PNAS COMMENTARY

SOS1 safeguards plant circadian rhythm against daily salt fluctuations

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The rotation of the Earth has generated cyclic changes in environmental factors. To fully exploit these predictable oscillatory factors for better fitness, plants have evolved an intricate endogenous timing system called the circadian clock. The plant circadian clock system perceives environmental cues like light and temperature through its input pathways. The input pathways then deliver these environmental cues to the core oscillators which are composed of interlocked transcription–translation feedback loops, generating self-sustaining circadian rhythms in various physiological processes through the output pathways (1, 2). Since most of the core oscillators are transcription factors, the plant circadian clock influences global transcription profoundly. Comprehensive transcriptomic studies suggest that 89% of transcripts in *Arabidopsis* cycle under certain conditions and the circadian clock system is responsible for the oscillatory expression of more than one-third of the genes in *Arabidopsis* (3). Strikingly, a study on plant transcriptome changes in response to salt, osmotic, and cold stresses suggests that 68% of the circadian oscillatory genes are involved in stress responses (4). It is now increasingly clear that the plant circadian clock plays an indispensable role in diverse plant stress responses (5). In PNAS, Cha et al. discover that SALT OVERLY SENSITIVE 1 (SOS1), a plasma membrane (PM) Na^+/H^+ antiporter, interacts with and stabilizes GIGANTEA (GI), a core circadian clock component, to realize period compensation of plant circadian clock under daily fluctuating salt levels (6).

Due to inappropriate irrigation practices, misuse of fertilizer, and industrial pollution, salt stress has become one of the major hurdles curbing the yield of various crops, affecting over 6% of the world's total land area (7). In response to the ionic toxicity triggered by salt stress, plants rely on the SOS pathway to transport excessive Na^+ from the cytoplasm to the apoplast, thus ensuring endogenous ionic homeostasis. Although the bona fide sensor of Na^+ is yet to be identified in plants, elevated intracellular and intercellular Na^+ levels were found to trigger cytosolic Ca^{2+} signals, which are decoded by SOS3, an EF-hand Ca^{2+}-binding protein. SOS3 interacts with and activates SOS2, a serine/threonine protein kinase, in a Ca^{2+}-dependent manner. Activated SOS2 then phosphorylates SOS1, which releases the inhibition by the C-terminal autoinhibitory domain of SOS1, activating the Na^+/H^+ antiporter activity of SOS1. SOS1 transports excessive Na^+ outward of the cytoplasm in exchange for inward transportation of H^+. The H^+ gradient across the PM which drives the activity of SOS1 is generated by PM H^+-ATPase activated by salt stress (8).

The plant circadian clock gates plants' responses to salt stress. *Arabidopsis* seedlings are more sensitive to salt stress during the day (9). The diurnal differences in responses to salt stress are likely due to the diurnal differences in transpiration rate, which is significantly higher during the day than at night (10). *SOS1* transcript levels oscillate under both diurnal and circadian conditions. Salt causes a significantly higher induction in *SOS1* transcript level when treated during the day than during the night. Besides transcriptional regulation, the plant circadian clock also has posttranslational control on SOS1. While the SOS1 protein abundance in the SOS1 overexpression seedlings does not oscillate under basal conditions, salt treatment during the day leads to a robust diurnal oscillation of SOS1 protein abundance (9).

A posttranslational regulation on the SOS pathway by the plant circadian clock was previously reported by Kim et al. (11). GI was found to negatively regulate salt tolerance by sequestration of SOS2. In the absence of salt stress, GI cages SOS2 and inhibits SOS2-mediated phosphorylation of SOS1. Upon salt stress, GI is degraded through the 26S proteasome pathway. At the same time, salt stress and SOS3 also suppress the interaction between GI and SOS2. Consequently, SOS2 was released to phosphorylate SOS1, which both enhances the protein stability of SOS1 and activates its Na^+/H^+ antiporter activity to alleviate salt stress (Fig. 1).

While the plant circadian clock has an extensive impact on plant responses to salt stress, salt stress may also have feedback regulations on the circadian clock, as the bidirectional interplays between the plant circadian clock and various environmental factors are well-known. A diverse array of environmental Zeitgebers (aka time-givers) of plant circadian clock have been discovered including sugars like glucose and fructose, inorganic nitrogen like KNO_3 and NH_4NO_3, nitrogen assimilation products like glutamate and glutamine, and mineral elements like iron and magnesium (5). Further considering the diurnal oscillation of transpiration rate, the endogenous Na^+ concentration is likely to display a diurnal difference. Therefore, daily fluctuating salt levels may feed back to the plant circadian clock.

One of the core functions of the plant circadian clock is to anticipate and prepare plants for recurring cyclic environmental changes. It is critical for the clock system to

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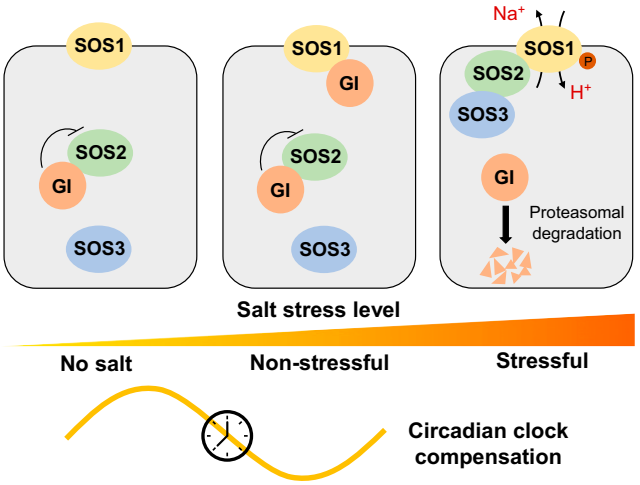

Fig. 1. Interplays between GI and SOS pathway under different salt stress levels. In the absence of salt, GI interacts with SOS2 to suppress SOS2-mediated SOS1 activation. Under nonstressful salt levels, SOS1-mediated stabilization of GI allows salt compensation of the circadian clock. Under salt stress, GI undergoes proteasomal degradation, releasing its inhibition on SOS2. SOS2 forms complex with SOS3 and phosphorylates SOS1 to activate its Na^+/H^+ antiporter activity.

distinguish the periodically recurring environmental cues and the abruptly occurring stress signals. GI appears to be a bridge connecting the plant circadian clock and responses to salt stress. Besides the involvement in the posttranslational regulation of the SOS pathway, as a core circadian clock component GI also plays a key role in the posttranslational regulation of the plant circadian clock. GI interacts with ZEITLUPE (ZTL), an F-box protein. This interaction promotes the stabilization and maturation of ZTL, contributing to the circadian oscillation of ZTL (5). Since ZTL mediates degradation of TIMING OF CAB EXPRESSION 1 (TOC1), PSEUDORESPONSE REGULATOR 5 (PRR5) (5), and CCA1 HIKING EXPEDITION (CHE) (12), salt-induced GI degradation may dramatically perturb the oscillatory expression patterns of ZTL, TOC1, PRR5, and CHE, causing severe defects in plant circadian clock. Therefore, how plants maintain a robust circadian rhythm under daily fluctuating yet nonstressful salt conditions is a pending question in the field.

In PNAS, Cha et al. report a period compensation mechanism of the plant circadian clock under daily fluctuating salt levels (6). Through luciferase imaging assay using *CAB2:LUC* and *CCA1:LUC* reporter lines, Cha et al. found that an elevated yet nonstressful level of NaCl treatment (25 mM) lengthens the period of the circadian rhythm only in the *sos1* mutant but not in wild type (WT) or *sos2* or *sos3* mutants. These results suggest that the WT seedlings can buffer the impact of the nonstressful salt levels while SOS1 may play a role in maintaining the robustness of the plant circadian clock. Through coimmunoprecipitation, bimolecular fluorescence complementation, and pull-down assays, Cha et al. (6) further show that SOS1 interacts with GI in a salt-dependent manner. Elevated salt levels promote the interaction between SOS1 and GI. Importantly, this interaction stabilizes GI, thus preventing degradation of GI under elevated yet nonstressful salt levels. Interestingly, neither the phosphorylation of SOS1 nor the SOS1-mediated salt tolerance is required for GI stabilization, since $SOS1^{DAPA}$, a mutated SOS1 that cannot be phosphorylated by SOS2, can still stabilize GI. Consistently, SOS1-stabilized GI leads to increased ZTL and reduced TOC1 protein abundance. Elevated NaCl levels bring about both ionic toxicity and osmotic stress (8). To determine the specificity of SOS1–GI-mediated period compensation effect, Cha et al. (6) applied mannitol and KCl to elicit osmotic and ionic stresses respectively. Neither mannitol nor KCl could phenocopy NaCl treatment. Therefore, SOS1–GI-mediated period compensation is specific to NaCl treatment. Further pharmacological studies suggested that salt-induced Ca^{2+} and reactive oxygen species (ROS) signals are involved in this period compensation mechanism. The interaction between SOS1 and GI can be promoted by Ca^{2+} and ROS inducer. Genetic studies demonstrated that the *gi-1* mutant is epistatic to the *sos1-1* mutant, as the period of *CAB2:LUC* is no longer responsive to 25 mM NaCl in the *sos1-1 gi-1* double mutant. Taken together, SOS1-mediated stabilization of GI maintains the proper basal oscillation of the plant circadian clock under daily fluctuating yet nonstressful salt levels (Fig. 1).

Elucidation of the SOS1–GI-mediated salt compensation pathway of the plant circadian clock in *Arabidopsis* enlightens a potential in agricultural application, as GI has also been implicated in salt tolerance in crops. Genetic analysis of heterogeneous inbred *Brassica rapa* lines identified causal nucleotide polymorphism in *B. rapa* GI responsible for the allelic variation in salt tolerance (13). Suppression of *GI* through RNA interference conferred enhanced halotolerance in *B. rapa* (14). EARLY FLOWERING 3 (ELF3), a constituent of the evening complex, promotes degradation of GI and halotolerance in *Arabidopsis* (15). Loss-of-function alleles of soybean *ELF3* homologous genes *J* contribute to decreased tolerance to salt stresses in soybean (16). Exploration of the mechanisms underlying salt compensation of circadian rhythms in crops may provide novel targets for breeding to improve halotolerance and yield.

Discovery of the interaction between SOS1 and GI also raises new questions. On the one hand, elevated yet nonstressful salt levels promote the interaction between SOS1 and GI, which can stabilize GI (6). On the other hand, stressful salt levels lead to the degradation of GI (11). A critical NaCl concentration that triggers the switch from stabilization to degradation of GI is yet to be determined. Whether this critical NaCl concentration has any physiological meaning remains to be investigated. While period compensation is secured by SOS1-mediated stabilization of GI, how stressful salt levels may affect the circadian rhythm and how the perturbed circadian rhythm may recover once the salt stress is resolved remain to be studied. Besides SOS1 and SOS2, GI also interacts with other partners such as ZTL and FLAVIN BINDING, KELCH REPEAT, F-Box 1 (FKF1) (17). How GI is partitioned among the interactions with these partners spatially and temporally and its relevance to both salt tolerance and maintenance of the circadian clock require quantitative analysis, e.g. quantification of the binding affinities and studies of the binding kinetics.

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1. M. A. Nohales, S. A. Kay, Molecular mechanisms at the core of the plant circadian oscillator. *Nat. Struct. Mol. Biol.* **23**, 1061–1069 (2016).
2. N. Creux, S. Harmer, Circadian rhythms in plants. *Cold Spring Harb. Perspect. Biol.* **11**, a034611 (2019).
3. T. P. Michael *et al.*, Network discovery pipeline elucidates conserved time-of-day-specific cis-regulatory modules. *PLoS Genet.* **4**, e14 (2008).
4. J. A. Kreps *et al.*, Transcriptome changes for *Arabidopsis* in response to salt, osmotic, and cold stress. *Plant Physiol.* **130**, 2129–2141 (2002).
5. X. Xu, L. Yuan, Q. Xie, The circadian clock ticks in plant stress responses. *Stress Biol.* **2**, 15 (2022).
6. J.-Y. Cha *et al.*, The Na^+/H^+ antiporter SALT OVERLY SENSITIVE 1 regulates salt compensation of circadian rhythms by stabilizing GIGANTEA in *Arabidopsis. Proc. Natl. Acad. Sci. U.S.A.* **119** (2022).
7. R. Munns, M. Tester, Mechanisms of salinity tolerance. *Annu. Rev. Plant Biol.* **59**, 651–681 (2008).
8. Y. Yang, Y. Guo, Unraveling salt stress signaling in plants. *J. Integr. Plant Biol.* **60**, 796–804 (2018).
9. H. J. Park, Z. Qiang, W. Y. Kim, D. J. Yun, Diurnal and circadian regulation of salt tolerance in *Arabidopsis*. *J. Plant Biol.* **59**, 569–578 (2016).
10. J. Sakurai-Ishikawa *et al.*, Transpiration from shoots triggers diurnal changes in root aquaporin expression. *Plant Cell Environ.* **34**, 1150–1163 (2011).
11. W. Y. Kim *et al.*, Release of SOS_2 kinase from sequestration with GIGANTEA determines salt tolerance in *Arabidopsis. Nat. Commun.* **4**, 1352 (2013).
12. C. M. Lee *et al.*, Decoys untangle complicated redundancy and reveal targets of circadian clock F-Box proteins. *Plant Physiol.* **177**, 1170–1186 (2018).
13. Q. Xie *et al.*, Allelic polymorphism of *GIGANTEA* is responsible for naturally occurring variation in circadian period in *Brassica rapa. Proc. Natl. Acad. Sci. U.S.A.* **112**, 3829–3834 (2015).
14. J. A. Kim *et al.*, Reduction of *GIGANTEA* expression in transgenic *Brassica rapa* enhances salt tolerance. *Plant Cell Rep.* **35**, 1943–1954 (2016).
15. Y. Sakuraba, S. Bülbül, W. Piao, G. Choi, N. C. Paek, *Arabidopsis EARLY FLOWERING3* increases salt tolerance by suppressing salt stress response pathways. *Plant J.* **92**, 1106–1120 (2017).
16. Q. Cheng *et al.*, The soybean gene J contributes to salt stress tolerance by up-regulating salt-responsive genes. *Front Plant Sci* **11**, 272 (2020).
17. M. Sawa, D. A. Nusinow, S. A. Kay, T. Imaizumi, FKF1 and GIGANTEA complex formation is required for day-length measurement in *Arabidopsis. Science* **318**, 261–265 (2007).