

Pumping Protons against Gradients into a Plant Vacuole

This report features the work of Yuh-Ju Sun, Rong-Long Pan, and their co-workers published in *Nature* **484**, 399 (2012).

A vacuole is a compartment in cells in which water and various molecules are stored.¹ In plants, a vacuole typically occupies most of the cell volume and is vital to plant life. One important function of a vacuole is to store protons transported from the cytoplasm, which maintains the pH of the cytoplasm balanced and the vacuole acidic. The pH in a vacuole for higher plants is generally 5.0 - 5.5, but in the lemon fruit it can decrease to 2.5. Accumulated protons create an electrochemical gradient that can serve to drive other transporters to import various molecules into a vacuole for storage or other purposes.

As the interior environment of a vacuole is acidic, pumping protons into it occurs against a chemical gradient. Transporters of two kinds, vacuolar H⁺-ATPase (V-ATPase) and H⁺-pyrophosphatase (H⁺-PPase), use ATP and pyrophosphate (P₂O₇⁴⁻, PP_i), respectively, as energy sources to transport protons into a vacuole in plants.

To understand the function and the mechanism of H⁺-pyrophosphatase, Dr. Sun's group determined the structure of H⁺-pyrophosphatase (VrH⁺-PPase) from mung bean (*Vigna radiata*) with X-ray protein crystallography.² The task is daunting because the hydrophobic nature of H⁺-pyrophosphatase makes it difficult to handle in aqueous solution for protein purification and crystallization. It was the first all- α helical membrane protein solved in Taiwan; the result was published in *Nature*. This work used beamlines **BL13B1/BL13C1** at

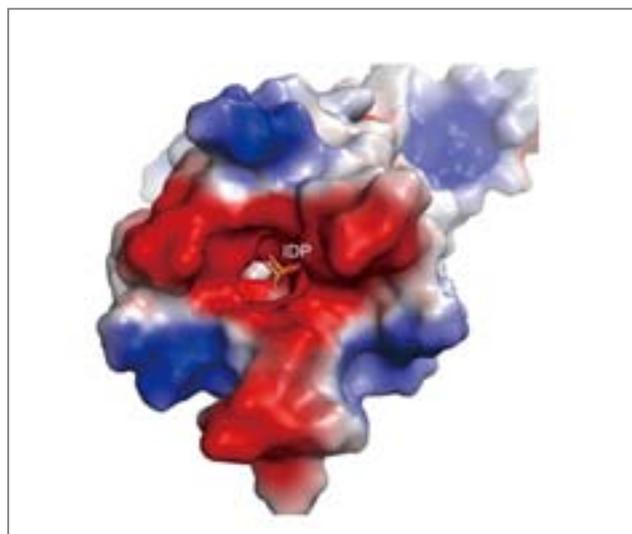


Fig. 2: Molecular surface with electrostatic potential of H⁺-pyrophosphatase shows IDP in the acidic environment rendered in red. (courtesy of Prof. Yuh-Ju Sun)

NSRRC, Taiwan, and **BL44XU/BL12B2** at SPring-8, Japan.

H⁺-pyrophosphatase is a homodimer sitting side by side on the membrane. Each monomer has 16 trans-membrane helices forming two concentric walls: six and ten helices at inner and outer circles, respectively, in a counterclockwise manner (Fig. 1). A non-hydrolyzable pyrophosphate analogue, imidodiphosphate (IDP), is co-crystallized with H⁺-pyrophosphatase and is found in a funnel-shaped pocket on the inner circle near the cytosolic end facing outside the vacuole (Fig. 2). This pocket has an atypically acidic environment, which facilitates the hydrolysis of pyrophosphate. One potassium and five magnesium ions surrounding the IDP are explained with the roles of the potassium ion as stimulator of type I H⁺-pyrophosphatase and the necessity of magnesium ions in H⁺-pyrophosphatase activity. A detailed comparison of the pyrophosphate-binding pocket between *Vigna radiata* H⁺-pyrophosphatase

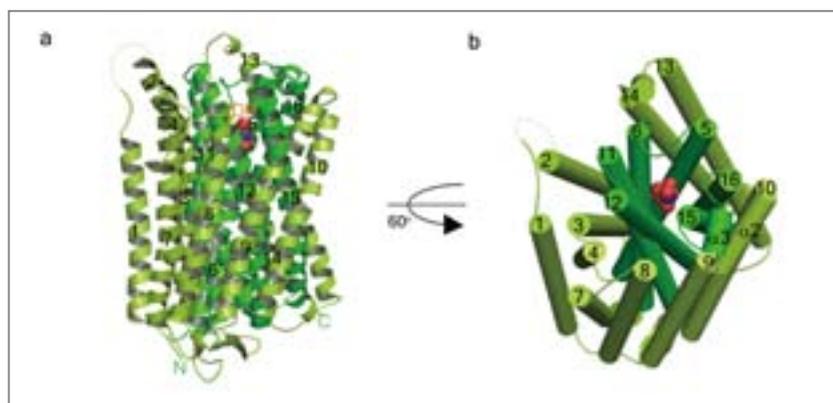


Fig. 1: Overview of H⁺-pyrophosphatase with IDP (red). Sixteen helices form two concentric walls and the proton channel locates in the center. (courtesy of Prof. Yuh-Ju Sun)

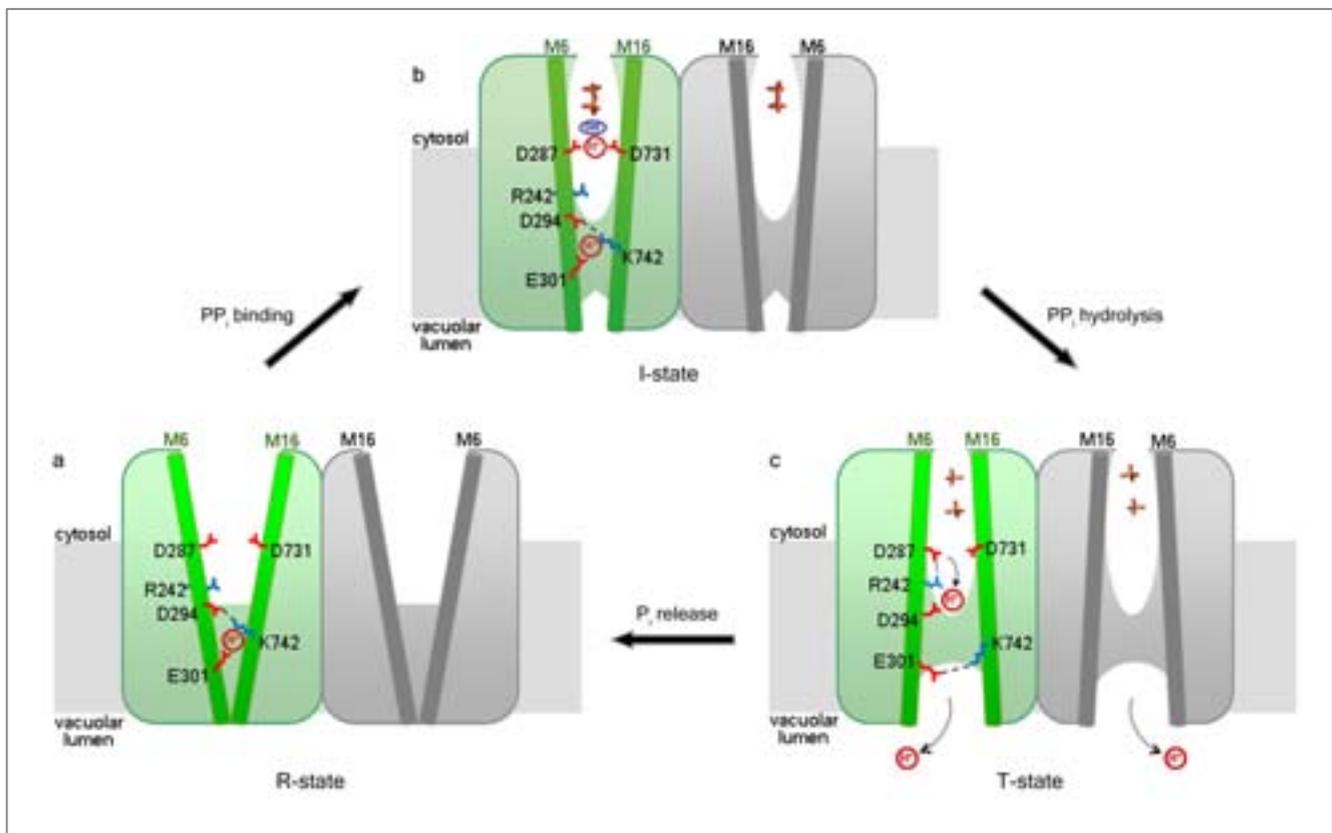


Fig. 3: A proposed pathway of proton transportation coupled with pyrophosphate hydrolyzation. At the resting state (R-state), H⁺-pyrophosphatase is open to cytosol. When pyrophosphate comes in and binds to the active site, it goes to the initiated state (I-state). During the hydrolyzation of pyrophosphate, it changes to the transient state (T-state) and release the bound hydrogen to vacuolar lumen. Finally, it goes back to R-state when P_i is released. (courtesy of Prof. Yuh-Ju Sun)

and water-soluble *Escherichia coli* pyrophosphatase (EcPPase) indicates that membrane-bound H⁺-pyrophosphatase might apply a strategy to trap the nucleophile for pyrophosphate hydrolysis different from that of the water-soluble one.

The proton pathway is found not at the dimerization interface, as previously thought, but in a narrow channel inaccessible to water from the pyrophosphate binding pocket on the cytosolic side to the luminal side toward the inside of the vacuole. Several acidic and basic amino-acid residues align in the front section of this channel; a glutamate at position 301 (Glu301) locates at the most narrow point and serves as the gatekeeper. Beyond this glutamate are several hydrophobic residues that maintain protons at a large concentration inside the vacuole away from entering the channel. Similar to other proton-pumping proteins, two water molecules are confined in the channel of H⁺-pyrophosphatase to assist the passing of protons. Through protonation and deprotonation in series among acidic and basic amino acids and bound water molecules in the channel, pro-

tons can be translocated from donors and acceptors in a sequential manner.

A model of proton translocation can be based on this structure and other biochemical knowledge (Fig. 3). In the R-state, the proton channel is open to the cytoplasm and the luminal part when the channel is closed. When the pyrophosphate binds to the acidic pocket in the I-state, the cytosolic side of the proton channel is closed and the luminal side is semi-closed. The hydrolysis of pyrophosphate in the T-state triggers a sequential transfer of protons through the channel, which are then released into the vacuole on the opening of the luminal side. This proton-pumping mechanism provides researchers with direct information to understand the working of a plant vacuole.

References

1. S.-M. Lin, J.-Y. Tsai, C.D. Hsiao, Y.-T. Huang, C.-L. Chiu, M.-H. Liu, J.-Y. Tung, T.-H. Liu, R.-L. Pan, and Y.-J. Sun, *Nature* **484**, 399 (2012).
2. L. Taiz, *J. Exp. Biol.* **172**, 113 (1992).