Voltage-gated K Channels-Gating II

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Does the Linker in Ci-VSP Function as a PI(4,5)P2 Binding Domain?

Susy C. Kohout1, Sarah C. Bell1, Qiang Xu2, Daniel L. Minor2, Ehud Y. Isacoff1.

1University of California, Berkeley, Berkeley, CA, USA, 2University of Chicago, Chicago, IL, USA.

Proteins are often composed of multiple domains which confer specialized functions to the full length protein. The voltage sensing domain (VSD) from voltage-gated ion channels is one such domain. Originally thought to be restricted to ion channels, we now know that it can also confer voltage control to enzymes. The Ciona intestinalis voltage sensor-containing phosphatase (Ci-VSP) is composed of the unique combination of a VSD coupled to a lipid phosphatase. The coupling between these two domains is intriguing since it means that a modular VSD can control two very different effectors, a pore or an enzyme. The original characterization of Ci-VSP suggested that the inter-domain linker played a role in activating the protein while more recent work has shown evidence supporting the hypothesis that the inter-domain linker functions as a phosphatidylinositol 4,5-bisphosphate (PI(4,5)P2) binding domain (PBM) (1,2). This function is similar to that of the N-terminus of PTEN, a well-known lipid phosphatase that shares a high degree of homology to Ci-VSP. We chose disease-causing mutations from PTEN, introduced them into Ci-VSP, and probed Ci-VSP’s voltage-dependent movements and phosphatase activity using voltage clamp fluorometry, two electrode voltage clamp, and biochemical methods. We find that the linker composition is critical for activity where single amino acid mutations either decrease or eliminate activity. We also found that upon PI(4,5)P2 depletion, the voltage dependent motions of the VSD were altered when the linker was intact, but not when the linker was mutated. Our data suggests that the linker both couples to the two domains and also serves as a PBM, regulating via its interaction with PI(4,5)P2. 1. Murata, Y., et al, (2005) Nature 435, 1239-1243 2. Villalba-Galea, C. A., et al, (2009) J Gen Physiol 134, 5-14

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Sensing Charges of Ci-VSP

Carlos A. Villalba-Galea1,2, Ludivine Frezza3, Ernesto Vargas4, Francisco Bezanilla5.

1Virginia Commonwealth University, Richmond, VA, USA, 2The University of Chicago, Chicago, IL, USA.

The Ciona intestinalis Voltage Sensor-containing Phosphatase (Ci-VSP) is a member of the Voltage Sensitive Phosphatase family that exhibits membrane potential-controlled enzymatic activity. Alignments of the amino acid sequence of Ci-VSP against Voltage Gated Channels (VGC) indicate that its Voltage Sensing Domain (VSD) is formed by four transmembrane segments. The putative fourth segment (S4) of Ci-VSP extends between the arginine (R) in position 217 and the glutamine (Q) in position 239, containing five arginines, which might be the voltage sensing charges. Although it has been shown that R229 and R232 are critical for voltage sensing in Ci-VSP (Murata et al., 2005), the role of the remaining charges is still unclear. To address this issue, we have performed a partial Histidine Scanning of the S4 of Ci-VSP, following the paradigm established for the VGC Shaker (Starace and Bezanilla, 2004). The voltage dependence of the sensing current of the R217H mutant was modulated by pH. Decreasing the external pH shifted the Q-V curve towards positive potentials, while a pH increase had the converse effect, consistent with the finding that neutralizing R217 (R217Q) produces a negative shift of the voltage dependence of Ci-VSP (Kohout et al., 2008). However, the total net charge of R217H did not change with pH, indicating that R217 does not participate in sensing the membrane potential. When the second arginine is replaced by histidine, the resultant mutant (R223H) exhibits a voltage dependent proton current which closes at positive potentials, resembling the current recorded from Shaker-W434F with its first gating charge replaced by histidine (R362H). This result strongly suggests that R223 has access to both the intra- and the extracellular media depending on voltage. Taken together, our results indicate that R223 is the most extracellularly located sensing charge of the Ci-VSP S4 segment. (Support: NIHGM030376)