Dynamic changes in phosphoinositide levels control ion channel activity

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In the last decade, following the initial reports by Hilgemann and Ball (*Science* 273:956–959, 1996) and Fan and Makielski (*J. Biol. Chem.* 272:5388–395, 1997), dynamic changes in the levels of plasma membrane phosphoinositides have been shown to control the activity of most ion channels and transporters. This form of regulation of the activity of membrane proteins complemented the well-studied phosphoinositide control of the localization of cytosolic proteins, such as in vesicular trafficking, cytoskeletal remodeling, recruitment to the plasma or to the nuclear membranes. In this special issue, we first examine phosphorylation and dephosphorylation of phosphoinositides, as well as functional consequences of such modifications, the atomic level interactions of phosphoinositides with a number of different proteins, the evidence for local PIP_2 signals, and the dynamic way in which changes in plasma membrane phosphoinositide levels can be visualized by introducing novel approaches. We next turn to phosphoinositide regulation of the activity of a number of different ion channels, such as potassium inward rectifiers (Kir), a two-pore domain potassium ($\text{K}_{2\text{P}}$) leak, the voltage-gated human ether-a-go-go related gene (HERG) and muscarinic (M)-type potassium, the hyperpolarization-activated cyclic

nucleotide-gated (HCN), the voltage-gated Ca^{2+} , TRP, the epithelial sodium (ENaC), and purinergic P2X receptors.

Yuntao Mao and Helen Yin review the evidence showing that the phosphatidylinositol 4-phosphate 5 kinases (PIP5Ks), which make $\text{PIP}(4,5)\text{P}_2$ (or PIP_2) from $\text{PI}(4)\text{P}$, show spatial and temporal regulation and isoform-specific functions, such as actin polymerization, receptor-mediated endocytosis, focal adhesion dynamics, synaptic vesicle physiology, membrane ruffling, phagocytosis, etc.

Dora Visnjic and Hrvoje Banfic review recent studies of nuclear phosphoinositide signaling, which has been documented in the nuclei of many different cell types. They focus on phosphatidylinositol-specific phospholipase C (PI-PLC) and phosphoinositide 3-kinase (PI3K) by examining their subnuclear localization, mechanisms of activation, and possible physiological roles in the regulation of cell cycle, survival, and differentiation.

Daniel Biero and colleagues in Christophe Erneux's laboratory review the different phosphoinositide phosphatases and illustrate the importance of these enzymes in cell regulation by their involvement in several human diseases such as Lowe syndrome, X-linked myotubular myopathy, cancer, diabetes, and bacterial infection.

Avia Rosenhouse-Dantsker and Diomedes Logothetis review 25 crystallographic complexes of phosphoinositides with different proteins and summarize molecular features that characterize the binding, such as the types and numbers of residues that bind phosphoinositides, the determinants of binding affinity and specificity, and the protein conformational changes induced by phosphoinositide binding. They also consider implications that these generalizations may have for phosphoinositide binding to ion channels, where no structures of complexes have become available yet.

Don Hilgemann reviews cases in which PIP_2 is proposed to function in a localized way, and he considers different

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mechanistic models to account for local PIP_2 action. Such considerations require restrictions in PIP_2 diffusion and a number of mechanisms to achieve this are discussed, such as the action of the cytoskeleton, the binding to interacting proteins and specialized membrane domains. It is speculated that molecular PIP_2 signaling could take place in the absence of PIP_2 gradients, and possibilities are discussed.

Peter Varnai and Tamas Balla review tools that have been developed, particularly through use of engineered protein domains, to visualize and manipulate phosphoinositide dynamics in live cells. They report on lipid-binding domains as tools to monitor phosphoinositide dynamics and discuss the different ways in which the localization of these protein domains to the membrane has been quantified. In manipulating phosphoinositide dynamics, they discuss how to target lipid-binding domains to different cellular compartments.

Diomedes Logothetis and colleagues review work on inwardly rectifying K^+ channels that aims to obtain molecular insights on how channel- PIP_2 interactions control channel activity and gating. They map onto the three-dimensional structure of Kir2.1 (also known as IRK1) the position of basic residues identified through site-directed mutagenesis to be involved in PIP_2 sensitivity onto the three-dimensional structure of Kir2.1 (or IRK1). The localization of these putative PIP_2 -interacting residues has given rise to a model of gating in which PIP_2 is thought to act as the glue to tether the N and C termini of the channel to the membrane and exert a tangential force that pulls on the inner helices and accomplishes mechanical gating.

Jean Chemin and colleagues in the Honoré and Lazdunski laboratories present original work to study the regulation of the two-pore domain channel TREK-1 by PIP_2 and other membrane phospholipids. They find that intracellular phospholipids inhibit basal channel activity and activation by membrane stretch, intracellular acidosis, and arachidonic acid. Poly-lysine treatment targeting endogenous anionic phospholipids reduces the inhibition and reveals channel stimulation by exogenous phosphoinositides. The distal C-terminal domain is implicated in this effect.

Jin-Song Bian and Thomas McDonald review the evidence for PIP_2 interactions with the voltage-gated HERG channel, a rapidly activating delayed rectifier K^+ current (I_{Kr}), and its potential biological significance in the autonomic regulation of HERG channels that proceeds through the Gq-coupled receptor to change membrane PIP_2 levels.

Simon Hughes and colleagues in the Tinker and Brown laboratories explore the mechanism of M-type K^+ current inhibition by the peptide bradykinin (BK) using original work. It was thought that BK inhibited M currents via an IP_3 -mediated release of Ca^{2+} from intracellular stores rather

than by a depletion of PIP_2 levels. Yet PLC δ -PH-GFP translocation from the membrane to the cytosol showed a similar pattern to that caused by a muscarinic agonist, which has been shown to inhibit M currents by PIP_2 depletion. Hughes et al. utilized a better tool reporting on PIP_2 levels, tubby-R332H-cYFP, which showed that BK produced about one third the translocation seen by the muscarinic agonist. This difference between the two agonists could be abolished when a BK-induced synthesis of PIP_2 could be blocked.

Phillip Pian and colleagues in Steve Siegelbaum's laboratory explored through original work the modulation of HCN currents by signals that activated phospholipase C and, thus, hydrolyzed PIP_2 . PIP_2 enhanced HCN activity by causing a rightward shift of voltage-dependent gating as was previously shown. Stimulation of receptors coupled to PLC had a similar effect on the voltage dependence of activation as PIP_2 . This paradoxical effect could be accounted for if, as in the study by Hughes and colleagues on the BK-mediated inhibition of M currents, PIP_2 synthesis was blocked.

Ioannis Michailidis, Yun Zhang, and Jian Yang reviewed phosphoinositide regulation of high voltage-activated Ca^{2+} channels. Phosphoinositides have been shown to stabilize Ca^{2+} channel activity but also to produce a voltage-dependent inhibition that can be antagonized by protein kinase A (PKA) phosphorylation. PIP_2 depletion and arachidonic acid together are responsible for the slow voltage-independent inhibition of Ca^{2+} currents seen with Gq/11-coupled receptor stimulation. A sufficient level of PIP_2 seemed to also be necessary for $\text{G}\beta\gamma$ -mediated inhibition. PIP_3 promoted trafficking of Ca^{2+} channels to the plasma membrane.

Tibor Rohacs and Bernd Nilius review regulation of various TRP channels (e.g., TRPMs, TRPCs, TRPVs) in the context of PIP_2 regulation of other ion channels. They discuss the possibility that PIP_2 is a common regulator of mammalian TRP channels and focus on novel tools to study the diverse effects of PIP_2 on these channels.

He-Ping Ma and colleagues in his and Douglas Eaton's laboratories explore regulation of ENaC by phosphoinositides. PIP_2 and PIP_3 activate ENaC. Activation of PLC by purinergic (P_2Y) receptor stimulation or EGF-induced activation reduce membrane PIP_2 and consequently decrease channel activity. In contrast, stimulation of insulin-like growth factor-1 (IGF-1) receptor by aldosterone of insulin produces PIP_3 and increases channel activity. Interactions of PIP_2 and PIP_3 with the N and C termini of distinct ENaC subunits of the channel are investigated. The implications of these agonist-induced effects to disease are discussed.

Qi Zhao, Diomedes Logothetis, and Philippe Seguela explored the regulation of purinergic P_2X receptors by phosphoinositides. Two receptors, P_2X_2 and P_2X_7 , have

previously been studied. Basic residues in the C-terminal region proximal to the membrane seem to be involved in interactions with PIP_2 in both P2X_2 and P2X_7 channels. Moreover, in original data from P2X_3 receptors found in DRG neurons, the authors showed that block of PIP_2 levels inhibited P2X_3 currents.

Although the field of phosphoinositide regulation of membrane proteins has exploded in recent years, it is clear that we are still at the early stages of understanding the

fundamental principles by which these important signaling phospholipids are able to control the activities of so many ion channels. To meet this challenge, novel tools that will enable us to directly monitor changes in phosphoinositides need to be developed. Because hot spots of putative PIP_2 -interacting residues are shared among very different channel types, it is likely that the mechanism by which specific channel– PIP_2 interactions occur and how they lead to channel gating will be shared among different channel types.