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ARTICLE ADDENDUM

SlPRA1A/RAB attenuate EIX immune responses via degradation of LeEIX2 pattern recognition receptor

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ABSTRACT

Pattern recognition receptors (PRR) are plasma membrane (PM) proteins that recognize microbe-associated molecular patterns (MAMPs), triggering an immune response. PRR are classified as receptor like kinases (RLKs) or receptor like proteins (RLPs). The PM localization of PRRs, which is crucial for their availability to sense MAMPs, depends on their appropriate trafficking through the endomembrane system. Recently, we have identified SlPRA1A, a prenylated RAB acceptor type-1 (PRA1) from *S. lycopersicum*, as a regulator of RLP-PRR localization and protein levels. SlPRA1A overexpression strongly decreases RLP-PRR protein levels, particularly those of LeEIX2, redirecting it to the vacuole for degradation. Interestingly, SlPRA1A does not affect RLK-PRRs, indicating its activity to be specific to RLP-PRR systems. As PRA1 proteins stabilize RABs on membranes, promoting RABs activity, we aimed to identify a RAB target of SlPRA1A. Screening of a set of *A. thaliana* RABs revealed that AtRABA1e is able to mimic SlPRA1A activity. Through live cell imaging, we observed that SlPRA1A enhances AtRABA1e localization on SlPRA1A positive punctuated structures. These results indicate that AtRABA1e is a putative target of SlPRA1, and a co-regulator of LeEIX2 trafficking and degradation.

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Recognition of microbe-associated molecular patterns (MAMPs) depends on plasma membrane (PM) receptors termed pattern recognition receptors (PRRs), which lead to activation of signal transduction upon microbe perception/recognition.[1,2] PRRs traffic from the endoplasmic reticulum, where they are synthesized, through different endomembrane compartments to the PM, were they function to bind MAMPs. Therefore, PRR localization at the PM is pivotal to enable a proper and efficient immune response.[3] PRRs are classified in two groups; receptor like kinases (RLKs) and receptor like proteins (RLPs), according to the presence or absence of a kinase domain, respectively.[4]

Recently, we have identified SlPRA1A, a prenylated RAB acceptor type-1 (PRA1A) protein from *Solanum*,[3] as a component of the trafficking machinery involved in PRR-trafficking and immunity [5]). SlPRA1A regulates trafficking of RLP-PRRs and LeEIX2 in particular, but not of RLK-PRRs such as FLS2, demonstrating SlPRA1A specificity for PRR regulation.[5] LeEIX2 is a *S. lycopersicum* RLP-PRR that recognizes the fungal MAMP – EIX, triggering immune responses characterized by oxidative burst, induction of ethylene production and hypersensitive response.[6–9] We have demonstrated that LeEIX2 PM localization and protein level are highly diminished upon SlPRA1A overexpression, due to redirection of LeEIX2 to the vacuole where it is degraded.[5] Consequentlly, LeEIX2 depletion mediated by SlPRA1A, strongly decreases LeEIX2s sensing capabilities, impairing the plant immune response to this MAMP.

RABs are small GTPases which play an important role in endomembrane trafficking, being implicated in vesicle fusion at the target compartment, where they are accumulate.[10] Endomembrane trafficking of proteins is highly dynamic and highly dependent on RAB function.[10,11] Overexpression or loss of function of these proteins can generate significant changes in protein trafficking, cell functioning and plant physiology.[11] Several reports show the significant role that RABs play in plant immunity; in secretion of defense components and in hypersensitive response execution.[12–15] Indeed, RABs can be target for inhibition by bacterial effectors, secreted by pathogens [16] or targeted for hijacking during viral infection.[17] PRA1 proteins regulate RABs by stabilizing their location at cell membranes.[18] Promoting RAB activity and consequently the trafficking mediated by them.[19] In this context, it will beintriguing to identify the putative RAB target regulated by SlPRA1A and establish its regulatory role in LeEIX2 trafficking and degradation.

We have performed a screen, overexpressing a set of RAB proteins from *Arabidopsis thaliana*, searching for RABs which can mimic the effect of SlPRA1A overexpression on EIX defense responses.[5] Oxidative burst after EIX exposure was measured to test RABs effect on LeEIX2 mediated defense responses, (Figure 1). Among analyzed AtRABs, AtRABA1e, an early endosomal/Trans-Golgi Network (EE/TGN) RAB,[20] showed a diminished response to EIX treatment, resembling the effect of SlPRA1A (Figure 1A). Interestingly, another EE/TGN RAB, RABD2b that highly colocalized with SlPRA1A,[5] did not affect the oxidative burst triggered by EIX (Figure 1A). Additionally, AtARA6 and AtARA7, two extensively studied late endosomal RABs,[21,22] did not significantly affect EIX induced defense

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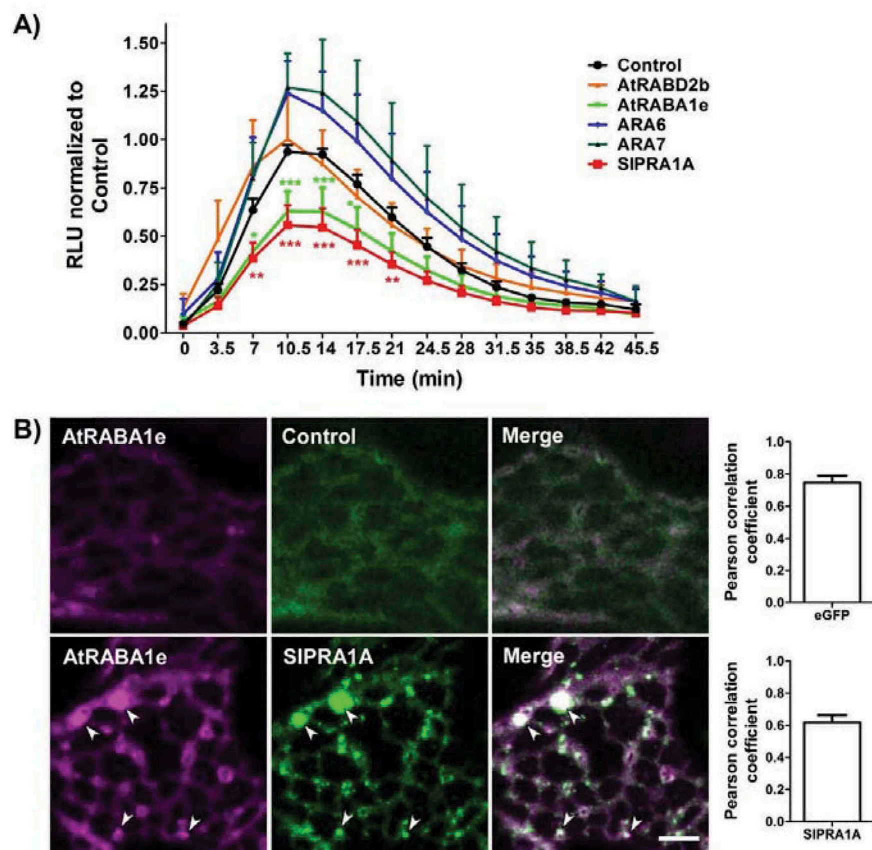

Figure 1. Effect of RABs on EIX induced oxidative burst. A) ROS oxidative burst was measured in *N. tabacum* transiently expressing free mCherry (control), SlPRA1A-mCherry, AtRABA1e-mCherry, AtRABD2b-mCherry, ARA6-GFP or ARA7-GFP using a luminol luminescence-based system. ROS production is normalized to the peak value of the control. Asterisks represent statistical significance (* p-value ≤ 0.05, ** p-value ≤ 0.01, *** p-value ≤ 0.001) in two-way ANOVA and Bonferroni post-tests. Data are represented as mean ± SEM. B) Confocal microscopy images of *N. benthamiana* epidermal cells transiently expressing SlPRA1A-GFP or free-eGFP as control (green) and AtRABA1e-mCherry (magenta). Representative images are shown. Scale bar 5 µm. White arrowheads point to SlPRA1A compartments co-localizing with AtRABA1e. Pearson correlation coefficient of the co-localization between SlPRA1A and the markers (N = 15). Data presented as mean ± SEM.

responses (Figure 1A). Previous studies using the PRR FLS2 showed that FLS2 is localized in AtARA7 and AtARA6 compartments after elicitation, and that ARA7 function is needed for FLS2 endocytic trafficking.[12,23] In our experiments we observed a slight (not significant) increase in the oxidative burst when AtARA6 and AtARA7 are overexpressed (Figure 1A). Further experiments should be undertaken to explore the role of these two RABs in EIX induced defense responses.

The oxidative burst results suggest that AtRABA1e, may be a specific candidate for SlPRA1A regulation. AtRABA1e is involved in cell plate formation.[24] However, its role in immune defense has not been described so far. Using live cell imaging, we observed high colocalization between SlPRA1A and AtRABA1e, providing a subcellular platform where they could interact (Figure 1B). Interestingly, while AtRABA1e is mainly localized in the cytoplasm in control conditions, we observed that co-expression with SlPRA1A strongly increased AtRABA1e localization in punctuated structures that SlPRA1A positive structures (Figure 1B). The shift in AtRABA1e localization, supports a possible role of SlPRA1A in stabilization of AtRABA1e at the membrane, promoting its activity. Taken together the role of SlPRA1A in driving LeEIX2 to vacuolar degradation [5] the effect of AtRABA1e on EIX induced oxidative burst (Figure 1A) and the effect of SlPRA1A on AtRABA1e localization (Figure 1B) lead us to hypothesize that AtRABA1e is a target of SlPRA1A regulation, and together they regulate LeEIX2 trafficking and degradation.

Here we identified a putative target of SlPRA1A regulation using *A. thaliana* RABs. We now intent to isolate the *S. lycopersicum* ortholog of AtRABA1e and determine its role as a SlPRA1A target in an endogenous system. We seek to continue deciphering the trafficking machinery regulating LeEIX2 at the protein and sub-cellular levels and investigate its linkage with EIX immune responses.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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