Keeping a Lid on Shoot Regeneration: SIZ1 Suppresses Wound-Induced Developmental Reprogramming

Plants have the remarkable ability to regenerate tissues and organs in response to wounding. This process relies on the formation of callus, a mass of unorganized cells that provides protection to the wounding site while allowing cell reprogramming and proliferation. Callus can be formed in vitro by incubating explants on auxin-rich callus-inducing medium (CIM), which stimulates reactivation of lateral root formation (Sugimoto et al., 2010). Subsequently, callus can be transferred to cytokinin-rich shoot-inducing medium (SIM), which triggers reprogramming of callus cells to shoot meristem identity and allows regeneration of a whole plant. Extensive transcriptional networks involving a wide range of transcription factors, hormones, and additional regulators have been identified that control each step of these developmental transitions. However, it has remained unknown if posttranslational regulation occurs during these wounding and developmental processes.

Various posttranslational protein modifications increase the size and functionality of proteomes, enabling exquisite control of stress responses and signaling pathways. SUMOylation, the conjugation of the SMALL UBIQUITIN-LIKE MODIFIER (SUMO) to target proteins, is a well-characterized protein modification that controls the activity of many important transcriptional regulators in plants (Augustine and Vierstra, 2018). SUMO is conjugated to proteins by an enzymatic cascade involving a SUMO-activating E1 enzyme, a SUMO-conjugating E2 enzyme, and SUMO E3 ligases. The Arabidopsis (*Arabidopsis thaliana*) genome encodes two SUMO E3 ligases: METHYL METHANESULFONATE-SENSITIVE21 (MMS21) and SAP AND MIZ1 (SIZ1). Of these, SIZ1 is the predominant E3 ligase responsible for the majority of SUMO ligation to target proteins (Rytz et al., 2018). While SUMOylation has been implicated in some developmental processes, it is unknown whether it functions in wounding-induced regeneration.

In this issue of *Plant Physiology*, Coleman et al. (2020) report that SIZ1 suppresses in vitro shoot regeneration in Arabidopsis. The authors compared the shoot regeneration capacities of wild-type and *siz1* mutant plants by incubating explants on CIM before transferring them to SIM. Shoot regeneration was dramatically enhanced in *siz1* mutants, with new shoots appearing earlier and in greater numbers than in wild type. Expression of a *SIZ1* transgene under its native promoter rescued this enhanced shoot generation phenotype of *siz1* plants. At the time of transfer between CIM and SIM, callus size and morphology were indistinguishable between wild type and *siz1* mutants. These results suggest that SIZ1 negatively regulates in vitro shoot regeneration.

To uncover any transcriptomic differences between wild type and *siz1* mutants, the authors performed RNA-sequencing at different time points during shoot regeneration. The difference in global gene expression was most apparent immediately after cutting, with 1,375 genes upregulated and 912 genes downregulated in *siz1* compared to wild type. After 4 d of incubation on CIM, the difference between genotypes was much less pronounced, but after transferring the cuttings to SIM, *siz1* plants again showed profound differences in gene expression compared to wild type at both 4 and 6 d posttransfer. The misexpressed genes in *siz1* plants were mostly distinct after either cutting or incubation on CIM or SIM. Gene ontology analyses revealed that genes upregulated in *siz1* after cutting were largely associated with stress responses. The authors compared these genes to those identified as upregulated in intact *siz1* plants in other studies (Catala et al., 2007; Rytz et al., 2018) and established that most of the upregulated genes in the Coleman et al. (2020) study were unique to cutting. Together, these data suggest that *siz1* mutants display a heightened stress response to wounding. Accordingly, in the absence of any external hormone treatment, *siz1* mutants displayed enhanced callus formation compared to wild-type plants after cutting of hypocotyls.

Wounding responses in plants are regulated by a range of phytohormones including salicylic acid (SA), jasmonic acid, abscisic acid, and ethylene. Many genes representing gene ontology categories related to all of these hormones were upregulated in *siz1* mutants after cutting. It was previously shown that the dwarf phenotypes and autoimmunity of *siz1* mutants were due to high levels of SA, and these phenotypes were reversible by expression of a bacterial salicylate hydrolase, *NahG*, which degrades SA (Lee et al., 2007). To determine if the enhanced shoot regeneration phenotype of *siz1* is a result of high SA levels, Coleman et al. (2020) compared *siz1* plants expressing *NahG* to *siz1* single mutants. No difference in shoot regeneration was observed between these genotypes, suggesting that the enhanced shoot regeneration of *siz1* mutants is independent of SA signaling.

The ability of plant tissues to form callus and acquire regeneration capacity after wounding is promoted by the transcription factor WOUND-INDUCED DEDIFFERENTIATION1 (WIND1; Iwase et al., 2011). Coleman et al. (2020) report that expression of both *WIND1* and its

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homolog *WIND2* are elevated in *siz1* mutants after cutting. To determine if the enhanced shoot regeneration observed in *siz1* mutants was due to elevated *WIND1* expression, the authors expressed a dominant-negative form of WIND1 fused to a SUPERMAN repression domain (SRDX) in *siz1* plants. These *siz1-3 WIND1-SRDX* plants showed less shoot regeneration than *siz1-3* plants, but this phenotype was not rescued to wild-type levels. This suggests that the enhanced shoot regeneration capacity of *siz1* plants is partially due to enhanced WIND1 activity. The authors further investigated this by comparing genes upregulated in *35S::WIND1* seedlings from a previous study (Iwase et al., 2011) to those upregulated in *siz1* at 4 d on CIM. There was a substantial overlap of genes, further suggesting that the enhanced shoot regeneration of *siz1* plants is due to a hyperactive wounding response.

The authors also examined transcriptional responses to hormones that are involved in both the acquisition of regeneration capacity and in vitro shoot regeneration. While overall transcriptional responses to auxin were unaffected in *siz1*, transcription of cytokinin-responsive genes was altered in *siz1* plants. This altered transcription included enhanced expression of the key regulator of shoot meristem induction *WUSCHEL* (*WUS*). Compared to wild type, *siz1* mutants were hypersensitive to exogenously applied cytokinin. Together, these data suggest that SIZ1 is involved in regulating the cytokinin response in the context of shoot meristem regeneration.

In summary, this exciting study by Coleman et al. (2020) shows that in vitro shoot regeneration is suppressed by SIZ1-mediated regulation of the transcriptional wounding response. While loss of SIZ1 function does not appear to affect auxin-induced callus formation or pluripotency, it enhances cytokinin-induced shoot meristem regulators. It is unclear how SIZ acts on this pathway, and further work should focus on identifying SUMO substrates modified by SIZ1 during the different stages of in vitro shoot regeneration. This study did not examine any effects of the loss of SIZ1 function on global SUMOylation during shoot regeneration or identify any candidate proteins that might be SUMO substrates during this process. These experiments will be necessary in the future to understand how SIZ1 directly influences in vitro shoot regeneration and may uncover additional targets of SUMOylation that can be modulated to engineer shoot regeneration potential and/or stress tolerance in plants.

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