

**COMMENTARY**

# With PIPs, you get ZIPs and blips

 Eric N. Senning 

Two-pore  $\text{Na}^+$  channel 3 (TPC3) is a voltage-gated ion channel with a topology resembling that of the superfamily of voltage-gated ion channels. A distinguishing feature of members within the TPC family is that two functional voltage-gated channel units are concatenated to form a subunit, which dimerizes to assemble as a channel. Each TPC3 subunit contains a voltage sensor and pore (S1-S6) domain followed by a second voltage sensor and pore domain. Previous research on TPCs has revealed an activation mechanism that appears to rely on the binding of phosphoinositide phosphates (PIP<sub>s</sub>; [Cang et al., 2014a,b](#); [She et al., 2019](#)). A structure of TPC1, together with mutagenesis studies, has suggested that the first voltage sensor/pore domain (I) of TPCs acts as the PIP<sub>s</sub> sensor and the second voltage sensor/pore domain (II) senses the voltage across the membrane ([She et al., 2018](#)). In this issue of the *Journal of General Physiology*, [Shimomura and Kubo](#) report that, although both TPC1 and TPC2 interact with phosphoinositide-(3,5)bisphosphate (PI(3,5)P<sub>2</sub>) in order to gate their pores, TPC3 opening is contingent on PI(3,5)P<sub>2</sub> or PI(3,4)P<sub>2</sub> being in the membrane.

Cellular membranes dominate our description of cellular architecture. Whether through brightfield microscopy or the detailed descriptions of electron micrographs, we perceive the cell as bounded and compartmentalized by membranes. Although such images may imbue a sense of permanence, lipid membranes are actually highly dynamic, constantly exchanging and rearranging their integral components. We need look no further than the life cycle of a membrane protein to appreciate the dynamic nature of membranes. Membrane proteins are synthesized in the ER before they visit numerous compartments on their journey toward expression in a target membrane followed by their eventual demise in a lysosome ([Stryer, 1995](#)). As a membrane protein progresses through each compartment, a small part of the lipid bilayer is transferred with the protein in an unrelenting descent toward entropy.

Despite the lipid bilayer mixing process during trafficking, which would eventually dissipate any unique lipid composition of organelles, each compartment's lipid character is maintained by highly regulated lipid enzymes and lipid-associating proteins

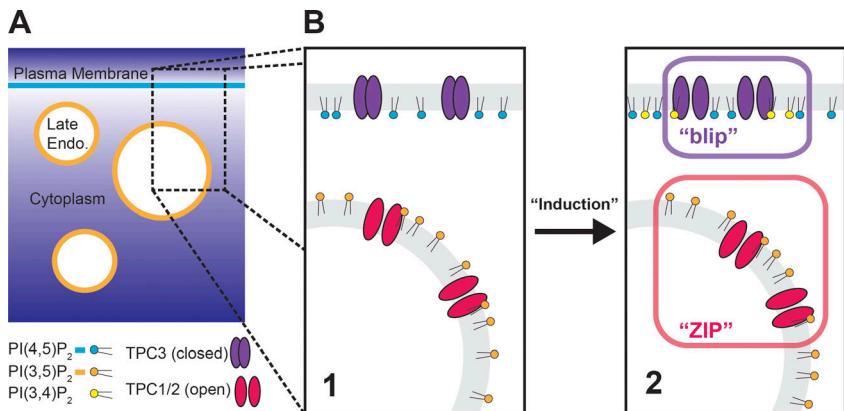
([Behnia and Munro, 2005](#); [Lippincott-Schwartz and Phair, 2010](#)). This means that specific PIP<sub>s</sub> provide a unique identifier for particular organelar membranes. The maintenance of certain PIP<sub>s</sub> as compartmental markers has been interpreted as a cellular address system—the “ZIP codes,” if you will—where protein functionality in a membrane is specified by the correct PIP being present ([Di Paolo and De Camilli, 2006](#)). In such a view, the unique PIP profile of each cellular membrane determines the protein activity at its surface, thereby ensuring homeostatic function. On the other hand, short-lived fluctuations in PIP concentration (“blips”) also confer an acute stimulus for modulation of signaling pathways or direct interaction with target proteins ([Balla, 2013](#)). Signaling through G<sub>αq</sub>-coupled receptors relies on the degradation of PI(4,5)P<sub>2</sub> into inositol-(1,4,5)trisphosphate and diacylglycerol ([Falkenburger et al., 2010b](#)). In addition, conversion of PI(4,5)P<sub>2</sub> into PI(3,4,5)P<sub>3</sub> on the plasma membrane is an important cue for cell survival and cell migration ([Lien et al., 2017](#)). Lest we contend that PI(4,5)P<sub>2</sub> is an innocuous bystander in lipid signaling, it serves well to be reminded that PI(4,5)P<sub>2</sub> is essential for the gating of various Kir, KCNQ and TRP channels, to name just a few ([MacGregor et al., 2002](#); [Rohács et al., 2005](#); [Klein et al., 2008](#); [Jensen et al., 2009](#); [D'Avanzo et al., 2010](#)). PIP<sub>s</sub> may therefore be considered as regulatory elements for a diverse array of proteins by (1) designating a specific membrane location for activity and (2) exerting dynamic control over protein function through local, transient fluctuations in PIP levels.

The report on TPC3 regulation by [Shimomura and Kubo \(2019\)](#) highlights an interesting development in the context of “ZIP” and “blip” types of PIP regulation. In the case of TPC1 and TPC2, their expression in late endosomes and regulation by PI(3,5)P<sub>2</sub>, which is enriched in these compartments, conforms to the “ZIP” code type of regulation. However, TPC3 is expressed on the plasma membrane. [Shimomura and Kubo \(2019\)](#) deduce that an “induction” process leads to TPC3 activation by raising PI(3,4)P<sub>2</sub> levels, suggestive of the “blip” type of regulation ([Fig. 1](#)). The authors support their conclusions with evidence collected from fluorescence imaging experiments, whole-cell

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**Figure 1. PIP regulation of the TPC family.** (A) Small region near the edge of a cell. Relative to the other PIPs, the plasma membrane has high concentrations of PI(4,5)P<sub>2</sub> (blue), and late endosomes have high concentrations of PI(3,5)P<sub>2</sub> (orange). (B) Expanded section of A illustrates the molecular details of PIP interactions with the TPCs. Channels open or close depending on association with their respective activating PIP. In subpanel 1, TPC1/2 are activated by the abundant PI(3,5)P<sub>2</sub> in late endosomes, but TPC3 is not activated by the PI(4,5)P<sub>2</sub> in the plasma membrane. After induction through a prolonged depolarization, subpanel 2 shows that PI(3,4)P<sub>2</sub> (yellow) is elevated in the plasma membrane. The transient fluctuation in PI(3,4)P<sub>2</sub> activates TPC3 by a "blip" type of regulation. The activation of TPC1/2 is determined only by the continued, local presence of PI(3,5)P<sub>2</sub>, conforming to the "ZIP" code type of regulation.

electrophysiology, and inside-out patch clamp electrophysiology with direct application of PIPs onto the intracellular leaflet of patches. Their efforts testify to the necessary lengths required for understanding the specificity of PIP regulation of ion channel function.

As previously reported by Cang et al. (2014a), TPC3 from the zebrafish *Danio rerio* can be exogenously expressed in mammalian and amphibian expression systems and is experimentally tractable by electrophysiology because the channel is present in the plasma membrane. These authors found that a long depolarizing pulse in a TPC3-expressing oocyte increases macroscopic currents by a process termed "induction." This led them to speculate that induction of native Na<sup>+</sup> currents in oocytes, which exhibit slow activation kinetics, high activation thresholds, and are noninactivating, may be due to an endogenous TPC3 channel (Baud and Kado, 1984). Shimomura and Kubo (2019) confirmed the electrophysiological properties of TPC3 from *Xenopus tropicalis* expressed in *Xenopus* oocytes. After characterizing a smaller induction current in untransfected *Xenopus* oocytes, which would not interfere with the interpretation of much larger induction currents in xtTPC3-expressing oocytes, Shimomura and Kubo (2019) proceeded with their study of TPC3 function.

PIP regulation of ion channel function bridges cell signaling pathways and cellular excitability (Falkenburger et al., 2010a). Shimomura and Kubo (2019) took advantage of this connection by beginning their investigation into PIP regulation of TPC3 activity with a pharmacological approach. The effect of wortmannin, a membrane-permeable inhibitor of lipid kinases, on TPC3 currents in oocytes implicates PIP fluctuations as a plausible mechanism for induction. To narrow down the field of PIP candidates for TPC3 regulation, the authors rely on rapid interconversion of PIP species with a voltage-sensitive phosphatase (VSP), *Ciona intestinalis* VSP (Ci-VSP), followed by verification using direct application of specific PIP species on excised patches from oocytes that express TPC3.

VSPs are generally thought to rapidly dephosphorylate the phosphate at the fifth position of the inositol ring on a PIP (Murata and Okamura, 2007). However, as recently communicated by the Hille, Kruse, and Kohout groups, there is growing

evidence that VSPs will also dephosphorylate the third position of the ring (Kruse et al., 2019). The phosphatase activities of Ci-VSP and *D. rerio* VSP, as well as the dynamic levels of PIPs, have been extensively studied in different cell types by the Hille group and others using PIP sensors coupled to fluorescent proteins. A remarkable aspect of VSP activation and changes to PIP levels is the rapid recovery to prestimulus levels through the actions of endogenous kinases and phosphatases (Halaszovich et al., 2009; Jensen et al., 2009; Falkenburger et al., 2010c).

When perturbing the steady-state levels of PIPs, it is essential to show that the PIP that one seeks to study is veritably affected (Goulden et al., 2019). To accommodate the rapid dynamic interconversion of PIPs within the cell and directly follow the fluctuations of distinct species, Shimomura and Kubo (2019) used spectroscopic measurements of the FRET sensors F-TAPP and F-PLC, respectively probing for PIP(3,4)P<sub>2</sub> and PIP(4,5)P<sub>2</sub> (Grimm and Isacoff, 2016). Fluorescent sensors of PIP levels permit synchronous measurements of PIP accumulation or depletion in the membrane while recording ion channel currents after activation of Ci-VSP (Falkenburger et al., 2010c). The simultaneous measurements of TPC3 currents and F-TAPP FRET showed that PI(3,4)P<sub>2</sub> levels tracked with changes in the current. On the other hand, PIP(4,5)P<sub>2</sub> levels, acquired through the respective sensor F-PLC, did not track with TPC3 current, providing the first clue that PIP(3,4)P<sub>2</sub> is a regulatory PIP for TPC3.

The Ci-VSP and PIP2 FRET electrophysiology experiments by Shimomura and Kubo (2019) reveal an unambiguous link between endogenous PI(3,4)P<sub>2</sub> accumulation in the plasma membrane and TPC3 activation. By directly applying PI(3,4)P<sub>2</sub> and other PIPs onto excised patches of TPC3-expressing oocyte membrane, the specificity of each lipid was tested. These experiments showed that, besides PI(3,4)P<sub>2</sub>, PI(3,5)P<sub>2</sub> was sufficiently capable of activating TPC3.

Shimomura and Kubo (2019) brought their investigation of TPC3 full circle through a series of mutagenesis experiments. Using a homology model of TPC3 based on the structure of TPC1, mutations of residues that are critical for mmTPC1 binding to PI(3,5)P<sub>2</sub> were introduced to TPC3. Several of these mutants partially or fully abolished TPC3 induction when long depolarizations were applied in two electrode voltage clamp

experiments using oocytes. In excised patches, direct application of both PIP(3,4)P<sub>2</sub> and PIP(3,5)P<sub>2</sub> did not activate these TPC3 induction mutants, supporting the hypothesis that induction of TPC3 is mediated through an interaction between the channel and either PI(3,4)P<sub>2</sub> or PI(3,5)P<sub>2</sub>.

The PIP regulation and mutagenesis studies of TPC3 evince a structural similarity between binding of PIPs to TPC1 and TPC3. This information should pique our curiosity considering that the former appears to subscribe to the “ZIP” location type of regulation and the latter to the “blip” fluctuation type. From a mechanistic perspective, there need not be a sharp distinction between the “ZIP” and “blip” types. It could be that channels segregate between the two types by exhibiting a sensitivity to PIPs across different physiological ranges. Whatever the case may be, the TPC family of channels has the potential to elucidate mechanistic peculiarities between these two types of channel regulation. With novel fluorescence tools that are increasing our dynamic range and sensitivity to PIP fluctuations, as well as techniques that inform us about the specificity of PIP interactions with their targets, great discoveries about the fascinating interactions between lipid membranes and ion channels are just around the corner.

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