学 位 論 文 の 要 約

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学 位 論 文 題 名

Quantitative imaging dissects contributions of SnRK2 and ABI3 on plasmodesmatal permeability in *Physcomitrella patens*
(原形質連絡を介した高分子の細胞間拡散を抑制する SnRK2 と ABI3 の役割の定量イメージング解析)

Cell-to-cell communication is tightly regulated in response to environmental stimuli in plants. Our laboratory previously used photoconvertible fluorescent protein Dendra2 as a model reporter to study this process. This experiment revealed that intercellular macromolecular trafficking is suppressed in response to abscisic acid (ABA) in protonemal cells of *Physcomitrella patens*. However, it remains unknown what and how ABA signaling components contribute to this suppression. I established an experimental system to quantify the process of Dendra2 movement between cells and its change by ABA treatment, based on the previous experimental system. I here clarified that the Dendra2 movement between cells is a simple diffusion process, and that among ABA signaling components, a protein kinase SUCROSE NON-FERMENTING 1-RELATED PROTEIN KINASE 2 (PpSnRK2) and a transcription factor ABA INSENSITIVE 3 (PpABI3) play roles in regulating ABA-induced suppression of Dendra2 diffusion between cells (ASD) as an essential and a promotive factor, respectively. My quantitative imaging analysis further revealed that disruption of *PpSnRK2* resulted in a defect of ASD onset itself, whereas disruption of *PpABI3* caused an 81-min delay in initiation of ASD. Live-imaging of callose with aniline blue staining showed that callose deposition on cross walls was constant during the progression of ASD irrespective of the absence or presence of *PpABI3*, suggesting that PpABI3-mediated ABA signaling facilitates ASD in a callose-independent manner. Given that ABA is an important phytohormone to cope with abiotic stresses, I next explored cellular physiological responses. I found that PpABI3 promoted acquisition of salt stress tolerance in a similar timescale of tens of minutes as ASD. These results suggest that PpABI3-mediated ABA signaling may effectively coordinate cell-to-cell communication with acquisition of salt stress tolerance. To examine this, I tested the effect of mannitol-induced hyperosmotic conditions, as one of ABA-related abiotic stresses, on Dendra2 diffusivity between cells. I found that the Dendra2 diffusivity was decreased as the degree of hyperosmolarity increased, further supporting my idea that there is a coordinated regulation between cell-to-cell communication and stress tolerance by ABA signaling. Interestingly, the effect of hyperosmolarity on the Dendra2 diffusivity was clearly detected in the disruptant of an ABA biosynthetic enzyme *ZEAXANTHIN EPOXIDASE/ABA DEFICIENT 1* (*PpABA1*), whereas it was alleviated in the disruptants of *PpSnRK2* and *PpABI3*. This suggests that ABA signaling chiefly contributes to hyperosmolarity-induced suppression of Dendra2 diffusion between cells than ABA biosynthesis, and that ABA signaling components can work without elevation of endogenous ABA levels. Finally, I present preliminary data about effects of cycloheximide (CHX), salicylic acid (SA), chitin oligosaccharide (NA-COS-Y), and FeEDTA treatments on Dendra2 diffusion between cells. Although CHX was used in an attempt to test whether ASD is elicited even without a transcriptional regulation, CHX by itself reduce PD permeability. SA, NA-COS-Y or FeEDTA treatment was performed to test whether molecular movement between cells is decreased as reported in *Arabidopsis thaliana*. Among these treatments, only NA-COS-Y treatment obviously results in decrease in intercellular Dendra2 diffusivity. These results will support our quantitative understanding in ABA signaling mechanism and function in response to various abiotic stresses.