acad. Sci. USA.* 95:1307–1312.

Tucker, S.J., and T. Baukrowitz. 2008. How highly charged anionic lipids bind and regulate ion channels. *J. Gen. Physiol.* 131:431–438.

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

Xie, L.H., M. Horie, and M. Takano. 1999. Phospholipase C-linked receptors regulate the ATP-sensitive potassium channel by means of phosphatidylinositol 4,5-bisphosphate metabolism. *Proc. Natl. Acad. Sci. USA.* 96:15292–15297.

Zhang, H., C. He, X. Yan, T. Mirshahi, and D.E. Logothetis. 1999. Activation of inwardly rectifying K^+ channels by distinct PtdIns(4,5)P2 interactions. *Nat. Cell Biol.* 1:183–188.

Zhang, H., L.C. Craciun, T. Mirshahi, T. Rohács, C.M. Lopes, T. Jin, and D.E. Logothetis. 2003. PIP_2 activates KCNQ channels, and its hydrolysis underlies receptor-mediated inhibition of M currents. *Neuron.* 37:963–975.