Khalili, K., Del Valle, L., Muralidharan, V., Gault, W.J., Darbinian, N., Otte, J., Meier, E., Johnson, E.M., Daniel, D.C., Kinoshita, Y., et al. (2003). Mol. Cell. Biol. *23*, 6857–6875.

Kwon, S., Barbarese, E., and Carson, J.H. (1999). J. Cell Biol. *147*, 247–256.

Ranum, L.P.W., and Cooper, T.A. (2006). Annu. Rev. Neurosci. *29*, 259–277.

Sofola, O.A., Jin, P., Qin, Y., Duan, R., Liu, H., de Haro, M., Nelson, D.L., and Botas, J. (2007). Neuron *55*, this issue, 565–571.

Sobczak, K., de Mezer, M., Michlewski, G., Krol, T., and Krzyzosiak, W.T. (2003). Nucleic Acids Res. *31*, 5469–5482.

Timchenko, L.T., Miller, J.W., Timchenko, N.A., DeVore, D.R., Datar, K.V., Lin, L., Roberts, R., Caskey, C.T., and Swanson, M.S. (1996). Nucleic Acids Res. *24*, 4407–4414.

PIP_2—the Master Key

Liora Guy-David[1] and Eitan Reuveny[1,*]
[1] Department of Biological Chemistry, Weizmann Institute of Science, Rehovot 76100, Israel
*Correspondence: e.reuveny@weizmann.ac.il
DOI 10.1016/j.neuron.2007.08.001

The function of inwardly rectifying K^+ (Kir) channels is highly diverse and therefore is tightly regulated by various environmental factors. In their article in this issue of *Neuron*, Rapedius et al. recognize a conserved structural mechanism for Kir channels gating by both pH and PIP_2. In light of these findings and accumulated knowledge, PIP_2 is suggested to have a common coregulatory role in the gating of Kir channels by all their soluble modulators.

Inwardly-rectifying K^+ (Kir) channels are an important class of K^+ channels involved in the regulation of membrane excitability, heart rate, vascular tone, hormone secretion, and in the control of body salt balance (Bichet et al., 2003). Kir channel activity was shown to be affected by several factors, including pH, Na^+ and Mg^{2+} ions, ATP, polyamines, G proteins, and phosphatidylinositol-4,5-bisphosphate (PIP_2) (Reimann and Ashcroft, 1999). Current model for Kir channel gating claims that conformational rearrangements in the cytoplasmatic domains, promoted by environmental factors or ligand binding, are transduced to the transmembrane domains (TMs), which in turn rotate to allow opening of the channel pore (Bichet et al., 2003).

For any biological system with convergent regulation by multiple factors, questions can be asked about mutual and hierarchical interactions among these factors and the specific role each contributes to the overall modulation. Indeed, in the case of Kir channel regulation, several studies were aimed at revealing the relevance of multiple interactions among the currently known modulators to channel gating. For example, both G protein and Na^+ ions were shown to stabilize GIRK(Kir3.x)-PIP_2 interaction (Huang et al., 1998). Similarly, in K_{ATP}(Kir6.x) channels, binding of PIP_2 reduces the channel sensitivity to ATP inhibition (Baukrowitz et al., 1998). Furthermore, the notable structural resemblance among Kir channels as opposed to the diverse type of chemical entities that modulate the channels implies that the final step in the cascade of channel regulation may involve a specific mediator, common to all channels. Understanding the biochemical and structural basis underlying channel modulation may clarify the nature of the complex regulation of Kir channels. Yet, highly tuned and reliable techniques are required to provide the desired temporal resolution, which would allow distinctive characterization of the different stages of channel activation and modulation.

In this issue of *Neuron*, Rapedius et al. (2007) provide new insights into a conserved mechanism of Kir channel gating to establish that both low pH and PIP_2 act through a common downstream element that controls the stabilization of the closed or open channel conformations, respectively (Rapedius et al., 2007). Using two independent kinetic measurement methodologies, the authors were able to establish that the rate-limiting step in channel activation is not the binding or unbinding of PIP_2 but the conformational rearrangement that follows PIP_2 binding. The latter event is much slower and can be dramatically enhanced by the disruption of a hydrogen bond between the ε-nitrogen of K80 located in TM1 and the carbonyl oxygen of A177 located in TM2, suggesting that this TM1-TM2 hydrogen bond stabilizes the closed state of the channel and its rupture may be one of the rate-limiting steps in channel transition between the closed to the open conformations. Interestingly, this mechanism of stabilization of the closed state was also found in other members of the IR channel family, hence suggesting a general conserved mechanistic basis for the closed-channel conformation. PIP_2, the main modulator for channel activation, is the one involved in disrupting this hydrogen bond to support channel activation or stabilization of the open conformation. In addition, Rapedius et al. (2007) provide

further support for the significant and broad involvement of PIP_2 regulation in Kir channel gating. By using a fast-application system, the authors were able to successfully measure pH-dependent gating kinetics of different Kir channel mutants. Their results demonstrate strong correlations between the kinetics of channel opening by increasing pH or by the application of PIP_2 and the propensity to form a hydrogen bond between TM1 and TM2. It may thus appear that proton- and PIP_2-dependnet gating relies on a common structural element. How can this be achieved?

In general, PIP_2 is considered as merely one member among the still-growing group of Kir channel modulators. Recently, evidence has been accumulating, assigning PIP_2-channel interactions a unique coregulatory role. It has been shown that binding of some channel modulators give rise to an increased PIP_2 affinity, leading to stabilization of the open state of the channel, as in the case of G proteins, Na^+ ions, and PKA (Huang et al., 1998; Zeng et al., 2003). On the other hand, other channel modulators have been shown to reduce PIP_2 affinity, as in the case of ATP and pH, consequently stabilizing the channel in its closed state (Baukrowitz et al., 1998; Schulze et al., 2003). (For a detailed review, see Xie et al. [2007].) Since PIP_2 directly regulates all Kir channels, it is possible that PIP_2 serves as a common moderator in the regulation of Kir channel gating by various environmental cues (Du et al., 2004).

While the effect of channel modulators is chemically diverse, they can still support the same structurally conserved channel rearrangements at the ion conduction region. It is thus reasonable to assume that PIP_2 may be the molecule that can actively control this process. Since the end point of this cascade is the control of the interaction between TM1 and TM2, via hydrogen bonding or hydrophobic interactions, it is plausible that PIP_2 binding supports the open conformation and its absence promotes channel closing, controlled by rearrangement at the TM1-TM2 interface. In other words, PIP_2 might possess a suprarregulatory role, as the ''translator'' of binding or unbinding events of various environmental cues by the virtue of its interaction with the channel (Logothetis et al., 2007). The decision whether a channel would open or close in response to specific modulators, might then be dependent on the steric interactions between the binding sites of the channel modulators and PIP_2. Likewise, the sensitivity of the channel to a specific modulator might be dependent on the basal affinity of PIP_2 to that channel. Indeed, it appears that channels that show high affinity for PIP_2 are hardly modulated (e.g., Kir2.1), while channels that exhibit moderate (e.g., Kir2.3) to low (e.g., Kir3) affinity for PIP_2 are relatively sensitive to modulatory signals (Logothetis et al., 2007).

The results presented by Rapedius et al. (2007) give additional insights into the possibility that PIP_2 serves as an end effector regulating channel gating and that the differences among Kir channels in their sensitivity to a given modulator may reflect differences in their affinity to PIP_2 binding. Unfortunately, there are no solved structures at atomic resolution of Kir channels with bound PIP_2 and therefore limiting our understanding of how PIP_2 stabilizes the open conformation. Similarly, despite the 4-fold symmetry of the IR channels and the assumed four potential binding sites for PIP_2, we still do not know the stochiometry of PIP_2 channel interaction that supports full channel opening.

REFERENCES

Baukrowitz, T., Schulte, U., Oliver, D., Herlitze, S., Krauter, T., Tucker, S.J., Ruppersberg, J.P., and Fakler, B. (1998). Science *282*, 1141–1144.

Bichet, D., Haass, F.A., and Jan, L.Y. (2003). Nat. Rev. Neurosci. *4*, 957–967.

Du, X., Zhang, H., Lopes, C., Mirshahi, T., Rohacs, T., and Logothetis, D.E. (2004). J. Biol. Chem. *279*, 37271–37281.

Huang, C.L., Feng, S., and Hilgemann, D.W. (1998). Nature *391*, 803–806.

Logothetis, D.E., Lupyan, D., and Rosenhouse-Dantsker, A. (2007). J. Physiol., in press.

Rapedius, M., Fowler, P.W., Shang, L., Sansom, M.S.P., Tucker, S.J., and Baukrowitz, T. (2007). Neuron *55*, this issue, 602–614.

Reimann, F., and Ashcroft, F.M. (1999). Curr. Opin. Cell Biol. *11*, 503–508.

Schulze, D., Krauter, T., Fritzenschaft, H., Soom, M., and Baukrowitz, T. (2003). J. Biol. Chem. *278*, 10500–10505.

Xie, L.H., John, S.A., Ribalet, B., and Weiss, J.N. (2007). Prog. Biophys. Mol. Biol. *94*, 320–335.

Zeng, W.Z., Li, X.J., Hilgemann, D.W., and Huang, C.L. (2003). J. Biol. Chem. *278*, 16852–16856.