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Technical Note:

Environmental Responses of the Membrane Trafficking Factor PATROL1 in the Arabidopsis Stomatal Complex

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The plant stomatal complex regulates the aperture of the stomatal pore in response to changes in environmental factors such as light, water, salinity, and atmospheric gasses. Stomatal opening is achieved by activating a plasma membrane proton pump ATPase (H^+ -ATPase) to induce plasma membrane hyperpolarization, which facilitates potassium influx to guard cells. A membrane trafficking factor named H^+ -ATPase translocation control 1 (PATROL1) was isolated as a key regulator of stomatal opening in *Arabidopsis thaliana* under low CO_2 conditions (Hashimoto-Sugimoto *et al.* 2013). PATROL1 has a MUN domain with synaptic vesicle priming activity, which is crucial for neurotransmitter release in animals. The *patroll* mutation impairs both the plasma membrane localization of the H^+ -ATPase AHA1 and stomatal opening, suggesting that PATROL1 delivers AHA1 to plasma membranes depending on environmental conditions (Hashimoto-Sugimoto *et al.* 2013). Confocal imaging revealed that green fluorescent protein (GFP)-tagged PATROL1 (GFP-PATROL1) localized to the cytoplasm and dot-like components on plasma membranes, and the GFP-PATROL1 dots appeared and remained at the same site for 2–10 s, implying exocytosis or endocytosis of plasma membrane components such as AHA1 (Higaki *et al.* 2014, Higaki 2015).

The cover shows a confocal image of GFP-PATROL1 and red fluorescent protein (RFP)-tagged AHA1 in the stomatal complex of an *A. thaliana* cotyledon. The cotyledon was detached from a 7-day-old transgenic seedling, and then mounted on a slide glass with a cover glass and a drop of buffer to avoid bubbles between the cotyledon and cover glass (Higaki 2015). The specimen was observed under a fluorescence microscope equipped with confocal spinning disk confocal laser scanning unit (CSU-W1; Yokogawa, Tokyo, Japan) and a complemen-

tary metal oxide semiconductor camera (Zyla; Andor, Belfast, UK). GFP and RFP were excited at 488 nm and 561 nm, respectively, with emission wavelengths of 510–550 nm and 624–640 nm, respectively.

Our live cell imaging revealed that the GFP-PATROL1 dot density reversibly changes in response to environmental conditions; the density in the guard cells increases when the stomata close and decreases when the stomata open (Hashimoto-Sugimoto *et al.* 2013, Higaki *et al.* 2014). In addition, we recently found that salinity stress diminished GFP-PATROL1 dots but induced unusual GFP-PATROL1-labeled large bodies and rod-like structures (Sato *et al.* 2021). Cells under the hyperosmotic condition had similar large bodies, but not rods, indicating that the rod-like GFP-PATROL1-labeled structures form specifically under salinity stress conditions (Sato *et al.* 2021). In addition, the primary root growth of the *patroll* mutant was sensitive to salinity stress, suggesting that PATROL1 also contributes to salinity stress tolerance (Sato *et al.* 2021).

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