

Regulation of NB-LRR-type UNI and its related signaling pathway

Signaling crosstalk and methodology for quick identification of related factors

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Activation of NB-LRR-related UNI proteins by *uni-1D* mutation, a gain-of-function mutation of the *UNI* gene, induces some pathogenesis-related responses and also affects morphology through modulation of meristem activities. In a recent study we reported that the *uni-1D* phenotypes require cooperative action of ERECTA (ER) receptor kinase family members in *UNI*-expressing cells, suggesting that an intracellular signaling crosstalk between ER-family-dependent and UNI-triggered signaling pathways plays a significant role in the phenotypes. Further we recently succeeded in the establishment of a methodology for rapid identification of factors involved in the UNI function. EMS-induced causal mutations that suppress the *uni-1D* phenotypes could be identified using whole-genome-sequencing technologies with much less labor compared with the conventional map-based cloning method that is generally time-consuming and labor-intensive. Thus it would be now possible to intensively identify factors that play significant roles in regulation of UNI proteins and/or UNI-related signaling pathways.

UNI Activation Affects Meristem Activities in a Non-Cell-Autonomous Manner

The *uni-1D* mutant of *Arabidopsis thaliana* harbors a gain-of-function and dominant mutation in the *UNI* gene, which encodes a member of the CC-NB-LRR (coiled-coil-nucleotide-binding-site-leucine-rich-repeat) family.¹ Because NB-LRR-type proteins are known to function as

molecular sensors to trigger intracellular signaling pathways upon stimulation by ligands/elicitors,²⁻⁴ the *uni-1D* mutation probably converts UNI proteins into an active state without an activation event, which triggers downstream intracellular signaling pathways; however, such a ligand or an elicitor for UNI activation have not yet been identified.

The heterozygous *uni-1D/+* plants show morphological alterations including early termination of inflorescence stem growth due to rapid consumption of stem cells with the shoot apical meristem (SAM) and formation of extra axillary meristems (AMs) at leaf axils.^{1,5} Interestingly, the *UNI* promoter is active only outside the meristems though *uni-1D/+* plants display meristem abnormalities⁵ (Fig. 1), suggesting that the SAM maintenance and AM formation might be modulated by non-cell-autonomous effects triggered by UNI activation. Expression of *WUSCHEL* (*WUS*), a central player in stem cell maintenance in SAMs,⁶ is severely reduced in *uni-1D/+* plants.⁵ Because *CLAVATA3* (*CLV3*) controls the expression pattern and level of *WUS*⁷ and there is a regulatory feedback loop between *WUS* and *CLV3*, UNI activation might affect the homeostasis of the feedback loop. Cytokinin is one of players affecting the *WUS-CLV3* regulatory loop⁸⁻¹⁰ and cytokinin-related regulation is modulated in *uni-1D/+* mutants,^{1,5} suggesting that cytokinin might be involved in the non-cell-autonomous effects observed in *uni-1D/+* mutants. We will describe the relationship between UNI signaling and cytokinin-related regulation in the next section.

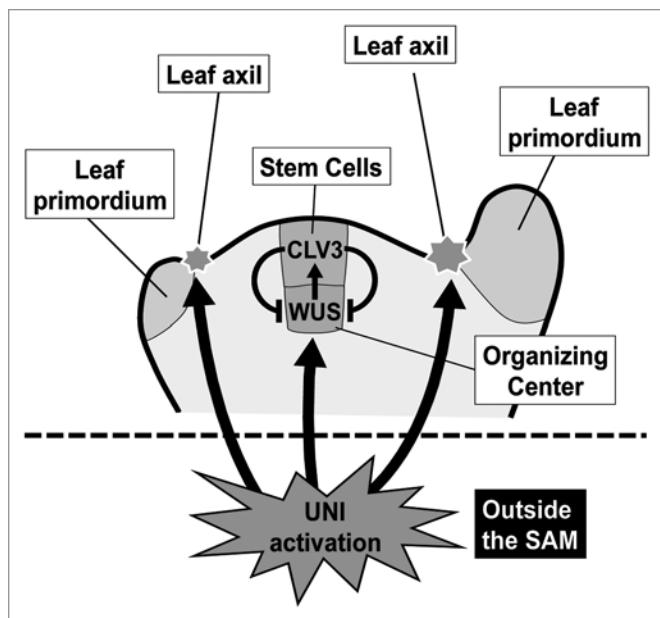


Figure 1. UNI activation non-cell-autonomously affects activities of both the SAM and axillary meristem. *UNI* promoter is active only outside the meristems. Activities of the SAMs, which are located at the top of stems, are largely attenuated in *uni-1D/+* plants and extra axillary meristems are formed at leaf axils of *uni-1D/+* plants. See the text for further explanation.

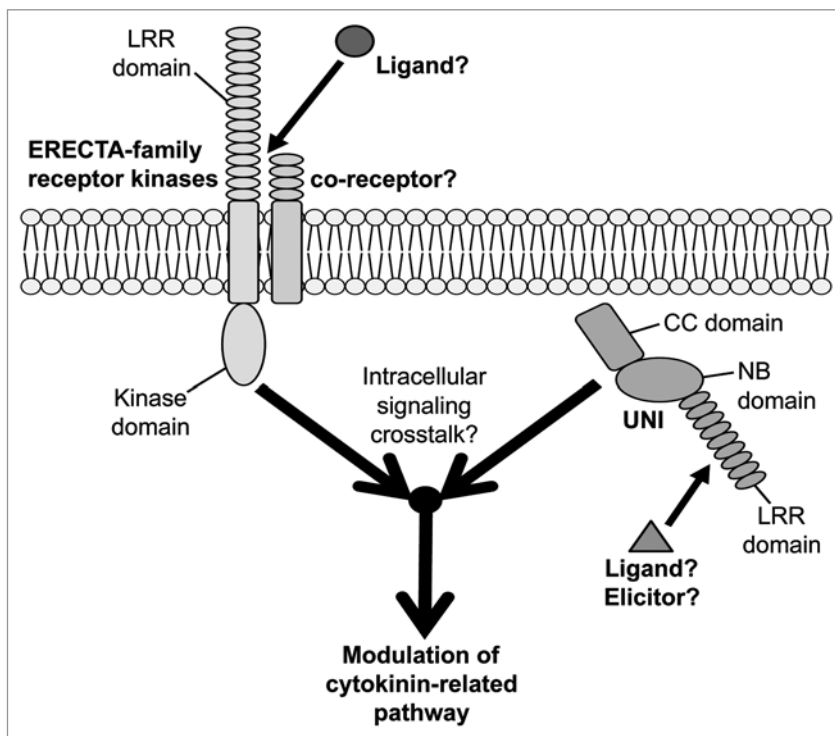


Figure 2. Intracellular signaling crosstalk between UNI-triggered and ER-family-dependent signaling pathways modulates cytokinin-related pathway. UNI harbors a CC (coiled-coil) domain, an NB (nucleotide-binding-site) domain and an LRR (leucine-rich repeat) domain. A ligand or an elicitor for activation of UNI proteins have not yet been identified. The ERECTA (ER) family members encode LRR (leucine-rich-repeat) receptor-like kinases in a subfamily of transmembrane-type receptors. Information about co-receptors that might form receptor complexes with ER-family members in UNI-related signaling and also about ligands for such receptor complexes is still lacking. Activities of ER-family members in *UNI*-expressing cells are required for *uni-1D/+* phenotypes and also for modulation of cytokinin-related pathway.

Intracellular Signaling Crosstalk between UNI-Triggered and ERECTA-Family-Dependent Signaling Pathways Modulates Cytokinin-Related Regulation

Trans-zeatin (tZ)-type cytokinins accumulate in *uni-1D/+* plants and, correspondingly, expression of the cytokinin-responsive genes is upregulated in *uni-1D/+* plants.¹ Artificial reduction of cytokinins in *uni-1D/+* mutants suppresses the abnormal morphologies of *uni-1D/+* plants, suggesting the significance of cytokinin pathways for the morphological alterations of *uni-1D/+* mutants.¹ tZ-type cytokinin production requires the *CYP735A* enzyme¹¹ and *CYP735A2* expression is upregulated in *uni-1D/+* mutants.¹ We recently reported that cooperative action of ERECTA (ER) family members in *UNI*-expressing cells is required for *uni-1D/+* morphological alterations and also upregulation of cytokinin response and *CYP735A2* expression.⁵ The ER family consists of ER, ER-LIKE 1 (ERL1) and ERL2¹² and encodes leucine-rich repeat receptor-like kinases in a subfamily of transmembrane-type signaling receptors in plants.^{12,13} Although it has been inferred that ER-family functions in receptor complexes through associations with multiple partners,¹⁴ information about such partners and about ligands for the receptor complexes in UNI-related signaling is still lacking. Because both ER-family members and UNI have protein structures that can act as starting points to trigger intracellular signaling pathways (Fig. 2), it would be interesting to investigate intracellular signaling crosstalk between signaling pathways triggered by ER-family proteins and those triggered by UNI proteins and also to analyze how these signaling pathways are connected to cytokinin-related regulation including regulation of *CYP735A2* expression.

Methodology for Rapid Identification of Factors Involved in UNI-Related Regulation by Whole Genome Sequencing

We recently established a methodology to rapidly identify the EMS-induced causal mutations that suppress the morphological

alterations of *uni-1D*^{+/+} mutants using one round of whole genome sequencing.¹⁵ This methodology requires much less labor compared with the conventional map-based cloning method that is generally time-consuming and labor-intensive. In our recent paper, we identified independent recessive mutations in *SGT1b* gene as candidates of causal mutations of two distinct *uni-1D*^{+/+} suppressor mutants (termed *sup#1* mutant and *sup#2* mutant, respectively).¹⁵ *SGT1b* is a core member of the chaperone complex that plays a significant role for NB-LRR-type proteins to exert their proper functions¹⁶⁻²⁵ and loss-of-function mutations in the *SGT1b* gene render NB-LRR proteins non-functional mainly through their destabilization. Here, as shown in **Figure 3A–C**, we confirmed the *sup#1* and *sup#2* are actually allelic to a reported *sgt1b* loss-of-function mutant. Also, as shown in **Figure 3D**, immunoblot analysis using anti-UNI antibody showed that UNI proteins are unable to stably exist in *sup#1* and *sup#2* mutants. These results demonstrate that causal mutations of *sup#1* and *sup#2* mutants are loss-of-function mutations in *SGT1b* gene, affecting the stability of UNI proteins.

Final Remarks

There are still a lot of unrevealed points about regulation of UNI protein functions and signaling pathways triggered by UNI activation.⁵ However it would be now possible to intensively identify factors that play significant roles in UNI-related regulation by our new methodology using next generation sequencing technologies.¹⁵ Many NB-LRR-type proteins have been reported to play significant roles in plant immunity as sensors for pathogen-derived factors to activate defense responses²⁻⁴ and *uni-1D* plants also show some aspects of pathogenesis responses.¹ Though so far there has been no evidence that UNI is actually involved in plant immunity, UNI is, at least partly, regulated by mechanisms common to immunity-related NB-LRR proteins.^{1,15,26} Identification of causal mutations of yet unanalyzed *uni-1D* suppressor mutants would contribute not only to analysis of the UNI function but also to further exploration into general

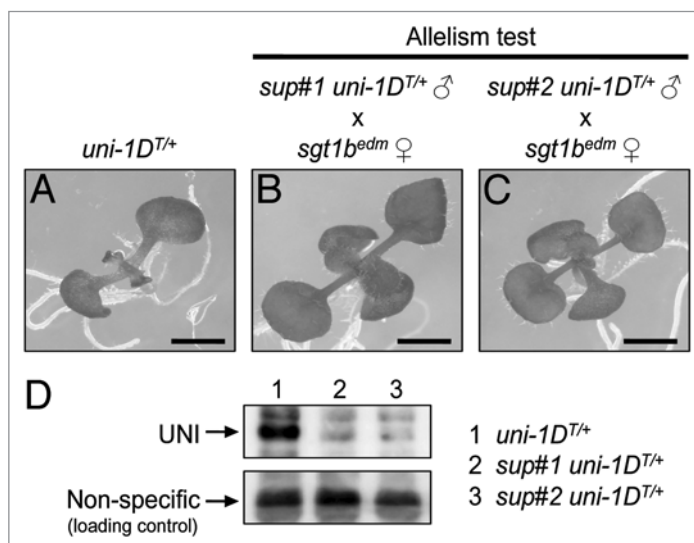


Figure 3. *sup#1* and *sup#2* mutants are allelic to *sgt1b* loss-of-function mutant and UNI proteins are unable to stably exist in both mutants. (A) 10-d-old seedling of the hemizygous transgenic plant harboring a *uni-1D* genomic fragment (hereafter, *uni-1D*^{+/+}).¹⁵ shows growth defects such as slower leaf formation and narrow leaf shape. Allelism test was performed by crossing *edm1*,²² an *sgt1b* loss-of-function mutant, with (B) *sup#1 uni-1D*^{+/+} or (C) *sup#2 uni-1D*^{+/+} plants. The resulting F1 seeds were sown on kanamycin-containing plates to ensure the presence of *uni-1D*⁺ transgene that contains a kanamycin-resistant gene. *edm* seeds were kindly gifted from Dr. Ken Shirasu (RIKEN). Bars = 1 mm. (D) Protein extracts from 10-d-old seedlings were separated by SDS-PAGE and then immunoblot using anti-UNI antibody was performed. Lane 1: *uni-1D*^{+/+}. Lane 2: *sup#1 uni-1D*^{+/+}. Lane 3: *sup#2 uni-1D*^{+/+}. Plants were grown on kanamycin-containing plates to ensure the presence of *uni-1D*⁺ transgene. Upper part shows UNI proteins and lower part shows non-specific bands detected on the same membrane as loading controls.

mechanisms that regulate NB-LRR proteins in plant immunity. In addition, it would be interesting to dissect relationships between immune responses and morphological regulation through such studies using *uni-1D* mutants.²⁶

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