Machinery for Activating the Drought-Tolerance Response in Plants

Phytohormone abscisic acid (ABA) mediates many plant responses to environmental stresses, particularly to water status such as drought. However, it has remained unclear for the past five decades how ABA switches the stress responses. Elucidation of the mystery has progressed rapidly with the recent discovery of ABA receptors. We determined the structure of the ABA receptor PYR1-like 1 (PYL1) bound with ABA and that of the ternary complex formed by the further binding of ABA-INSENSITIVE1 (ABI1), which is a member of type 2C protein phosphatase (PP2C) and acts as a negative regulator of the stress-response pathway. The structures show that ABA induces the structural change of PYL1 to create a binding surface for shutting down ABI1.

Phytohormone abscisic acid (ABA) functions to control environmental-stress responses in plants (Fig. 1). Under non-stress conditions, type 2C protein phosphatase (PP2C) represses the ABA-mediated stress-response pathway through the inactivation of SNF1-related protein kinase 2 (SnRK2). When plants are exposed to dry conditions, ABA is accumulated in plant cells and SnRK2 is activated to mediate the expression of stress-tolerant proteins. Recently, ABA receptors were reported by two independent research groups [1, 2]. They identified the Arabidopsis PYRABACTIN RESISTANCE/PYR1-like/REGULATORY COMPONENT OF ABA RECEPTOR (PYR/PYL/RCAR) family of StAR-related lipid transfer (START) proteins as ABA receptors that inhibit the activity of PP2C in response to ABA, and identified all the players involved in ABA-mediated signal transduction. We determined the crystal structures of ABA-bound PYR1-like 1 (PYL1) and its complex with ABA-INSENSITIVE1 (ABI1) (Fig. 1) by using the BL-5A and AR-NW12A (Proposal number 2008S2-001), thus unveiling the action mechanism of ABA receptors [3].

PYL1 possesses a large, internal, water-filled cavity, wherein an ABA molecule is almost completely trapped with its carboxyl group oriented toward the center of the receptor molecule (Fig. 2). ABA is recognized with a water-mediated hydrogen-bond network and van der Waals contacts with PYL1. Two loops of PYL1, referred to as the “gate” and the “latch” loops, seem to put a lid on the cavity by van der Waals contacts with ABA

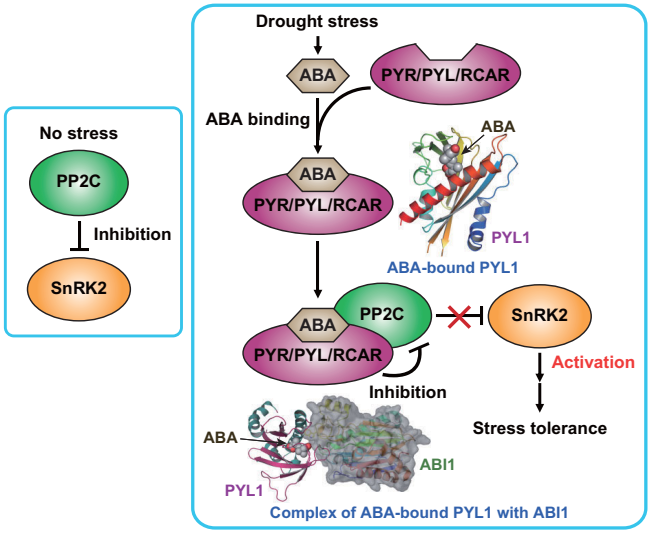

Figure 1
Schematic diagram of the ABA-mediated stress-response pathway. Crystal structures of ABA-bound PYL1 and its complex with ABI1 are also shown in this figure. ABA and PYL1 molecules are represented by sphere and ribbon diagrams, respectively. ABI1 is represented by a ribbon-and-surface diagram.

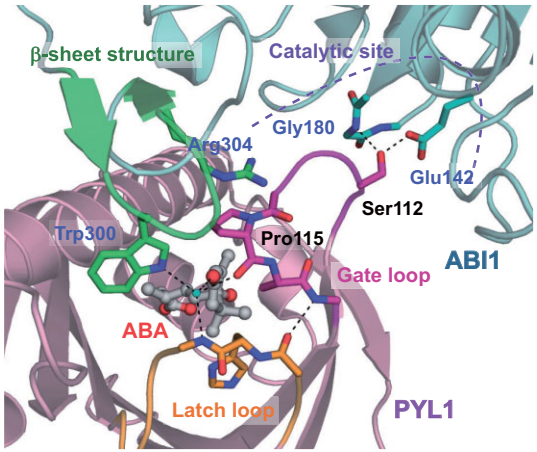

Figure 2
ABA-dependent ABI1 inhibition of PYL1. The cyan sphere and black dashed lines represent the water molecule and hydrogen bonds, respectively.

(Fig. 2). The structure of the loops covering the cavity would take an open conformation in an ABA-free form and become closed during ABA binding. In the ABA-bound state, a proline residue on the gate loop (Pro115) contacts ABA to close the gate on the cavity, whereas a serine residue on the gate loop (Ser112) is flipped out of the ABA-occupied cavity. In addition, the latch loop seems to lock the closed gate loop by a hydrogen bond and van der Waals contacts. These loops in the closed conformation then provide the surface for the interaction with ABI1 (Fig. 2). Thus, PYL1 switches on the ABA-mediated stress-response pathway by the open-to-close gating mechanism.

The ternary complex structure of ABA-bound PYL1 and ABI1 well defines the inhibition mechanism of ABI1 by PYL1 (Fig. 2). The catalytic site of ABI1 is sealed by the closed gate loop of PYL1. The serine residue (Ser112) exposed upon ABA binding forms a hydrogen bond between its side chain and one of the catalytic residues of ABI1 (Glu142). Thus, PYL1 is capable of competitively inhibiting the phosphatase activity of ABI1 in an ABA-dependent manner. On the other hand, the additional β-sheet structure of ABI1 provides the major binding interface with PYL1. There are two crucial residues for binding the closed gate and latch loops of PYL1 (Fig. 2). A tryptophan residue of ABI1 (Trp300) contacts the cleft between the two closed loops of the ABA-bound PYL1. The side chain forms a water-mediated hydrogen bond with the two closed loops and ABA. In addition, an arginine residue of ABI1 (Arg304) seems to hold down the gate loop of PYL1 by stacking between its side chain and the proline residue on the loop (Pro115). The gate loop is further locked in a closed conformation by these interactions, which makes the gate loop properly located into the active site of ABI1.

ABA-mediated signal transduction is a potent strategy for controlling responses to environmental stresses and developmental processes in plants. The structural basis of the ABA receptor provides a rational framework for the future design of alternative ligands and for engineering plants to control plant stress responses.

REFERENCES

[1] Y. Ma, I. Szostkiewicz, A. Korte, D. Moes, Y. Yang, A. Christmann and E. Grill, *Science* **324** (2009) 1064.
[2] S.Y. Park, P. Fung, N. Nishimura, D.R. Jensen, H. Fujii, Y. Zhao, S. Lumba, J. Santiago, A. Rodrigues, T.F. Chow, S.E. Alfred, D. Bonetta, R. Finkelstein, N.J. Provart, D. Desveaux, P.L. Rodriguez, P. McCourt, J.K. Zhu, J.I. Schroeder, B.F. Volkman and S.R. Cutler, *Science* **324** (2009) 1068.
[3] K. Miyazono, T. Miyakawa, Y. Sawano, K. Kubota, H.J. Kang, A. Asano, Y. Miyauchi, M. Takahashi, Y. Zhi, Y. Fujita, T. Yoshida, K. Kodaira, K. Yamaguchi-Shinozaki and M. Tanokura, *Nature* **462** (2009) 609.

BEAMLINES

5A and AR-NW12A

M. Tanokura, T. Miyakawa, K. Miyazono, K. Kubota and Y. Sawano (The Univ. of Tokyo)