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Keeping active channels in their place

Membrane phosphoinositides regulate TRPM channel activity in a compartment-selective manner

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We have long appreciated that the controlled movement of ions and solutes across the cell surface or plasma membrane affects every aspect of cell function, ranging from membrane excitability to metabolism to secretion, and is also critical for the long-term maintenance of cell viability. Studies examining these physiological transport processes have revealed a vast array of ion channels, transporters and ATPase-driven pumps that underlie these transmembrane ionic movements and how acquired or genetic disruption of these processes are linked to disease. More recently, it has become evident that the ongoing function of intracellular organelles and subcellular compartments also depends heavily on the controlled movement of ions to establish distinct pH or ionic environments. However, limited experimental access to these subcellular domains/structures has hampered scientific progress in this area, due in large part to the difficulty of applying proven functional assays, such as patch clamp and radiotracer methodologies, to these specialized membrane locations. Using both functional and immune-labeling assays, we now know that the types and complement of channels, transporters and pumps located within intracellular membranes and organelles often differ from those present on the plasma membrane. Moreover, it appears that this differential distribution is due to the presence of discrete tags/signals present within these transport proteins that dictate their sorting/trafficking to spatially discrete membrane compartments, where they may also interact with scaffolding proteins that help maintain their localization. Such targeting signals may thus operate in a manner analogous to the way a postal code is used to direct the delivery of a letter.

Over the past 20 years, it has also become apparent that the activity of membrane channels and transporters is influenced by the surrounding phospholipid bilayer, which serves as more than just an inert environment. In fact, it is now clear that the activity of many ion transport proteins at the cell surface depends upon the presence of phosphatidylinositol 4,5-bisphosphate [PtdIns(4,5)P_2], a phospholipid enriched in the inner leaflet of the plasma membrane (for review, see ref. 1). Moreover, different membrane compartments appear to be enriched with different species of phosphoinositides, for example, phosphatidylinositol 4-phosphate is concentrated in the Golgi membranes, phosphatidylinositol 3-phosphate is present in early and late endosomes, and phosphatidylinositol 3,5-bisphosphate is associated primarily with late endosomes and lysosomes.[2] These phosphoinositides may thus serve as biological signatures or "address labels" for distinct membrane compartments within a cell that support the correct trafficking and localization of ionic transport proteins required for the function of these compartments/organelles.

In the present study, the authors have examined the importance of compartment-associated membrane phosphoinositides on the activation and inactivation of TRPML1, a Ca^{2+} and Fe^{2+} permeable TRP channel family member that normally

resides in the lysosomal membrane, but may be also be transiently detected in the plasma membrane during events such as vesicle exocytosis. Experimentally, the authors recorded TRPML1 channel activity using whole cell and inside-out (I/O) patch clamp methodologies, along with channel recordings from intact lysosomal organelles. In giant I/O plasma membrane patches from HEK 293 cells expressing recombinant murine TRPML-1 channels, the authors observed that bath addition of phosphatidylinositol 3,5-bisphosphate [$PI(3,5)P_2$] robustly stimulated channel activity, and that this effect could be further enhanced by the selective small molecule activator SF-51. Interestingly, excision of plasma membrane patches from TRPML-1 expressing HEK 293 cells was also associated with "run-up" of TRPML-1 channel activity, which was strongly inhibited following bath addition of $PI(4,5)P_2$. This phosphoinositide also inhibited channel activity stimulated by SF-51 alone with an IC50 value of 0.2 μM, indicating a rather high affinity process. This finding thus appears to oppose a number of observations for plasma membrane-associated transport proteins, which seem to require $PI(4,5)P_2$ in the bilayer as a cofactor to support their activity at the cell surface. However, in the case of TRPML-1, a channel normally excluded from the plasma membrane, $PI(4,5)P_2$ clearly acts as an antagonist of channel activity. Using a mutated TRPML-1 channel with inactivated lysosomal targeting sequences, the authors observed more TRPML-1 activity at the plasma membrane, which could be positively and negatively modulated by addition of either $PI(3,5)P_2$ or $PI(4,5)P_2$, respectively. Inhibition of channel activity in the plasma membrane was also observed following bath addition of $PI(3,4)P_2$ and $PI(3,4,5)P_3$, whereas phosphoinositide monophosphates [i.e., PI(3)P, PI(4)P and PI(5)P] had very little effect on channel activity. Collectively, these data indicate that the phosphorylation pattern of the inositol ring critically determines the effect of phosphoinositides on TRPML-1 activity, which likely occurs via direct binding of the phosphoinositide to the channel itself.

Since addition of $PI(4,5)P_2$ was observed to negatively regulate TRPML-1 activity, the authors hypothesized that manipulations designed to lower $PI(4,5)P_2$ levels in the plasma membrane would elevate TRPML-1 channel activity. To test this prediction, cells were transfected with a plasma membrane targeted phospholipid phosphatase that would enzymatically dephosphorylate phosphatidyl inositols at the plasma membrane in response to the required stimulus. Using such a strategy to deplete the level of $PI(4,5)P_2$, the authors observed that TRPML-1 activity increased at the plasma membrane, whereas the same experimental manipulation robustly inhibited the activity of TRPM7, a related TRP channel normally present at the plasma membrane and dependent upon $PI(4,5)P_2$ for its activity. Structure-function analysis of the TRPML-1 channel revealed that mutating a cluster of seven basic amino acids (i.e., Arg and Lys residues) within the channel's cytoplasmic N-terminus could interfere with modulation of channel activity by membrane phosphoinositides. In particular, one group of basic residues was found to be important for channel activation by $PI(3,5)P_2$, whereas a neighboring stretch of residues was critical for inhibition by $PI(4,5)P_2$.

The opposing modulation of ion channels and transporters by membrane phosphoinositides, such as $PI(3,5)P_2$ and $PI(4,5)P_2$, may thus provide a molecular mechanism to ensure that these proteins function in their correct cellular compartment (i.e., lysosome vs. plasma membrane) and to prevent undesired solute transport activity when channels/transporters are mis-sorted to incorrect locations. In the case of TRPML-1, this modulation would prevent the channel from mediating unwanted entry of external Ca^{2+} that could have deleterious effects on cell function and/or health. Based on the results of this study, it appears that membrane phosphoinositides serve to regulate ion transport proteins in a compartment-specific manner, thereby ensuring that these activities occur in the correct subcellular locations and are prevented during routine trafficking steps or when these proteins contain mutations that misdirect their distribution within a cell. It will certainly be of interest to see how widespread this phosphoinositide-dependent regulatory process may be in the context of other membrane transport proteins.

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