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ALLOSTERY: A lipid two-step

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Abstract

A sensor of membrane depolarization controls the activity of a bound enzyme by a novel mechanism involving two sequential voltage-dependent transitions allosterically coupled to changes in substrate specificity of the catalytic domain.

Voltage-sensing domains (VSDs) are structural modules used by cells to switch biological processes on and off as a function of membrane potential. The controlled process depends on a distinct effector module connected to the VSD. When the effector is a pore domain, as in voltage-gated Na^+, K^+, and Ca^{2+} channels, the VSD controls ion permeation across the membrane. When the effector is an enzyme, as in voltage-sensitive phosphatases (VSPs), the VSD turns on and off enzymatic activity. In this issue, Grimm and Isacoff [1] report that the VSD can also make an effector enzyme switch between sequential on states with different substrate specificity, providing a novel mechanism by which membrane voltage can fine-tune the output of a chemical reaction under kinetic control.

In the voltage-sensitive phosphatase investigated, Ci-VSP from the sea squirt *Ciona intestinalis*,[2] the effector module is related to PTEN, a human tumor suppressor lipid phosphatase which is found mutated in many types of cancer.[3] Ci-VSP was previously found to be activated by membrane depolarization and to catalyze the removal of either the 3′ or 5′ phosphate in phosphatidylinositols containing two or three phosphate groups (PIP_2 and PIP_3, respectively). As a result of the Ci-VSP's mixed substrate specificity, PIP_2 can either be produced at the expense of PIP_3 or destroyed to form PIP.

Intrigued by earlier findings of an apparent separation in voltage dependence of PIP_2 production and destruction,[4,5] Grimm and Isacoff set out to investigate whether changes in conformation in the VSD of Ci-VSP could control changes in substrate specificity in the effector enzyme. In order to do so, they needed tools to monitor the conformational state of the VSD over time and to measure the production or destruction of PIP_2 in live cells, under conditions in which the membrane voltage could be controlled.

Although several fluorescent reporters for PIP_2 were available, none had a response-time short enough to follow the voltage-dependent reaction with adequate temporal resolution. Thus, Grimm and Isacoff developed two new fast PIP_2 reporters, named F-TAPP, and F-PLC, based on an earlier design.[6] These genetically-encoded sensors are constitutively bound to the plasma membrane and use CFP/YFP-based Föster Resonance Energy Transfer (FRET) and the PH (PI-binding) domains of TAPP1 or PLC1δ to report $PI(3,4)P_2$ or $PI(4,5)P_2$ levels, respectively.

Like the VSDs of voltage-gated ion channels, the VSD of Ci-VSP consists of four transmembrane segments folded in a helical bundle.[7] The fourth segment, known as S4, (shown in blue in Fig. 1) contains positively charged residues responsible for detecting the electrical field inside the membrane. To measure conformational changes in the VSD, and to control membrane voltage, Grimm and Isacoff used voltage-clamp fluorometry on the protein expressed in Xenopus oocytes.[8] The fluorescent reporter of VSD conformation was an environment-sensitive dye linked to an engineered cysteine located near the S4 segment. The PIP_2 reporters were also expressed in oocytes and their FRET signals recorded under voltage clamp conditions.

Under small membrane depolarizations, Ci-VSP induced the accumulation of PIP_2 species, but under larger depolarizations, the levels of PIP_2s first raised and then decreased. These findings suggested that, upon membrane depolarization, Ci-VSP switches sequentially from an inactive state (C) to two catalytic active states with distinct substrate specificity, an early PIP_3-preferring (A1) state and a later PIP_2-preferring (A2) state (Figure 1). On the other hand, kinetic analysis of the fluorescence signal from the reporter of VSD conformational changes showed an early fast component followed by a slower component. These two components were previously associated with two distinct movements of the VSD's S4 segment.[9]

The temporal correlation between the fluorescence and FRET signals indicated that the early (fast) VSD conformational change could drive the switch of the effector enzyme from the C state to the A1 state, while the later (slow) VSD conformational change could drive the switch from the A1 to the A2 state. This idea was supported by the finding that mutations that stabilize an intermediate, activated state of the VSD selectively depressed the second component of the FRET signals from the PIP_2 reporters, indicating that the effector enzyme was stabilized in the A1 conformation. In addition, a mutation that facilitates the full activation of the VSD shifted the voltage dependence of the two components of the FRET signals to more negative potentials, consistent with a facilitation of both the C → A1 and A1 → A2 transitions in the effector enzyme.

While further studies are required to understand the molecular events mediating the coupling between the VSD and the catalytic site of the phosphatase domain and their physiological implications,[3,10] the work from Grimm and Isacoff reveals a new mechanism by which the cell can fine-tune the levels of phosphoinositide lipids over time, following membrane depolarization and offers an example of allosteric control of substrates selectivity which could be relevant to other enzymes composed of catalytic and regulatory modules.

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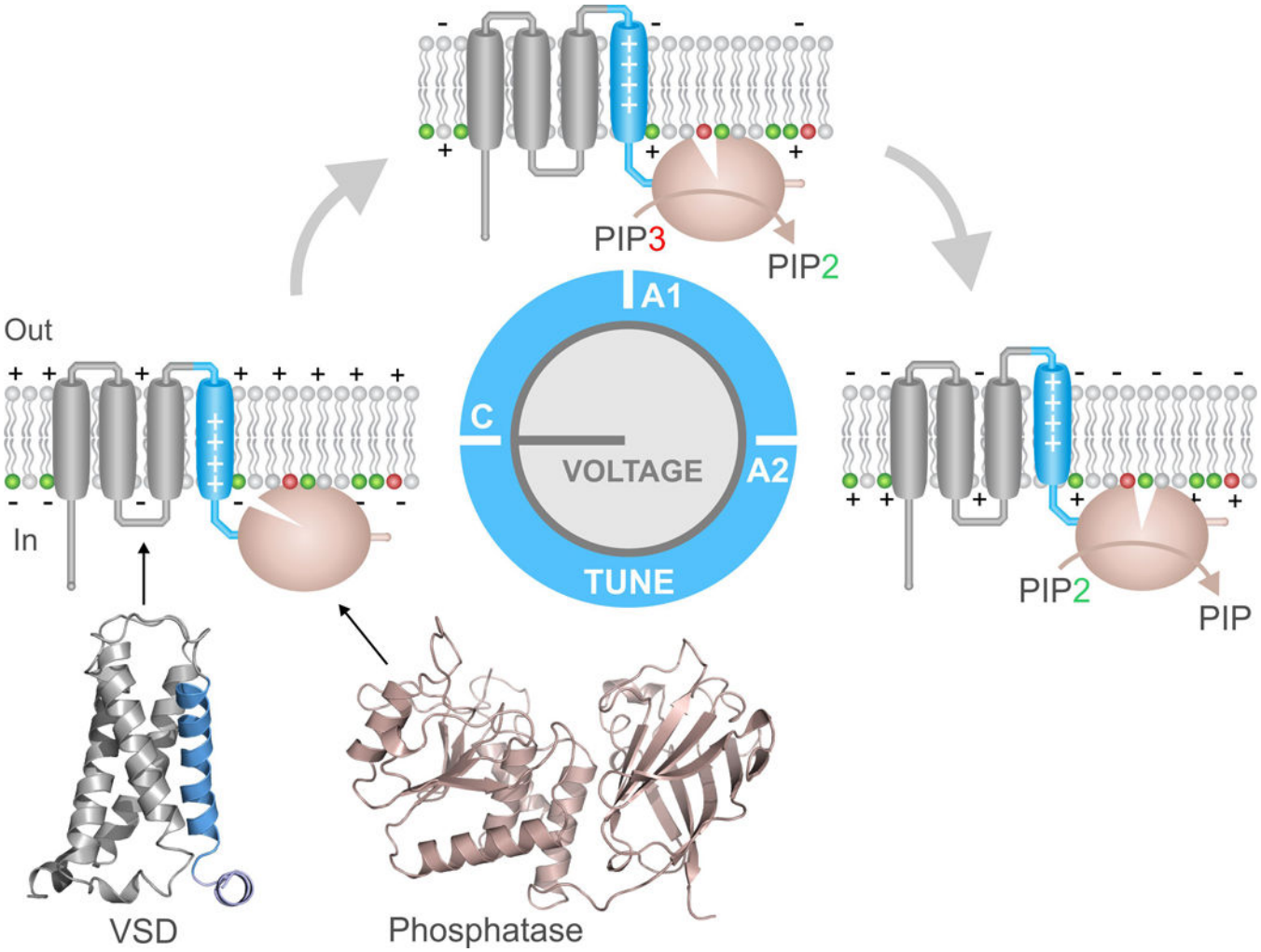

Figure 1.

Ci-VSP substrate specificity tuned by membrane voltage. Distinct conformational changes in the VSD, here represented as two sequential upward movements of the S4 segment (blue helix), allosterically control the transitions of the phosphatase module from the inactive state C to the activated states A1 and A2. A1 hydrolyzes preferentially PIP_3 while A2 preferentially hydrolyzes PIP_2. Lipid headgroups are shown as red spheres for PIP_3 and as green spheres for PIP_2. The structure of the VSD is from ref. ([7]). The structure of the phosphatase module (including phosphatase & C2 domain) is from ref. ([11]).