Quantitative imaging dissects contributions of SnRK2 and ABI3 on plasmodesmatal permeability in Physcomitrella patens

Author(s)
友井 拓実

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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 photocyclable 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.