Mechanism of Hormone and Effector Recognition by the Gibberellin Receptor

Plant hormones are a group of structurally different small molecules essential for various developmental processes in higher plants. In the last decade, receptors for all classical plant hormones have been identified and several emerging hormones have been recognized [1]. Current structural biology would be able to contribute toward the better understanding of the molecular mechanisms of plant hormone actions at an atomic resolution.

In the 1920s, gibberellin (GA) was initially identified as a fungus toxin from *Gibberella fujikuroi*, which causes the bakanae disease of rice, characterized by super-elongation and male sterility that reduces the rice harvest. In the 1950s, it became known that GAs were synthesized by plants, and thus GAs were accepted as an endogenous hormone. A plausible model of GA actions, however, has emerged only recently, with the remarkable findings of GA receptor GID1s, its direct downstream DELLA proteins and an F-box protein, SLY1. Here, we report the structural studies on the basis of gibberellin-induced DELLA recognition by *Arabidopsis* GID1 [2].

Gibberellins stimulate plant growth and development including seed germination, stem growth and floral development. GAs regulate the turnover of DELLA proteins, the negative transcriptional regulators, through the ubiquitin-proteasome pathway. In the absence of GA, DELLA proteins repress the GA responses. GA binds to its receptor GID1, which leads to a direct interaction with DELLA proteins. This interaction triggers the DELLA protein recognition by the ubiquitin E3 ligase SCF (SKP1-CULLIN-F-box) complex. Ubiquitylation of DELLA proteins results in the degradation of DELLA proteins at 26S proteasomes, and then the transcriptional factors liberated from the negative control by DELLA proteins start GA responses [3]. In *Arabidopsis thaliana*, there are three homologues of GA receptors, GID1A, GID1B and GID1C and five DELLA proteins, GAI, RGA and RGL1,2,3. DELLA proteins bind to GID1 through its N-terminal region referred to as DELLA domain, whereas a GRAS domain located in the C-terminus is involved in transcriptional regulation. We have succeeded in determining the structure of the ternary complex of *Arabidopsis thaliana* GID1A, bioactive GAs, GA_3 or GA_4, and the DELLA domain of GAI at 1.8 Å resolution using the data collected at **BL41XU** beamline [2].

The GID1A comprises a core domain (GID1 core) that adopts an α/β hydrolase fold with the N-terminal extension (GID1 N-Ex), which consists of three

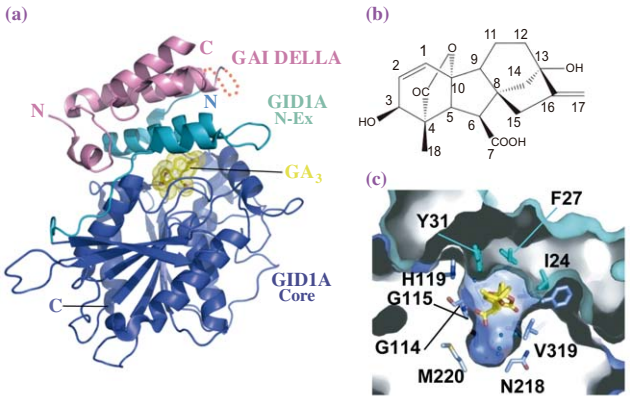

Fig. 1. Structure of the GA_3–GID1A–DELLA complex. **(a)** Ribbon representation of the GA_3–GID1A–DELLA complex, with the GAI DELLA domain (pink), GID1A N-terminal extension (N-Ex, cyan) and GID1A α/β core domain (light blue). The bound GA_3 molecule is represented in yellow. **(b)** Chemical structures and numbering of GA_3. **(c)** Binding pocket of GID1A.

α-helices loosely packed with each other (Fig. 1). The structure of the GID1 core resembles carboxylesterase as predicted, harboring a pocket for the substrate, although GID1A has no esterase activity owing to the alteration of the essential histidine residue forming a catalytic triad. Gibberellins are confined in the binding pocket between the GID1 core and GID1 N-Ex (Fig. 1(c)). This binding pocket is suitable for GA perception, by forming hydrophobic walls that tightly fit the shape of GA aliphatic rings and placing the polar residues and water molecules inside to form hydrogen bonds with all four polar groups of GA (Fig. 1(c), Fig. 2).

Hydroxylation on GA C-2, which should cause a steric clash with Tyr 31 and lead to a disadvantageous conformation for GID1A binding, is well characterized as a metabolic deactivation of GA in plants (Fig. 2(b)).

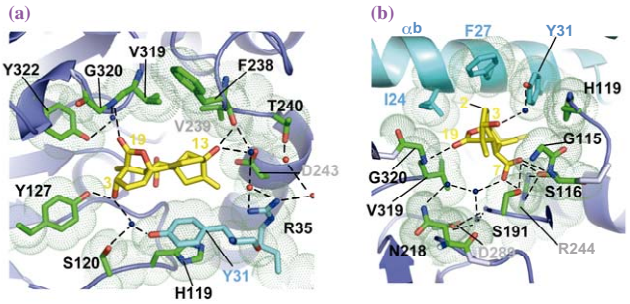

Fig. 2. Recognition of GA3 by GID1A. **(a)** Top view of the GID1A-bound GA_3. **(b)** Side view of the GID1A-bound GA3. GID1A residues that contact GA3 are highlighted in green (GID1 core) or cyan (GID1 N-Ex) and shown as stick models with van der Waals surfaces (dots) and hydrogen bonds (dotted lines).

Methylation of GA 7-carboxylate is also known to decrease the affinity by two to three orders of magnitude owing to the disruption of multiple hydrogen bonding and charge compensation by the oxyanion hole and GID1-specific arginine (Fig. 2(b)). Only a few GAs including GA_1, GA_3 and GA_4 serve as bioactive hormones although more than 100 natural gibberellins have been identified to date, which implies the rigorous GA recognition by the receptor. Our structures show the precise GA perception by the receptor and reveal the structure-activity relationship of bioactive and inactive GAs.

The DELLA domain of GAI forms four α-helices, αA to αD. The flat-shaped DELLA domain resembles a palm consisting of helices αB to αD, with helix αA being off-plane like a thumb (Fig. 3(a)). We define three conserved motifs of the DELLA domain, DELLA, LExLE and VHYNP motifs, which are essential for direct contact with the hormone receptor (Fig. 3(a)). The DELLA thumb helix αA is hooked onto the crevice of GID1A formed by the GID1A N-Ex and the GID1A core through nonpolar interactions and hydrogen bonds involving Asp 29 (Fig. 3(b)).

In view of the results from structural inspection and protease sensitivity assays, we propose that GID1 N-Ex serves as a conformational switch that senses GA. GID1 N-Ex adopts a relatively flexible conformation in the GA-unbound state, but once it perceives a GA molecule, it folds back to close the lid on the GA binding pocket and to generate the binding surface toward the DELLA domain. This conformational transition induces the structural conversion of the DELLA domain from a random coil to a helix bundle. This conversion may cause changes in the overall shape of DELLA proteins including the GRAS domain, which allows the F-box protein SLY1 to recognize DELLA proteins. GA functions as an allosteric effector of GID1A that closes the lid and triggers ubiquitylation of DELLA proteins by SCF complex without direct contact with DELLA proteins.

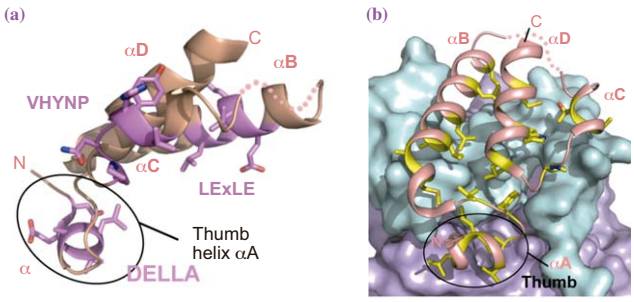

Fig. 3. Architecture of the DELLA domain of GAI and its recognition by GID1A. **(a)** A ribbon representation of DELLA domain of GAI. **(b)** Interaction between GAI and GID1. The hydrophobic palm (αB-αD) of GAI covers GID1 N-Ex by mainly hydrophobic interaction.

We refer to GID1 as a 'ubiquitylation chaperon' based on the function assisting a folding transition to stimulate a ubiquitylation by the SCF complex (Fig. 4).

Protein ubiquitylation is widely used as a degradation signal involved in diverse cellular activities as well as in several plant hormone signaling, including auxin, jasmonate and gibberellin. Notably, auxin and jasmonate receptors are F-box proteins. A recent structural study illustrates that auxin serves as the "molecular glue" that mediates contacts between the F-box protein and its substrate protein [4], whereas gibberellins, by contrast, act as the 'ubiquitylation chaperon'. Remarkably, both mechanisms are novel in that they generate an exposed degradation signal in the protein to be degraded, compared with other mechanisms that involve phosphorylation by protein kinases, unmasking by protein dissociation or creation of a destabilized N terminus by proteolysis. Thus, the structural investigation of plant hormone receptors has deepened our understanding of the degradation-signal-dependent regulation of cellular functions.

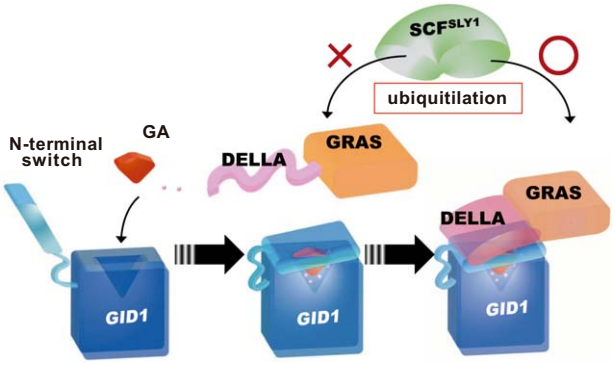

Fig. 4. A model of GA-regulated cell signaling by GID1–DELLA protein interactions.

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