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SHORT COMMUNICATION

Regulation of cellular VAMP721/722 abundance in arabidopsis

Yunjin Choi[a]*, Soohong Kim[a]*, Chian Kwon[a], and Hye Sup Yun[b]

[a]Department of Molecular Biology, Dankook University, Cheonan, Korea; [b]Department of Biological Sciences, Konkuk University, Seoul, Korea

ABSTRACT

Sessile plants are continuously threatened by biotic and abiotic environmental stresses. Since stress responses are in general accompanied by growth retardation, plants in nature should tightly control timing and duration of their stress responses for sustained growth. We previously reported that vesicle-associated membrane protein (VAMP) 721 and 722 are required for growth/development and stress responses in plants. It is suggested that plants regulate expression of *VAMP721/722* and/or drive VAMP721/722 to form distinct SNARE complexes with different plasma membrane (PM)-residing SNARE proteins in response to distinct stimuli. We here report that immune signaling triggered by the bacterial flg22 elicitor elevates VAMP721/722 levels in calreticulin 1 and 2 (CRT1/2)-lacking plants. Since VAMP721/722 amounts were reported not to be increased by an ER stress inducer, tunicamycin in *crt1/2* plants, our results suggest that ER stress and immune signalings distinctly control cellular abundance of VAMP721/722.

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As eukaryotes, plants require soluble *N*-ethylmaleimide-sensitive factor attachment protein receptors (SNAREs) to drive vesicle fusion events that are required for interactions among subcellular compartments, cell-to-cell communications, and responses to environmental stimuli.[1,2] SNAREs are engaged in vesicle fusion by forming the SNARE complex that facilitates merging of two different membranes of a target compartment and a vesicle, which is energetically unfavorable in nature. While four distinct SNAREs (Qa-, Qb-, Qc-, and R-SNAREs) form a quaternary SNARE complex for fusion between a vesicle and an endosomal compartment, three different SNAREs (Qa-, Qbc-, and R-SNAREs) do a ternary SNARE complex (in which Qbc-SNARE serves two SNARE domains) for a vesicle and the plasma membrane (PM).[1,2]

In plants, the first identified SNARE complex comprises the PM-localized SYP121 (also called PEN1) Qa-SNARE, the posttranslationally PM-attached and two SNARE domain-containing SNAP33 Qbc-SNARE, and the secretory vesicle-residing VAMP721/722 R-SNARE.[3,4] Although this SNARE complex was first found to play a role in defense against powdery mildew fungi,[3,4] later studies revealed that VAMP721/722 are additionally important for growth, development and abiotic stress responses.[4–6] However, how the same VAMP721/722 can be involved in such diverse biological processes is not clearly known yet. Interestingly, VAMP721/722 are known to form the SNARE complex with PM-localized SYP111 (KNOLLE), SYP121, SYP123, and SYP132 Qa-SNAREs.[4,5,7,8] This strongly suggests that plants spatiotemporally regulate abundance and/or the complex-forming activity of VAMP721/722 to finely control their engagement in a specific physiological process.

We previously found that VAMP721/722 levels were transcriptionally upregulated by biotic stresses,[4,8] whereas posttranslationally downregulated by abiotic stresses in Arabidopsis.[6,9] Growth inhibition by both types of stresses suggests that the growth-related VAMP721/722 are depleted either by lowering their abundance during abiotic stress responses or by re-distributing most of them to immune responses. We recently found that an ER stress inducer, tunicamycin, regulates *VAMP721/722* expression at posttranslational as well as transcriptional levels.[10] Interestingly, posttranslational maintenance of VAMP721/722 abundance in Arabidopsis requires calreticulin 1 and 2 (CRT1/2) during ER stress responses. Reduced VAMP721/722 protein levels but WT-like induced *VAMP721/722* transcript levels by tunicamycin in *crt1/2* plants, and tunicamycin-elevated interaction between CRT1/2 and VAMP722 suggest that CRT1/2 stabilize VAMP721/722 during ER stress responses.[10]

Since flg22 also induces *VAMP721/722* transcription resulting in elevated VAMP721/722 levels,[8] we therefore examined whether CRT1/2 are also required for maintenance of VAMP721/722 abundance during the flg22-triggered immune responses. For this, we treated liquid MS-grown WT and *crt1/2* plants with 1 μM flg22 for 24 h, and analyzed CRT1/2 and VAMP721/722 levels by immunoblot with anti-CRT1/2 and anti-VAMP721/722 antibodies. While flg22 slightly elevated CRT1/2 levels, it greatly increased VAMP721/722 levels in WT plants as previously reported (Figure 1(a)). Interestingly, we detected WT-comparable VAMP721/722 levels that were elevated by flg22 in *crt1/2* plants (Figure 1(a)). This suggests that unlike ER stress responses, CRT1/2 are dispensable to VAMP721/722 accumulation in immune responses. Since flg22 induces growth

CONTACT Hye Sup Yun hsyun@konkuk.ac.kr Department of Biological Sciences, Konkuk University, Seoul 05029, Korea
*These authors contributed equally to this work.

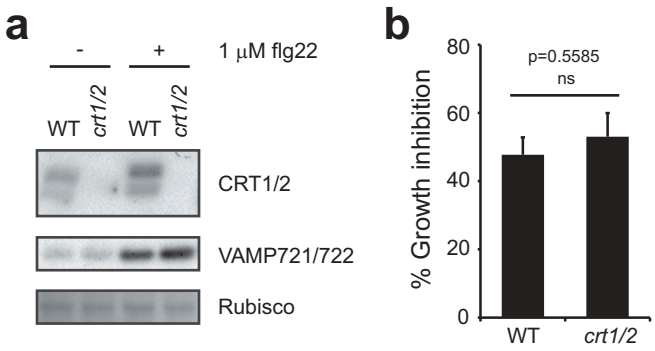

Figure 1. Dispensability of CRT1/2 in VAMP721/722 accumulation during immune responses. (a) CRT1/2 are not required for maintaining the flg22-elevated VAMP721/722 levels. Seven-d-old WT and *crt1/2* plants grown in liquid MS medium were treated with or without 1 μM flg22 for 2 d. Protein extracts were subject to immunoblot with anti-CRT1/2 and anti-VAMP721/722 antibodies. Equal loading was visualized by staining Rubisco with Coomassie. (b) No further growth inhibition by flg22 in *crt1/2* plants. Seven-d-old WT and *crt1/2* plants grown in liquid MS medium were treated with or without 1 μM flg22 for 5 d. Growth inhibition was measured by dividing weight of treated plants by that of untreated plants. Bars indicate mean ± SE (n = 4 biological replications, Student t-test).

inhibition for the cost of immune responses,[8] we additionally investigated growth retardation by flg22 in *crt1/2* plants. By comparing fresh weight of WT and *crt1/2* plants that were treated or not with 1 μM flg22 for 5 d, we measured the degree of their growth inhibition by flg22. With this, we found that the flg22-induced growth inhibition was indistinguishable between WT and *crt1/2* plants (Figure 1(b)). This further supports an irrelevant function of CRT1/2 in maintaining VAMP721/722 abundance in immunity, because VAMP721/722 are required for sustained growth during immune responses.

VAMP721/722 are R-SNAREs to direct secretory vesicles to the PM for exocytosis. Their involvement in cell division, growth/development, and responses to biotic and abiotic stresses[4-8] suggests that VAMP721/722 vesicles deliver distinct cargo depending on a cue. Interestingly, VAMP721/722-lacking plants show more reduced growth in response to the flg22 bacterial elicitor, the ABA abiotic stress hormone and the ER stress-inducing tunicamycin.[6,8,10] This suggests that plants sustain growth even during biotic, abiotic and ER stress responses, which in general retard plant growth, by employing a part of VAMP721/722 likely for transporting growth-related molecules. For this steady growth, plants upregulate *VAMP721/722* transcription especially during biotic and ER stress responses. Intriguingly, plants additionally engage CRT1/2 to maintain elevated VAMP721/722 levels during ER stress responses, because their protein levels are no more elevated by tunicamycin in *crt1/2* plants.[10] However, our present results that flg22 induces WT-like elevation of VAMP721/722 levels even in *crt1/2* plants (Figure 1(a)) indicate that CRT1/2 are not required for VAMP721/722 accumulation during immune responses. ER stress greatly elevates CRT1/2 levels and relocates some of them to the cytosol.[10] Since CRT1/2 and VAMP721/722 are likely to interact in the cytosol,[10] this implies that plants may have evolved an additional checkpoint to regulate VAMP721/722 abundance during ER stress responses. Rather transient elevation of VAMP721/722 levels in the presence of tunicamycin[10] may suggest that CRT1/2 stabilize elevated VAMP721/722 for sustained growth during early ER stress responses possibly resulting from a harsh condition. Since ABA posttranslationally downregulates VAMP721/722 levels,[6] whether CRT1/2 are also involved in maintaining VAMP721/722 abundance during ABA responses is also of interest.

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