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Article Addendum

Regulation of Plant Innate Immunity by SUMO E3 Ligase

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Addendum to:

Salicylic Acid-Mediated Innate Immunity in Arabidopsis *is Regulated by SIZ1 SUMO E3 ligase*

Lee J, Nam J, Park HC, Na G, Miura K, Jin JB, Yoo CY, Baek D, Kim DH, Jeong JC, Kim D, Lee SY, Salt DE, Mengiste T, Gong Q, Ma S, Bohnert HJ, Kwak SS, Bressan RA, Hasegawa PM, Yun DJ

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ABSTRACT

Reversible posttranslational modification of proteins by the action of small ubiquitin-like modifier (SUMO) peptide (sumoylation) has been known to participate in various biological processes in eukaryotes. However, much less is known about the role of sumoylation in plants. In our recent paper to which we write this Addendum, we show that loss of SIZ1, a SUMO E3 ligase, results in a highly increased SA-mediated defense signaling through a PAD4-dependent pathway. This signaling leads to constitutively expressed pathogen related (PR) genes and to increased disease resistance to a virulent bacterial pathogen. These findings significantly increase our understanding of the role of sumoylation in the plant defense system.

Sumoylation is a posttranslational modification process that covalently conjugates the small ubiquitin-like modifier (SUMO) protein to the K (lysine) residues located at binding sites of sumoylation target proteins.[1,2] The SUMO conjugation/de-conjugation process occurs in a sumoylation cycle that is mediated by E1-activating protein, E2-conjugation protein, E3-ligase and SUMO protease.[2] The 3-D structure of SUMO peptide is highly similar to the ubiquitin peptide that affects many biological processes by mediating protein-degradation. Even though sumoylation and ubiquitination may, in some instances, affect the same target protein, the biological function of sumoylation can be substantially different from ubiquitination.[3] In yeast and animals, SUMO is linked to numerous subcellular processes such as cell cycle progression, DNA repair, nucleocytoplasmic trafficking, subnuclear targeting, ubiquitination antagonism, and transcriptional regulation.[2-5]

Although there are few examples of SUMO function in plants, in vitro and in planta biochemical evidence has established the existence of an Arabidopsis SUMO sumoylation/de-sumoylation system and this system appears to play an important role in various plant environmental responses including heat shock, oxidative stress, ABA signaling, flowering, and phosphate deficiency.[6-10] In addition to these responses, it has long been speculated that SUMO could have an important role in plant defense against pathogens. Even though bacteria do not contain a sumoylation/de-sumoylation system, they have proteins that are structurally similar to SUMO proteases (Fig. 1). The *Xanthomonase campestris* effectors, XopD and AvrXv4 migrate to the plant nucleus after injection by the type III secretion system, and have SUMO protease activity.[11-13] Disruption of SUMO isopeptidase activity of AvrXv4 prevents the hypersensitive response in tabacco.[13] There are three specific *SUMO* genes (*SUMO 1, 2, 3*) that encode SUMO peptides that are slightly different in size and sequence from each other. Interestingly, in vitro analyses reveal that XopD can cleave all three SUMO conjugates. However, Arabidopsis SUMO proteases ULP1C, ULP1D, and ESD4 cleave SUMO1, 2- but not SUMO3-conjugates.[14] These results indicate that the SUMO conjugation/de-conjugation cycle plays some role in the plant innate immunity response system. However, to this point, direct evidence of any details for the role of SUMO in plant defense responses have been lacking.

In our study, we examined the role of SIZ1, a SUMO E3 ligase, in plant pathogen defense. The *siz1* mutant plants have a reduced growth phenotype, overexpress PR genes and exhibit resistance to the pathogen *Pseudomonas syringae* pv. *tomato (Pst)*. These characteristics are seen in other mutant plants with high levels of salicylic acid (SA).[15] As we expected, endogenous SA levels in *siz1* plants are elevated. When the *nahG* gene, which encodes a salicylic acid hydroxylase, is introduced into *siz1* mutant plants, a decreased SA level, and a reversal of the other altered phenotypes to wild-type results.[15] SIZ1 controls PR gene expression and disease resistance through an epistatic interaction with PAD4. Further, *Pseudomonas* resistance of *siz1* plants is mediated specifically by a EDS1/PAD4-dependent TIR-NBS-LRR-type *R* gene pathway.[15] Based on these observations,

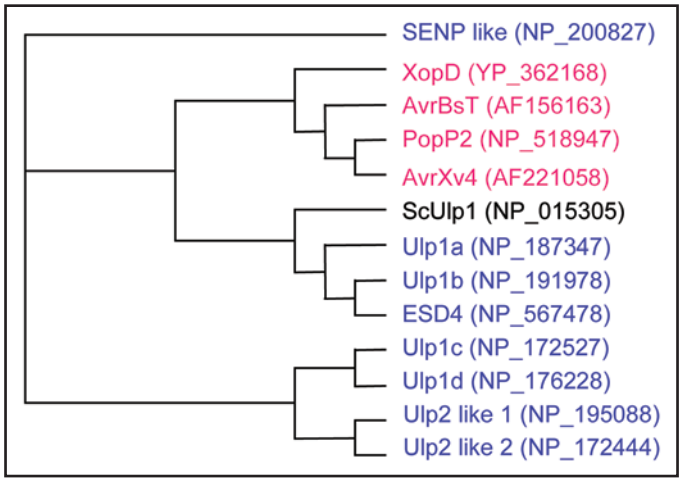

Figure 1. Phylogenic analysis of SUMO protease-like proteins from plant pathogens (red), Arabidopsis (blue), and yeast (black). Proteins were grouped into a phylogram using the Clustal W server at the http://www.ebi.ac.uk/clustalw/.

we concluded that SIZ1 SUMO E3 ligase negatively regulates SA-mediated pathogen defense signaling through a PAD4-dependent process in Arabidopsis.

We have recognized PAD4, EDS1, and SAG101 as possible targets of SIZ1. These proteins have conserved SUMO attachment motifs and regulate SA biosynthesis. Also, because the EDS1/PAD4-mediated specific *R* gene pathway is hyperactivated in *siz1* plants, it is possible that specific R protein(s) that regulate expression of PAD4/EDS1 might be direct or indirect targets of SIZ1. Recently, it has been reported that *Ralstonia. solanacerum* PopP2, a type III effector protein that structurally resembles SUMO protease, physically interacts with RRS1, a TIR-NBS-LRR type R protein, from *A. thaliana* (Fig. 1).[16] The identification of specific R proteins that are modified by sumoylation/de-sumoylation would reveal a new important regulatory mechanism that controls plant-pathogen interactions.

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