Molecular mechanisms of electrochemical conversion in voltage-sensing phosphatase (VSP)

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Abstract

Conserved among all organisms, cells utilize plasma membrane potential changes for homeostasis. In neuronal cells, voltage-gated ion channels (VGICs) sense an electrical signal by a voltage sensor domain (VSD) and translate it into an ionic flow through the plasma membrane. Interestingly, in sperm and enterocytes, voltage-sensing phosphatase (VSP) converts an electrical signal into phosphoinositide phosphatase activity, which is important for regulation of sperm motility and nutrient absorption, respectively. Despite its physiological importance, it remains unclear how an electrical signal is converted into a chemical one. In this study, we found that the interaction of the fourth transmembrane segment of the VSD (S4) with a hydrophobic part of the phosphatase region called the hydrophobic spine mediates the conversion from an electrical signal to a chemical one in VSP.

Background & Results

VGIC consists of the VSD and an ion permeation pore. VSP has a VSD similar to that of VGICs but does not have a pore. Instead, the VSD regulates the cytoplasmic phosphatase region with structural similarity to PTEN, a tumor suppressor enzyme, exhibiting the voltage-dependent phosphatase activity toward mainly $PI(4,5)P_2$. We have reported that the hydrophobic spine plays a critical role in the electro-chemical signal conversion. However, the detailed mechanism of the conversion remains unclear. To address this issue, we focused on the C-terminal end of S4 because at that part hydrophobic amino acids (isoleucine and phenylalanine) are conserved across animal species. We found that mutations of the hydrophobic part into hydrophilic amino acids diminished the conversion. By using *Ciona intestinalis* VSP (Ci-VSP), when we analyzed structural rearrangements of I233 and F234 replaced with a kind of fluorescent unnatural amino acid, Anap, using voltage clamp fluorometry (VCF) method on *Xenopus* oocytes, attenuation of I233Anap and F234Anap fluorescence upon membrane depolarization was more remarkable when tryptophan was introduced in the hydrophobic spine, consistent with the fluorescence quenching. When the hydrophobic spine and the C-terminal end of S4 were replaced by cysteines, restriction of the voltage-dependent S4 motion was induced upon repeated membrane depolarization, indicating that the disulfide bond was formed between two parts. These are consistent with the idea that voltage-induced upward motion of S4 brings its C-terminus into close proximity of the hydrophobic spine. The predicted full-length Ci-VSP structure also supports this interaction. Taken together, voltage-induced interactions of S4 with the hydrophobic spine play critical roles in translation from an electrical signal into a chemical one in VSP.

Significance of the research and Future perspective

Our findings present a new mechanism underlying information flow from transmembrane domain to cytoplasmic domain through helix to helix interaction. It will help to understand the mechanisms of voltage-gated ion channels of which pathological alteration leads to various diseases such as arrhythmia and epilepsy. In addition, findings of this work will be of significance for understanding of the regulatory mechanism of PTEN, an important tumor suppressor.

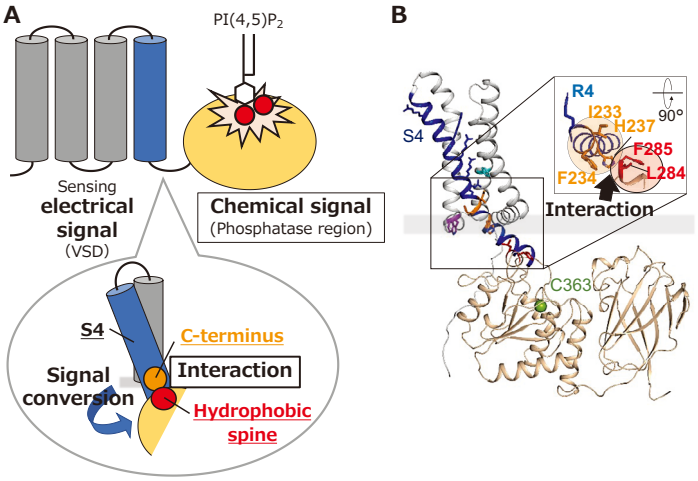

Fig. 1. Mechanisms underlying the signal conversion in VSP.
(A) Cartoon of VSP. (B) Predicted full-length structure of Ci-VSP.

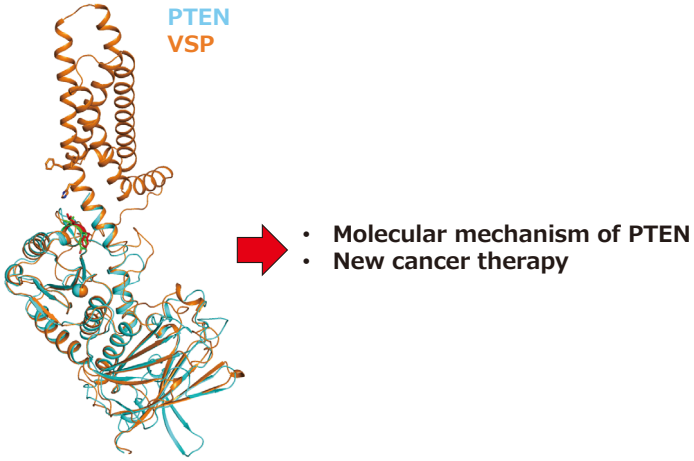

Fig. 2. Future perspective of this VSP study.
A remarkable structural similarity between VSP and PTEN might encourage understanding the molecular mechanism of PTEN and development of a new cancer therapy.

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Keyword ion channel, membrane potential, electrophysiology, electrochemical coupling, unnatural amino acid