

Mutations in the F-box gene *SNEEZY* result in decreased Arabidopsis GA signaling

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We previously reported that the *SLEEPY1* (*SLY1*) homolog, F-box gene *SNEEZY/SLEEPY2* (*SNE/SLY2*), can partly replace *SLY1* in gibberellin (GA) hormone signaling through interaction with DELLAs RGA and GAI. To determine whether *SNE* normally functions in GA signaling, we characterized the phenotypes of two T-DNA alleles, *sne-t2* and *sne-t3*. These mutations result in no apparent vegetative phenotypes, but do result in increased ABA sensitivity in seed germination. Double mutants *sly1-t2 sne-t2* and *sly1-t2 sne-t3* result in a significant decrease in plant fertility and final plant height compared to *sly1-t2*. The fact that *sne* mutations have an additive effect with *sly1* suggests that *SNE* normally functions as a redundant positive regulator of GA signaling.

This paper describes genetic evidence that the *SLEEPY1* (*SLY1*) homolog *SNEEZY/SLEEPY2* (*SNE/SLY2*) functions redundantly with *SLY1* to stimulate gibberellin signaling. GA responses such as seed germination, stem elongation and fertility are promoted by proteolysis of DELLA proteins, negative regulators of the GA signaling.¹ In the classic GA signaling model, GA binding to the GA receptor GID1 increases GID1 affinity for DELLA protein. This GID1-GA binding to DELLA causes *SLY1*, the F-box subunit of an SCF E3 ubiquitin ligase complex, to recognize, bind and ubiquitinate DELLA proteins thereby targeting them for destruction by the 26S proteasome. Thus, loss of *SLY1* function results in decreased GA responses, causing dwarfism, delayed

flowering, infertility and seed dormancy. The *sly1* mutants over-accumulate DELLA proteins due to failure to destroy them through the ubiquitin-proteasome pathway.

Overexpression of the *SLY1* homolog, *SNE*, partially rescues the germination, dwarfism and infertility of the *sly1-10* mutant.²⁻⁴ *SNE* overexpression in the *sly1-10* background is associated with reduced accumulation of DELLA proteins RGA and GAI, but not of DELLA RGL2. Co-immunoprecipitation assays demonstrated that *SNE* directly binds RGA protein as well as the cullin subunit of the SCF E3 complex. These recently published data suggest that *SNE* forms a functional SCF E3 ubiquitin ligase complex that negatively regulates a subset of the DELLA proteins regulated by *SLY1*.⁴

The finding that *SNE* overexpression rescues *sly1-10* phenotypes through down-regulation of DELLA RGA and GAI suggests that *SNE* is normally a positive regulator of GA signaling. If this is true, then we expect *sne* mutations to cause phenotypes resulting from reduced GA response including reduced germination, stature and fertility. To examine this hypothesis, three *sne* T-DNA mutants were identified: *sne-t1*, *sne-t2* and *sne-t3*. The *sne-t1* allele is a SALK line containing a T-DNA insertion 183-bp upstream of the coding region.⁵ This line showed no apparent phenotype and was not further characterized. The *sne-t2* allele is a Sussman T-DNA line^{4,6} containing a T-DNA insertion immediately before the ATG that is the *SNE* translational start codon (Fig. 1A). While this insertion does

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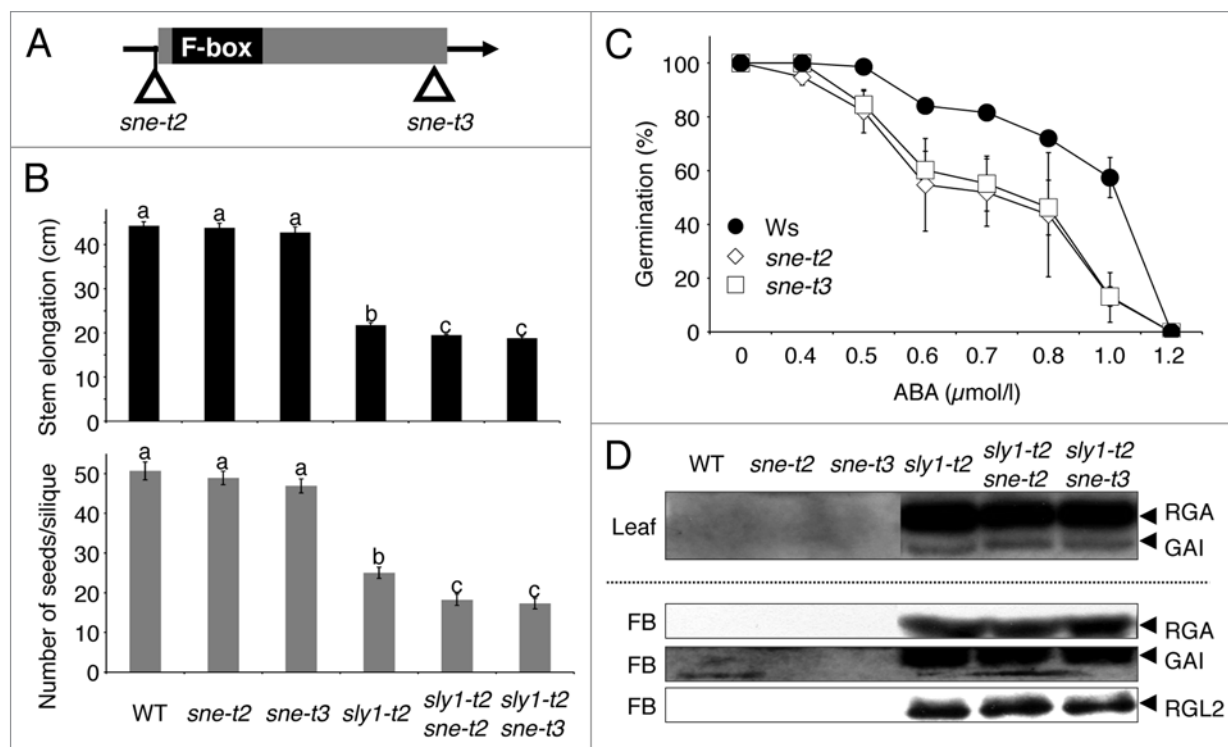


Figure 1. The phenotypes of *sne-t2* and *sne-t3* T-DNA mutants. (A) Schematic diagram of the *sne-t2* T-DNA insertion at position -1 bp and of *sne-t3* at position +435 bp with respect to the translation start site. (B) Final plant height (upper) and fertility (lower) of indicated genotypes. Letters indicate statistically different classes as determined by t-test. Bars represent standard error. (C) *sne* mutants show increase in ABA sensitivity. Seeds of wild-type Ws, *sne-t2* and *sne-t3* were after-ripened for 2 weeks then sown on MS-agar containing indicated concentrations of ABA as described by Steber et al.¹¹ Germination was scored based on radical emergence after incubating 3 days at 4°C followed by 14 days at 22°C. (D) Mutations in *SNE* cause no significant effect on DELLA RGA, GAI and RGL2 protein accumulation. Total protein was extracted from leaves of 12-d-old seedlings (Top) or flower buds (FB, bottom) and detected as described in Ariizumi et al.⁴

not disrupt the coding region, it likely disrupts SNE protein translation as the T-DNA contains multiple stop codons. The *sne-t3* allele contains a T-DNA insertion within the *SNE* ORF before amino acid 146 of the 157 amino acid predicted protein (FLAG_461E03).^{7,8} This allele should result in loss of the last 11 SNE amino acids. We know that loss of the last 8 SLY1 amino acids in *sly1-10* results in dwarfism, suggesting that loss of the last 11 SNE amino acids may also cause some loss of function in the small F-box protein. When the homozygous *sne-t2* and *sne-t3* lines were compared to wild-type Ws, no change was observed either in final plant height or fertility measured in seeds/silique (Fig. 1B). An ABA dose-response curve in seed germination detected a small but reproducible increase in ABA sensitivity during seed germination of *sne-t2* and *sne-t3* (Fig. 1C). The fact that the *sne-t2* and *sne-t3* mutants, like *sly1-2* and *sly1-10*, show increased ABA sensitivity suggests

that *SNE* and *SLY1* may have similar functions in GA signaling during seed germination.²

One possible explanation for the lack of apparent GA-insensitive phenotypes in *sne* T-DNA insertion lines, is that *SNE* function is redundant with *SLY1* in GA signaling.⁹ If so, we would expect *sly1 sne* double mutants to show stronger GA-insensitive phenotypes than the *sly1* single mutation. Double mutants were constructed containing either the *sne-t2* or *sne-t3* mutation in the *sly1-t2* null background. The *sly1-t2* allele was chosen because *sly1-t2*, *sne-t2* and *sne-t3* are all in the Ws ecotype. The *sly1-t2* allele contains a T-DNA insertion within the F-box domain resulting in severe GA-insensitive phenotypes including failure to germinate, reduced stature and infertility.¹⁰ The *sly1-t2 sne-t2* and *sly1-t2 sne-t3* double mutants showed a small but significant decrease in final plant height and fertility (seeds/silique) compared to *sly1-t2* (Fig. 1B). This increase

in phenotype severity was not associated with an apparent increase in DELLA RGA, GAI or RGL2 protein accumulation (Fig. 1D). It could be that DELLA protein levels in *sly1-t2* are so high that any slight increase due to *sne* mutations is undetectable. Our previous study of *SNE* overexpression lines showed that *SNE* has the ability to downregulate RGA and GAI protein accumulation. Figure 1 shows that the chromosomal *SNE* gene contributes to GA signaling presumably through ubiquitination of DELLA protein.

Taken together, the fact that *sne* mutants show only mild GA-insensitive phenotypes and that the natural *SNE* expression cannot compensate for lack of *SLY1*, indicate that *SLY1* is the main E3 ubiquitin ligase stimulating GA signaling (this study, reviewed in ref. 4). We cannot rule out the possibility that stronger *SNE* alleles would show either stronger GA response phenotypes or phenotypes that are unrelated to GA signaling. Indeed,

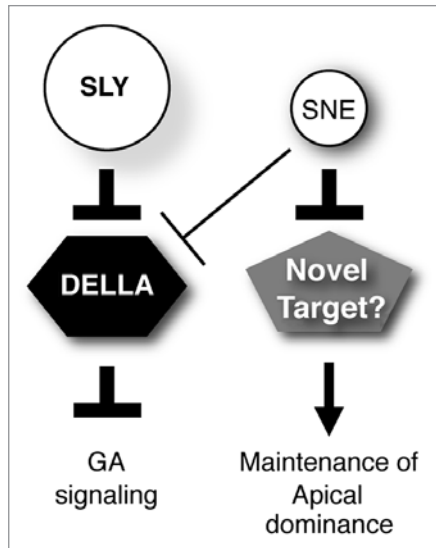


Figure 2. Model for SNE function in Arabidopsis. Both SLY1 and SNE act as positive regulators of GA responses via DELLA protein destruction. SNE may negatively regulate an unknown protein that maintains apical dominance.

there is evidence to suggest that SNE may have unique functions. The *sne-t3* (*sne-1*) allele results in a shortened root phenotype.⁸ That *SNE* is expressed in the endodermis and quiescent center of the root whereas *SLY1* is expressed in the stele, suggests that SNE may function independently in the root.⁸ Moreover, *SNE* overexpression, but not *SLY1* overexpression, results in decreased apical dominance and a prone growth habit suggesting that SNE may play a unique role in development.^{2,4} Our model is that in addition to regulating DELLA proteins RGA and GAI, SNE

may also regulate a yet unidentified target involved in apical dominance (Fig. 2). Future research will need to elucidate the role of *SNE* in Arabidopsis growth and development.

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