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HOP co-chaperones contribute to GA signaling by promoting the accumulation of the F-box protein SNE in *Arabidopsis*

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ABSTRACT

Gibberellins (GAs) play important roles in multiple developmental processes and in plant response to the environment. Within the GA pathway, a central regulatory step relies on GA-dependent degradation of the DELLA transcriptional regulators. Nevertheless, the relevance of the stability of other key proteins in this pathway, such as SLY1 and SNE (the F-box proteins involved in DELLA degradation), remains unknown. Here, we take advantage of mutants in the HSP70-HSP90 organizing protein (HOP) co-chaperones and reveal that these proteins contribute to the accumulation of SNE in *Arabidopsis*. Indeed, HOP proteins, along with HSP90 and HSP70, interact *in vivo* with SNE, and SNE accumulation is significantly reduced in the *hop* mutants. Concomitantly, greater accumulation of the DELLA protein RGA is observed in these plants. In agreement with these molecular phenotypes, *hop* mutants show a hypersensitive response to the GA inhibitor paclobutrazol and display a partial response to the ectopic addition of GA when GA-regulated processes are assayed. These mutants also display different phenotypes associated with alterations in the GA pathway, such as reduced germination rate, delayed bolting, and reduced hypocotyl elongation in response to warm temperatures. Remarkably, ectopic overexpression of SNE reverts the delay in germination and the thermally dependent hypocotyl elongation defect of the *hop1 hop2 hop3* mutant, revealing that SNE accumulation is the key aspect of the *hop* mutant phenotypes. Together, these data reveal a pivotal role for HOP in SNE accumulation and GA signaling.

Key words: HSP70-HSP90 organizing protein, seed germination, thermomorphogenesis, RGA, protein folding, protein stability

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INTRODUCTION

Gibberellins (GAs) are phytohormones that control diverse aspects of plant growth and development, including seed germination, stem elongation, leaf expansion, flowering time, and reproduction. The GA transduction pathway involves a short signaling cascade that leads to the degradation of the DELLA transcriptional regulators. This signaling involves the binding of GA to the receptor GA-INSENSITIVE DWARF1 (GID1), which stimulates the association of GID1 with the DELLAs, the recognition of the DELLAs-GID1 dimeric complex by the F-box components of specialized SCF (Skip1-cullin-F-box) E3 ligases, and, finally, the polyubiquitination and degradation of DELLAs by the 26S proteasome. This DELLA degradation leads to the induction of the GA-dependent transcriptional response (Daviere et al., 2008; Daviere and Achard, 2013; Ito et al., 2018).

One aspect that contributes to the control of different hormonal networks, but that is especially relevant in the regulation of GA signaling in the reference species *Arabidopsis thaliana*, is the functional diversification of essential components of the

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pathways. Indeed, five DELLA proteins have been characterized in *Arabidopsis*: repressor of *ga1-3* (RGA), GA-insensitive (GAI), and the RGA-like proteins 1–3 (RGL1, RGL2, and RGL3) (Peng et al., 1997; Silverstone et al., 1997; Dill and Sun, 2001; Lee et al., 2002; Wen and Chang, 2002). These proteins are encoded by different genes that show a differential expression pattern, which gives them a predominant role in different tissues and developmental transitions (Tyler et al., 2004). Furthermore, two *Arabidopsis* F-box proteins are involved in DELLA degradation: SLY1 (SLEEPY) and SNE (SNEEZY, also called SLY2) (Ariizumi et al., 2011). The existence of these two F-box proteins also increases the complexity of the GA pathway because the E3 ubiquitin ligase complexes defined by SLY1 and SNE show a certain degree of specificity for the DELLA proteins. In this way, SLY1 is involved in the efficient degradation of RGL2, RGA, and GAI, whereas SNE-containing E3 ligase complexes show an intrinsic specificity for degradation of RGA and GAI (Ariizumi et al., 2011). Hormonal output is also influenced by processes that regulate key components of the pathways (Blanco-Tourinan et al., 2020). For example, protein folding has been shown to influence auxin and JA signaling by controlling the accumulation/stability of the SCF F-box proteins and hormonal co-receptors TIR1/AFB2 and COI1, respectively. In these pathways, folding and stability of these co-receptors is mediated by the chaperone HSP90 (Zhang et al., 2015; Wang et al., 2016).

HSP90 is usually assisted in the folding of the specific client proteins by different co-chaperones (Mayer and Bukau, 2005; Li et al., 2012; Prodromou, 2012; Schopf et al., 2017; Radli and Rudiger, 2018). One family of HSP90 co-chaperones is HOP (HSP70-HSP90 organizing protein). The co-chaperones are proposed to enhance client protein folding by facilitating the transfer of target proteins from HSP70 to HSP90 (Johnson et al., 1998; Odunuga et al., 2004). In mammals,HOP function has been related to the folding of signaling proteins, probably influencing the selectivity of HSP90 for these specific clients (Bhattacharya et al., 2020); however, in plants, HOPs were initially associated with plant response to stress (Toribio et al., 2020). More recently, one member of this family in *Arabidopsis*, HOP3, was shown to be involved in JA signaling through the regulation of COI1 accumulation/stability (Muñoz et al., 2021), and the three members of this family were shown to participate in the auxin response through promoting the accumulation of TIR1 (Muñoz et al., 2022). Consistent with these findings, *hop3* and *hop1 hop2 hop3* mutants show a reduced sensitivity to JA and auxin, respectively, and display a higher susceptibility to *Botrytis cinerea* and *Tetranychus urticae* infections, in addition to alterations in several auxin-dependent developmental responses (Muñoz et al., 2021, 2022). Finally, HOP1 and HOP2, but not HOP3, were recently shown to participate in the brassinosteroid pathway under salt stress conditions by facilitating the nucleo-cytoplasmic partitioning of the HSP90–BIN2 complex (Zhang et al., 2022). However, the involvement of HOP in other hormonal networks, including the GA pathway, remains unresolved.

Despite the unknown role of HOP proteins in the GA network, it was previously speculated that the HSP90 cycle might have a role in the gibberellin response. Nevertheless, solid evidence for a possible role of HSP90 or its co-chaperones in this process was not provided (di Donato and Geisler, 2019). During the course of our previous studies, we obtained a battery of *hop* mutants, giving us the opportunity to analyze the role of HOPs in depth. In this work, we show that HOP co-chaperones are involved in GA signaling, contributing to the accumulation of the F-box protein SNE. We demonstrate that HOP proteins, HSP90, and HSP70 interact with SNE and that SNE accumulation is compromised in the absence of HOP. This reduced accumulation of SNE leads to multiple GA-associated defects in the *hop1 hop2 hop3* mutant plants, which display an increased sensitivity to GA inhibitors and a reduced response to GA. Interestingly, our findings also show that HOPs display a certain degree of specialization in the binding of specific GA-related F-box proteins, with SNE rather than SLY1 being the main target of HOP. These results extend the role of HOP proteins to different developmental and stress-related processes, uncover the essential role of HOP proteins in the precise accumulation of the F-box protein SNE, and describe new aspects of HOP target selectivity. Overall, this work provides novel information on the relevance of protein folding and quality control in modulating the abundance of key hormonal signaling proteins in plants.

RESULTS

hop mutants show phenotypes that could be associated with defects in the GA pathway

During the general characterization of the *hop1 hop2 hop3* mutant, we observed that it shows a delay in bolting initiation (Figure 1A and 1B), understood as a delay in initiating rapid elongation of the plant stem (Chen et al., 2019). Because bolting has been related to GA (Tyler et al., 2004; Han et al., 2016; Chen et al., 2019; Jung et al., 2020) and HSP90 (the protein assisted by HOP) was previously speculated to be involved in the GA pathway (di Donato and Geisler, 2019), we wondered whether the bolting delay in the *hop* mutant might be associated with defects in this hormonal network. To test this hypothesis, we analyzed whether this phenotype could be reverted by the addition of ectopic GA. Remarkably, as shown in Figure 1A and 1B, exogenous application of 100 μM GA fully rescued the late-bolting phenotype of the *hop1 hop2 hop3* mutant, providing the first link between HOP and this hormonal pathway.

In parallel, as part of the characterization of the role of HOPs in stress responses, we analyzed the growth of the *hop1 hop2 hop3* mutant under high salt conditions in a hydroponic system. As shown in Supplemental Figure 1, *hop1 hop2 hop3* plants remained smaller, had darker green leaves, and displayed a delay in senescence and bolting compared with their wild-type counterparts when challenged with increasing concentrations of NaCl. In addition, the germination rate of the *hop1 hop2 hop3* mutant was also markedly delayed when challenged with NaCl (Figure 1C). Interestingly, it was previously reported that high salt suppresses the expression of GA biosynthetic genes (Kim et al., 2008; Sun, 2010), which, along with the previous data, prompted us to test whether the salt-dependent germination defects of the *hop* mutant could be rescued by the addition of GA. Indeed, hypersensitivity of the *hop1 hop2 hop3* mutant to salt stress was largely suppressed when high concentrations (50 μM) of exogenous GA were added to the medium (Figure 1D), again suggesting that HOP proteins were somehow involved in the GA pathway.

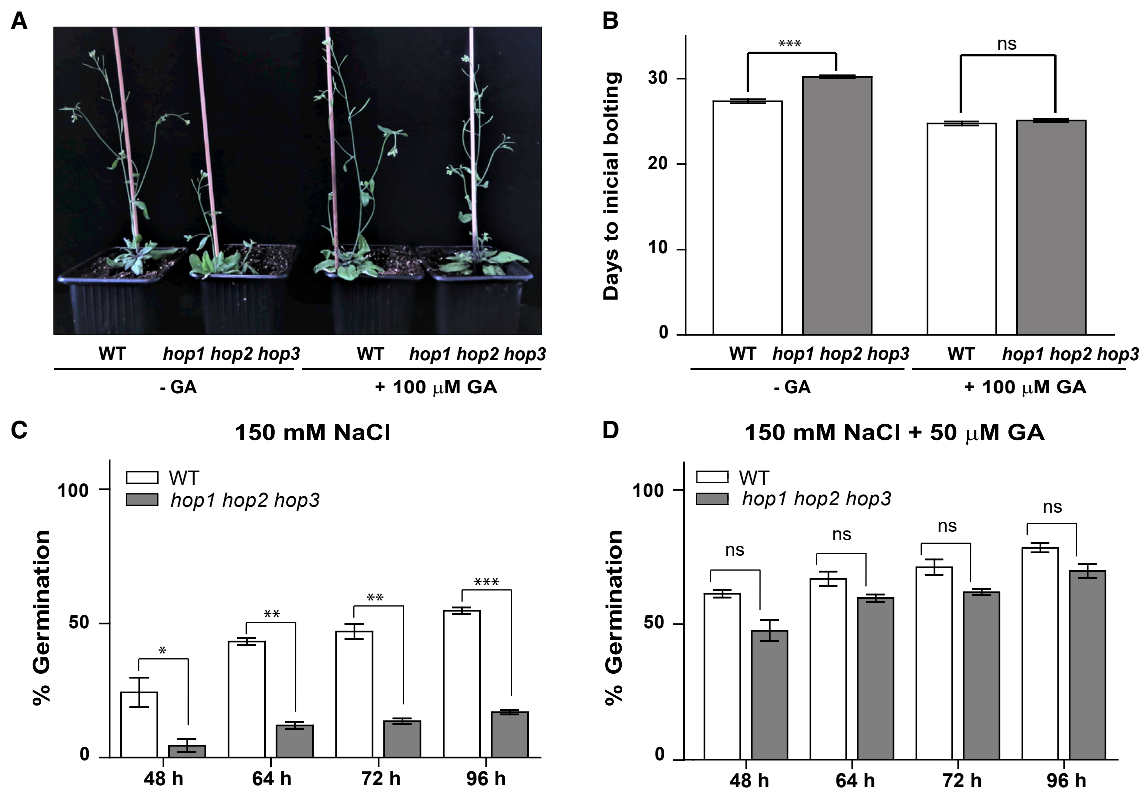

Figure 1. The *hop1 hop2 hop3* mutant shows developmental and stress-related phenotypes associated with possible alterations in the GA pathway.
(A) Representative photographs and **(B)** quantification of the days taken by wild-type (WT) and *hop1 hop2 hop3* mutant plants to initiate bolting in the absence or presence of 100 μM GA.
(C and D) Percentage of seed germination of the WT and the *hop1 hop2 hop3* mutant in the presence of **(C)** 150 mM NaCl or **(D)** 150 mM NaCl + 50 μM GA at different time points. In **(B–D)** data represent the mean and SE of n = 3 independent experiments, using for each experiment **(B)** 20 plants and **(C and D)** 50 stored seeds for each genotype and condition. Statistically significant differences were calculated using Student's t test (ns, nonsignificant; $*p < 0.05$; $**p < 0.01$; $***p < 0.001$).

To obtain further evidence in support of this hypothesis, we directly analyzed whether the *hop1 hop2 hop3* mutant showed alterations in germination under control conditions, as this developmental transition is well known to be regulated by GAs (Koornneef and van der Veen, 1980; Blazquez et al., 1998; Sun, 2010). As shown in Figure 2A, the *hop1 hop2 hop3* mutant displayed a delayed germination rate at the early stages. Taken together, these studies show that the *hop1 hop2 hop3* mutant displays several phenotypes potentially associated with the GA pathway.

HOP proteins seem to play a pivotal role in GA signaling

To directly assess the involvement of HOP proteins in the GA network, we repeated the germination assays in the presence of the GA biosynthesis inhibitor paclobutrazol (Böger and Sandmann, 1989). As shown in Figure 2B, germination inhibition was strongly accentuated in the *hop1 hop2 hop3* mutant in the presence of 5 μM PAC at all assayed time points (Figure 2B and Supplemental Figure 2), indicating that this mutant was hypersensitive to the inhibitor.

To further assess *hop1 hop2 hop3* response to GA, we evaluated whether the increased sensitivity to PAC of the *hop1 hop2 hop3* mutant could be reverted in the presence of GAs. For this purpose, we scored germination of Col-0 and *hop1hop2 hop3* seeds simultaneously in the presence of 10 μM PAC and 5 μM GA (Figure 2C, 2D, and Supplemental Figure 2). As shown in Figure 2C, moderate GA concentrations enhanced germination of the PAC-treated wild-type seeds at 32 h but did not significantly improve germination of the *hop1 hop2 hop3* mutant. At 48 h, addition of 5 μM GA fully reverted the PAC effects on germination in the wild type. Under such conditions, GAs also enhanced the germination rate of PAC-treated *hop1 hop2 hop3* seeds; nevertheless, a full reversion of the PAC effect was not observed in this mutant (Figure 2D). Interestingly, higher GA concentrations (50 μM GA) fully rescued the PAC-hypersensitive phenotype of this mutant (Supplemental Figure 3). These results indicate that the *hop1 hop2 hop3* mutant is partially insensitive to moderate concentrations of GAs.

To gain further insight into its GA sensitivity, we analyzed the germination rates of Col-0 and *hop1 hop2 hop3* seeds on different concentrations of GA (0–50 μM) at 24 h and 32 h. As shown in Figure 2E and 2F, the wild type responded to GA, reaching its maximum germination potential for each time point at low concentrations of GA. However, this effect was not observed in the *hop1 hop2 hop3* mutant, which was

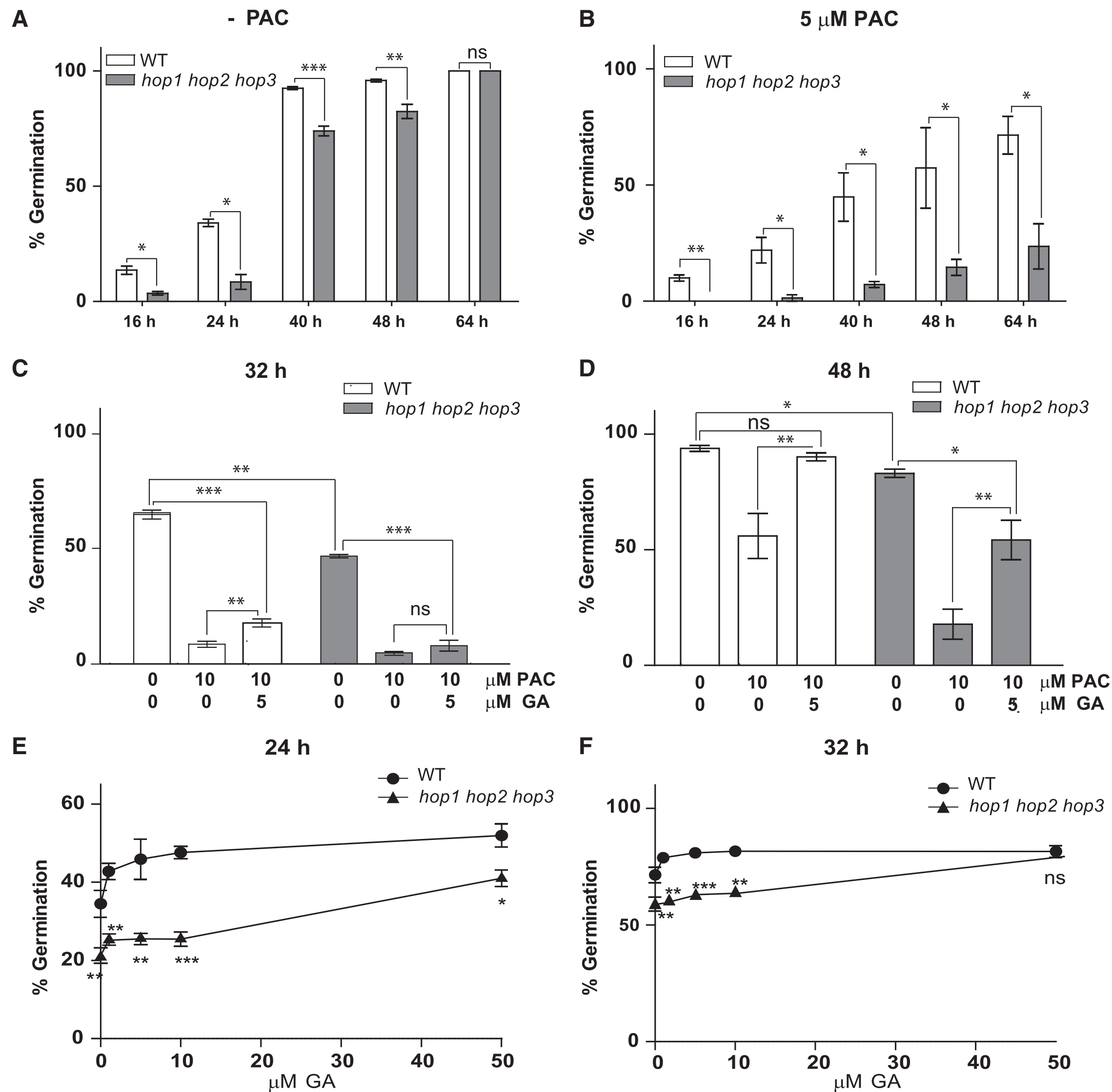

Figure 2. The *hop1 hop2 hop3* mutant shows an increased sensitivity to PAC and a reduced sensitivity to GA.

(A and B) Quantification of seed germination rate of the wild type (WT) and the *hop1 hop2 hop3* mutant with **(A)** no PAC (−PAC) or **(B)** 5 μM PAC at different time points.

(C and D) Quantification of the percentage of seed germination of the WT and the *hop1 hop2 hop3* mutant in the presence and absence of 10 μM PAC or 10 μM PAC + 5 μM GA at **(C)** 32 h and **(D)** 48 h.

(E and F) Quantification of the percentage of seed germination of the WT and the *hop1 hop2 hop3* mutant in the presence and absence of different GA concentrations at **(E)** 24 h or **(F)** 32 h. In all cases, data represent the mean and SE of n = 3 independent experiments, with 50 seeds for each genotype and condition in each experiment. Statistically significant differences were calculated using **(A, B, E, F)** Student's t test and **(C and D)** one-way ANOVA and the Newman-Keuls multiple comparison procedure (ns, nonsignificant; *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$).

mostly insensitive to low concentrations of GA and only attained levels of germination similar to the wild type at high GA concentrations (50 μM GA) (Figure 2F). Together, these data confirm that the *hop1 hop2 hop3* mutant is hypersensitive to PAC and partially sensitive to GA, suggesting that HOPs contribute to GA signaling.

HOP proteins interact with SNE in planta

The biochemical function of HOP is well defined in mammals: it is an HSP90 co-chaperone that assists the folding of specific proteins through the HSP70-HSP90 cycle. For this, HOP interacts simultaneously with HSP90 and HSP70 (the latter is already bound to the protein to be folded), forming a complex that enables transfer of the client proteins from HSP70 to HSP90 (Odunuga et al., 2004). Because the function of HOP is to assist in the folding of specific proteins, the GA signaling defect observed in the *hop* mutant may point to a drawback in the folding of one or more signaling regulators of the GA pathway. Hence, in an attempt to identify the client proteins of HOP in the pathway, we analyzed the interaction of HOP with different GA signaling regulators

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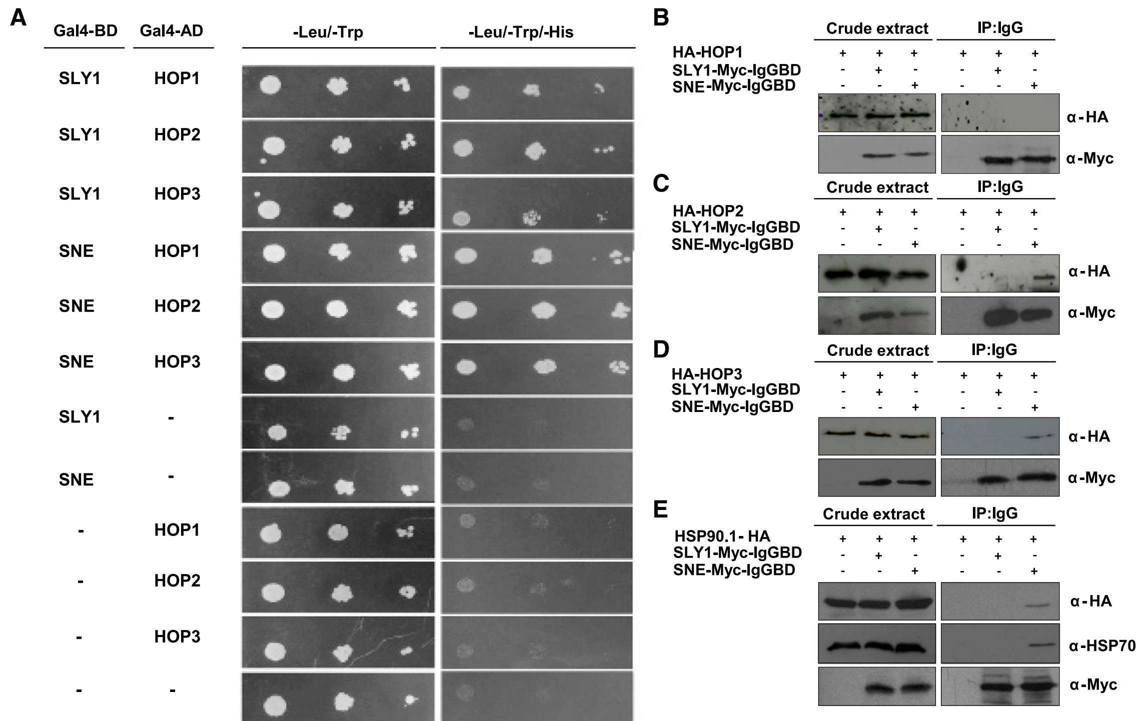

Figure 3. HOPs interact with the GA-related F-box protein SNE in the yeast two-hybrid system and *in planta*.
(A) Analysis of the interaction by yeast two-hybrid assays. Growth in nonselective medium (-Leu/-Trp) or autotrophy-selective medium (-Leu/-Trp/-His) of serial dilutions of yeast clones co-transformed with constructs expressing the indicated proteins fused to the GAL4-binding domain (Gal4-BD) or the GAL4-activation domain (Gal4-AD). The empty Gal4-BD and Gal4-AD vectors (-) were used as negative controls. No 3-amino-1,2,4-triazole (3-AT) was used in the assays.
(B–E) Co-immunoprecipitation analysis in *N. benthamiana*. Protein extracts (crude extracts) from *N. benthamiana* leaves transiently expressing, under the control of the *35S* promoter, different combinations of SLY1-Myc-IgGBD and SNE-Myc-IgGBD with **(B)** HA-HOP1, **(C)** HA-HOP2, **(D)** HA-HOP3, and **(E)** HSP90.1-HA were subjected to affinity purification using IgG beads. The presence of the corresponding fusion proteins in the crude extracts and in the eluted fractions from the immunoprecipitations (IP:IgG) was analyzed by western blotting using anti-HA (α-HA) or anti-Myc (α-Myc) antibodies. In **(E)**, the presence of endogenous NbHSP70 in the crude extracts and in the eluted fractions was analyzed by western blotting using a plant-specific anti-HSP70 antibody (α-HSP70). All experiments were repeated independently three times with similar results.

It is established that HOP proteins contribute to the folding/stabilization of COI1 and TIR1, two F-box proteins involved in the JA and auxin pathways, respectively (Muñoz et al., 2021, 2022). For this reason, we decided to analyze the interaction of HOP with the GA F-box proteins SLY1 and SNE. Directed two-hybrid analysis with these F-box proteins showed that the three members of the *Arabidopsis* HOP family interact with SLY1 and SNE (Figure 3A). To verify this interaction *in vivo*, we performed co-immunoprecipitation assays on *Nicotiana benthamiana* leaves co-expressing tagged versions of HOP2 (FLAG-HOP2) and SLY1 or SNE (SLY1-HA and SNE-HA). In these experiments (Supplemental Figure 4), we observed a band corresponding to SNE-HA but not a band corresponding to SLY1-HA in the eluates of FLAG-HOP2, suggesting that HOP2 interacts preferentially with SNE *in vivo*. To provide further evidence for this selective interaction and expand the study to all members of the HOP family, we carried out additional immunoprecipitation studies. This time, HOP1, HOP2, or HOP3 co-chaperones tagged with the HA epitope were co-expressed in *N. benthamiana* leaves with SLY1 and SNE proteins multitagged with Myc and the immunoglobulin G binding domain (IgG-BD). In these studies, we purified in parallel either SLY1 or SNE, taking advantage of the IgG-BD, and analyzed whether HA-HOP1 (Figure 3B), HA-HOP2 (Figure 3C), or HA-HOP3 (Figure 3D) were detected in the purified fractions. As shown in Figure 3B, we were unable to detect HA-HOP1 in the eluates from either SLY1 or SNE. However, HA-HOP2 and HA-HOP3 were specifically pulled down in the presence of SNE but not in the presence of SLY1 (Figure 3C and 3D). Together, these data indicate that although all three family members bind both F-box proteins in the yeast two-hybrid system, HOP2 and HOP3 display preferential binding affinity for the F-box SNE *in planta*.

HOP proteins are known to assist in the folding of specific proteins through the HSP70-HSP90 cycle. Indeed, the *in vivo* interactions of HOP proteins with HSP70 and, especially, with HSP90 have been exhaustively described in plants (Chen et al., 2010; Fellerer et al., 2011; Fernandez-Bautista et al., 2017, 2018; Toribio et al., 2020). Therefore, to obtain further evidence for the possible role of HOP in the folding of SNE through the HSP70/HSP90 cycle, we analyzed whether SNE interacts with these two major chaperones *in vivo*. To do so, we expressed different combinations of tagged versions of HSP90 and SNE (HSP90.1-HA and SNE-Myc-IgG-BD) in *N. benthamiana* leaves and immunoprecipitated SNE using IgG beads. As a control, we performed a similar parallel experiment with SLY1, a protein

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that does not seem to be a target of HOP co-chaperones according to our previous results. As shown in Figure 3E, a clear band corresponding to HSP90.1-HA was detected in the eluates of SNE, indicating that SNE interacts with HSP90 *in vivo*. Furthermore, we also observed an accumulation of endogenous HSP70 in these eluates (Figure 3E). Interestingly, in our hands, no clear interaction between SLY1 and HSP90.1 or HSP70 was detected *in planta*. All these data indicate that SNE, but not SLY1, preferentially interacts with HSP90 and HSP70 *in vivo*. These results, along with the interaction of SNE with HOP members, suggest that SNE may form a complex with HSP70-HSP90 and HOP proteins (especially HOP2 and HOP3).

The specific binding of SNE to HOP2 and HOP3, rather than HOP1, also points to a certain specialization or a predominant role of HOP2 and HOP3 among the members of the HOP family in the GA pathway. This specialization has previously been observed in other processes, such as alleviation of the endoplasmic reticulum (ER) response or JA signaling, in which HOP3 activity seems to play a predominant role (Toribio et al., 2020). Hence, to gain deeper insight into the contribution of each HOP family member to GA signaling, we analyzed the germination rates of the *hop* single mutants (*hop1*, *hop2,* and *hop3*) along with the *hop1 hop2 hop3* triple mutant. As shown in Supplemental Figure 5A, all *hop* single mutants showed a significant delay in germination at 24 h. At 32 h, this delay was observed only in the *hop2* and *hop3* single mutants but not in *hop1*. These results suggest that all HOP family members may participate in the early stages of germination, whereas HOP2 and HOP3, consistent with their stronger interactions with SNE, seem to have a more predominant role in the later stages of this process.

In addition, we analyzed the sensitivity of the single mutants to PAC and to the combined action of PAC and GA during germination (Supplemental Figure 5B). At the selected time point of 32 h, the *hop1* mutant showed a germination rate similar to that of the wild-type counterpart. Furthermore, similar to the wild type, the *hop1* mutant responds to the addition of GA in the presence of PAC. By contrast, like the *hop1 hop2 hop3* mutant, the *hop2* and *hop3* single mutants did not show a significant response to moderate concentrations of GA in the presence of PAC. These data, along with the interaction assays, demonstrate the predominant role of HOP2 and HOP3 relative to HOP1 in the GA response.

The role of HOPs in GA signaling is associated with SNE accumulation

The previous data show that SNE interacts with HSP70, HSP90, and HOP proteins. Because these chaperone/co-chaperone complexes are known to bind specific client proteins to assist with their folding in other eukaryotes (Bhattacharya and Picard, 2021), these results strongly suggest that HOPs may assist in the folding of SNE in plants.

It is well known that protein homeostasis in eukaryotes is maintained by the quality control (QC) pathway, which recognizes unfolded proteins and, among other functions, ensures that proteins that have not achieved their functional conformation are degraded by the proteasome (Houck et al., 2012; Shiber and Ravid, 2014). We therefore reasoned that if HOP proteins assist in the folding of SNE, SNE folding would be compromised in the *hop1 hop2 hop3* mutant, leading to the accumulation of misfolded SNE. These unfolded SNE molecules would then be recognized by the QC and degraded, which could lead to a reduced SNE accumulation in the *hop1 hop2 hop3* mutant background. To test this hypothesis, we transformed Col-0 and the *hop1 hop2 hop3* mutant with the *p35S*:*GFP-SNE* construct and analyzed *GFP* transgene expression by qRT-PCR and accumulation of the GFP-SNE protein by western blotting with the anti-GFP antibody in both backgrounds. As shown in Figure 4, although *GFP-SNE* mRNA levels were similar in the homozygous lines in both backgrounds (Figure 4A), levels of the ectopic SNE-GFP protein were significantly reduced in the *hop1 hop2 hop3* mutant (Figure 4B and 4C), indicating a role for HOP in the accumulation, and most probably the folding, of SNE.

Interestingly, Figure 4B and 4C show that although the level of SNE-GFP is reduced in the *hop1 hop2 hop3* mutant, some ectopically expressed protein still accumulates in this background. Therefore, we wondered whether such extra levels of SNE protein (resulting from expression of the *p35S*:*GFP-SNE* construct) could be sufficient to partially restore normal SNE levels and rescue the delayed germination of this mutant. To test this hypothesis, we scored the germination rate of the *p35S*:*GFP-SNE* seeds in the *hop1 hop2 hop3* and Col-0 backgrounds. As shown in Figure 4D, the delayed germination observed in the *hop1 hop2 hop3* background was overcome when SNE was overexpressed, providing evidence that the germination defect in the mutant is due to reduced SNE accumulation.

The results above, along with the conserved function of HOP in eukaryotes, seem to suggest that HOP proteins are involved in SNE folding/accumulation in such a way that, in the absence of HOP, SNE becomes unstable and accumulates to lower levels, leading to the GA-insensitive phenotypes of the *hop* mutant. If this is the case, lines showing reduced accumulation of SNE should also be affected in germination. Therefore, we further analyzed the germination potential of *sne* seeds to study to what extent a reduction in SNE affects germination, a process that is currently accepted to be dependent mainly on RGL2 and SLY1 (Tyler et al., 2004). As shown in Figure 4E and Supplemental Figure 6A, *sne* mutant seeds show a delay in germination under control conditions. Furthermore, these seeds show a reduced response to GA (Figure 4F and Supplemental Figure 6B). These altered responses closely mimic the germination phenotype observed in the *hop1 hop2 hop3* mutant. Together, these data reinforce the contribution of HOP to GA signaling and suggest that this function is associated with SNE accumulation. Furthermore, these results highlight the important influence of the control of SNE accumulation/stability during germination.

RGA protein accumulates in the *hop1 hop2 hop3* mutant

SNE function was previously associated with GA-dependent degradation of the DELLA proteins RGA and GAI in *Arabidopsis* (Ariizumi et al., 2011). Because SNE accumulation is reduced in the *hop1 hop2 hop3* mutant, we wondered whether this reduction would affect the accumulation of RGA. We therefore crossed the reporter line *pRGA*:*GFP-RGA* in the Col-0

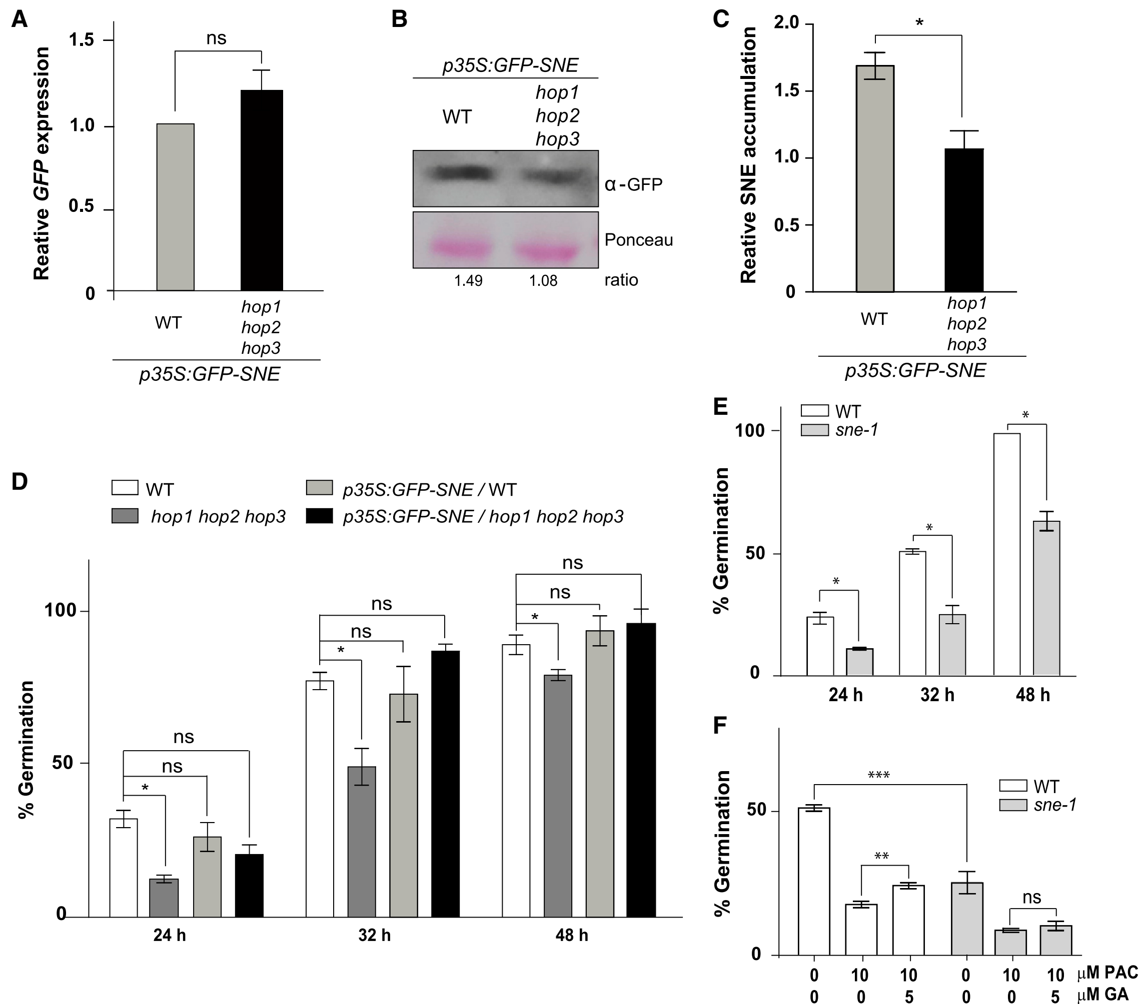

Figure 4. HOP proteins contribute to SNE accumulation, and this accumulation is essential to maintain a fast seed germination rate.
(A) qRT-PCR analysis of the expression of the *GFP-SNE* transgene, **(B)** representative western blot (using an anti-GFP antibody, α-GFP), and **(C)** quantification of the accumulation of GFP-SNE fusion protein in 7-day-old seedlings expressing the *p35S:GFP-SNE* reporter construct in the wild-type (WT) or *hop1 hop2 hop3* mutant background. Ponceau staining of the membrane is provided as a loading control. For each background, relative SNE protein accumulation is defined as the ratio between the GFP-SNE and loading control signals.
(D) Quantification of the percentage of seed germination of *p35S:GFP-SNE* lines in the WT (*p35S:GFP-SNE/WT)* and *hop1 hop2 hop3* (*p35S:GFP-SNE/ hop1 hop2 hop3)* backgrounds.
(E and F) Quantification of seed germination rate of the WT and the *sne-1* mutant **(E)** at different time points under control conditions or **(F)** in the presence or absence of 10 μM PAC or 10 μM PAC + 5 μM GA at 32 h.
In **(A, C–F)** data represent mean and SE of n = 3 independent experiments. Statistically significant differences were calculated in **(A, C, and E)** using Student's t test and in **(D and F)** using one-way ANOVA and the Newman-Keuls multiple comparison procedure (ns, nonsignificant; *p < 0.05; **p < 0.01; ***p < 0.001).

background with the *hop2-1* mutant, then analyzed expression levels of the *GFP-RGA* transgene and protein levels of the GFP-RGA fusion protein (as a measure of RGA accumulation) in both homozygous backgrounds. Whereas levels of *RGA-GFP* transgene expression were similar in the wild-type and mutant backgrounds (Supplemental Figure 7, qRT-PCR analyses), abundance of the GFP-RGA protein was significantly increased in the *hop2* mutant (Figure 5A, western blot with anti-GFP). To obtain additional evidence, we directly analyzed endogenous RGA protein levels by western blotting with an anti-RGA antibody in the wild-type and triple mutant backgrounds. As shown in Figure 5B, compared with Col-0, the *hop1 hop2 hop3 mutant showed significantly increased* RGA levels.

HOP proteins participate in the thermomorphogenic response by modulating GA signaling

GAs not only play an important role in different developmental processes but also participate in plant adaptation to different environmental conditions. One of these conditions is a moderate increase in temperature. Plant adaptation to high ambient temperature induces different morphological changes known as the thermomorphogenic response, which includes greater elongation of the plant hypocotyl (Quint et al., 2016). The DELLA protein RGA plays an important role in this process. Indeed, it is well documented that RGA levels decrease during the shift to higher temperature, which contributes, along with other hormonal

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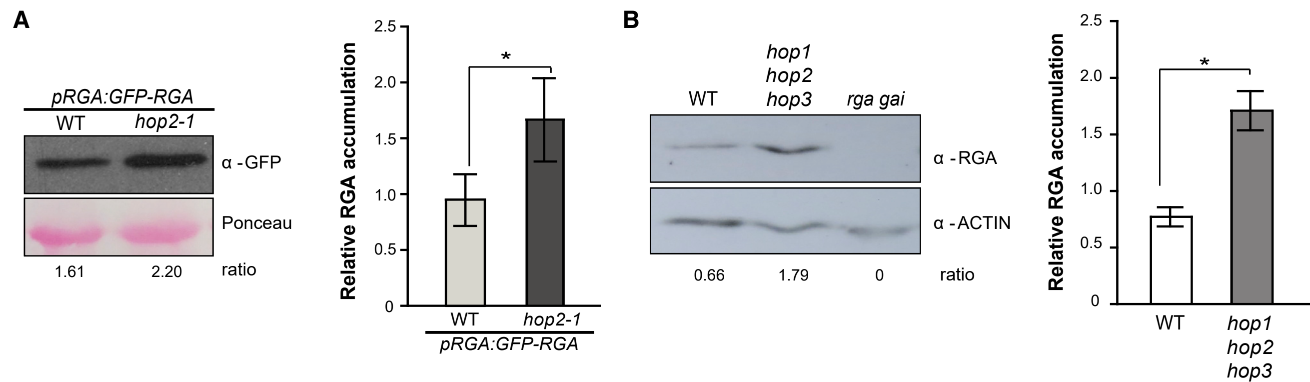

Figure 5. RGA protein is highly accumulated in the *hop2-1* and *hop1 hop2 hop3* mutants.

(A) Representative western blot (using an anti-GFP antibody, α-GFP) and quantification of the accumulation of ectopic GFP-RGA protein in 7-day-old seedlings expressing the *pRGA*:*GFP-RGA* reporter construct in the wild-type (WT) or in the *hop2-1* mutant backgrounds. Ponceau staining of the membrane is provided as a loading control.

(B) Representative western blot (using an anti-RGA antibody, α-RGA) and quantification of RGA protein accumulation in 7-day-old seedlings of the WT and the *hop1 hop2 hop3* mutant. Actin accumulation (α-ACTIN) is provided as a loading control. The *rga-28 gai-td1 (rga gai)* mutant is included as a negative control. In **(A)** and **(B)**, for each background, relative RGA protein accumulation was defined as the ratio between the RGA and loading control signals. Data are presented as mean and SE of n = 3 and n = 4 independent experiments, respectively. Statistically significant differences were calculated using Student's t test (ns, nonsignificant; *$p < 0.05$).

pathways, to increasing hypocotyl elongation under warm temperatures (Stavang et al., 2009). Because it is known that the *hop1 hop2 hop3* mutant shows reduced hypocotyl elongation in response to moderate increases in temperature (Muñoz et al., 2022), we decided to investigate whether the altered thermomorphogenic response of the *hop1 hop2 hop3* mutant could also be related to a defect in the GA pathway. To do so, we analyzed hypocotyl elongation in the Col-0 and *hop1 hop2 hop3* mutant backgrounds during the shift to 29°C in the presence or absence of GAs. Consistent with earlier reports, the mutant showed reduced hypocotyl elongation at 29°C. Nevertheless, this phenotype was partially rescued by the presence of high exogenous concentrations of GAs, suggesting that the altered thermomorphogenic response of the *hop* mutant is due, at least in part, to defects in the GA pathway (Figure 6A).

Based on the results described above, the impaired hypocotyl elongation may be due to a reduced accumulation of SNE in the mutant, which may have an influence on the GA output. To test this hypothesis, we took advantage of the *p35S*:*GFP-SNE* lines and directly analyzed accumulation of the ectopic GFP-SNE protein in the Col-0 and *hop1 hop2 hop3* mutant backgrounds at 20 and 29°C. As shown in Figure 6B, accumulation of GFP-SNE was reduced in the *hop1 hop2 hop3* mutant at both 20 and 29°C. As in the case of germination, the defect in hypocotyl elongation at 29°C was partially rescued by overexpression of SNE in the *p35S*:*GFP-SNE* mutant lines (Figure 6C and 6D), again highlighting the role of SNE accumulation/stability in different processes related to the GA pathway.

DISCUSSION

HOP plays a major role in GA signaling, promoting accumulation of the F-box protein SNE

The role of HOP in the glucocorticoid pathway was documented some years ago in mammals (Chen et al., 1996); however, the possible role of HOP in hormonal signaling in plants has remained elusive. Recently, evidence for the involvement of HOP co-chaperones in JA and auxin signaling has begun to emerge (Muñoz et al., 2021, 2022). In this article, we provide multiple lines of evidence showing that HOPs are involved in different processes associated with the GA pathway in *Arabidopsis*. Indeed, the *hop1 hop2 hop3* mutant displays delays in germination and bolting under control conditions. Our data also reveal a GA-dependent role of HOP proteins in hypocotyl elongation under warm temperature. These defects are consistent with a reduced response to moderate concentrations of GA in the mutant and with its marked hypersensitivity to inhibitors of GA biosynthesis such as PAC and high salt concentrations. These phenotypes, along with the identification of SNE as an interactor of HOP, strengthen the notion that HOPs are involved in GA signaling, probably by contributing to SNE folding and therefore to SNE accumulation/stability. Interestingly, the partial sensitivity of the *hop1 hop2 hop3* mutant to GA is masked at high GA concentrations, probably because such high concentrations force the GA signaling machinery (including SLY1, whose accumulation seems to be unaffected in the *hop1 hop2 hop3* mutant). The GA-related phenotypes described here and the partial response to moderate levels of GA are consistent with the reduced SNE protein levels in the *hop* mutants, which in turn lead to increased accumulation of the SNE target RGA. The involvement of HOP in SNE folding/accumulation is also reinforced by the fact that SNE overexpression rescues the delayed germination and hypocotyl elongation defects of the *hop1 hop2 hop3* mutant under moderate thermal stress.

The relevance of SNE folding/accumulation in the GA pathway

Protein activity depends on protein conformation and, in this regard, protein folding is an essential step in achieving overall protein function. During translation, some polypeptide chains may adopt the protein native conformation by spontaneous folding. However, in many other cases, acquisition of protein native conformation and, therefore, of protein function is assisted by

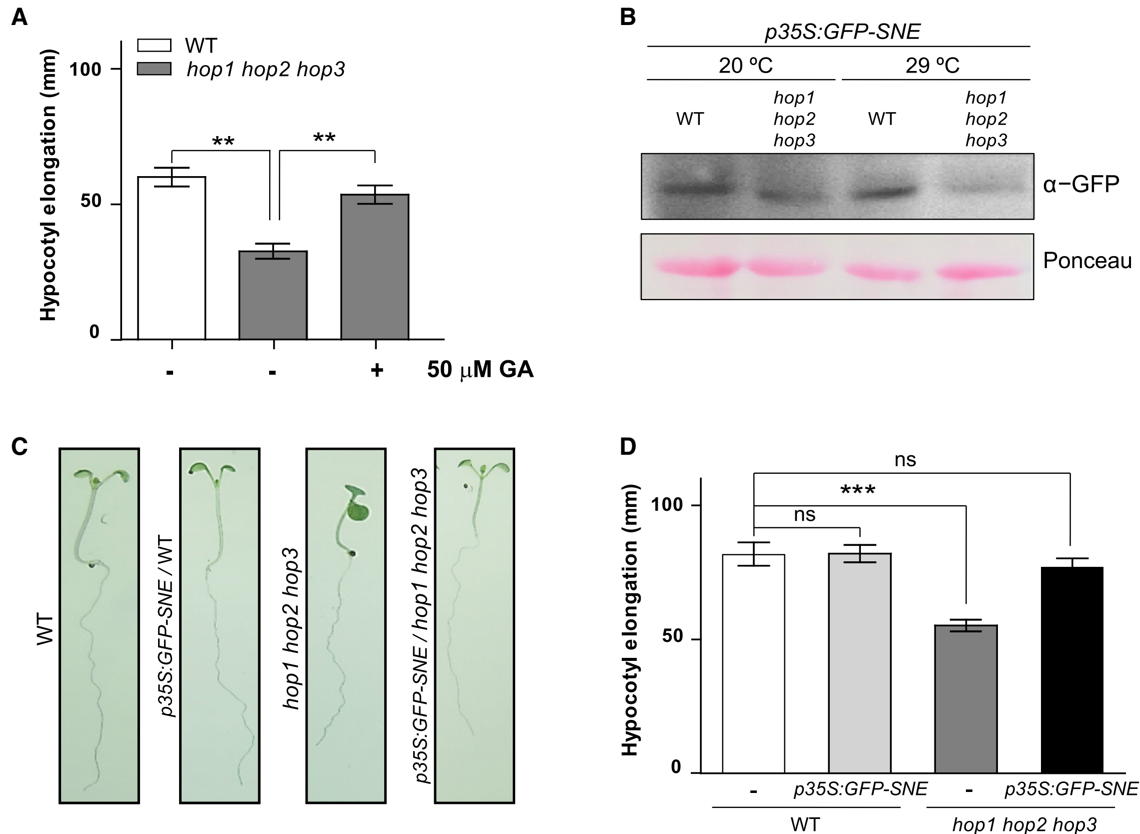

Figure 6. HOP contributes to hypocotyl elongation during thermomorphogenesis through the accumulation/stabilization of the GA-associated E3 ligase SNE.

(A) Quantification of hypocotyl elongation in wild-type (WT) and *hop1 hop2 hop3* triple mutant seedlings transferred from 20°C to 29°C in the presence or absence of 50 μM GA.

(B) Analysis of GFP-SNE protein accumulation by western blotting (using an anti-GFP antibody, α-GFP) in 7-day-old seedlings expressing the *p35S:GFP-SNE* reporter construct in the WT or *hop1 hop2 hop3* mutant background and grown under long-day conditions at 20°C or 29°C. Ponceau staining of the membrane is provided as a loading control.

(C) Representative pictures and **(D)** quantification of hypocotyl growth at 29°C of *p35S:GFP-SNE* lines in Col-0 (*p35S:GFP-SNE*/WT) and *hop1 hop2 hop3* backgrounds (*p35S:GFP-SNE/hop1 hop2 hop3*). Parental lines are also included.

In **(A and D)**, data represent mean and SE of n = 30 seedlings for each genotype and condition. Statistically significant differences were calculated in **(A)** using Student's t test and in **(D)** using one-way ANOVA and the Newman-Keuls multiple comparison procedure (ns, nonsignificant; $^{**}p < 0.01$; $^{***}p < 0.001$). In all cases, experiments were repeated three times with similar results.

different chaperones and chaperone/co-chaperone complexes (Moran Luengo et al., 2018; Toribio et al., 2020). In plants, it is well established that the chaperone HSP90 plays a major role in the folding/stability of the COI1 and TIR1/AFB2 co-receptors, thereby ensuring efficient JA and auxin signaling, respectively. In this process, HSP90 is assisted by the co-chaperones SGT1b and HOPs, which, as for the auxin response, form part of the same complexes and play a non-redundant role in TIR1 accumulation (Muñoz et al., 2022).

Despite the relevance of protein folding in the stability/accumulation of key proteins in the JA and auxin networks, it is important to note that the involvement of HSP90 and/or HSP90 co-chaperones in the GA pathway has not previously been demonstrated in plants. Indeed, the only available evidence for participation of HSP90 co-chaperones in this route involved a different co-chaperone (SGT1b) and was rather circumstantial. It was based on the observation that, upon flg22 treatment, expression of the JA-responsive *pCYP71A12-GUS* reporter was repressed by GA, whereas its expression was maintained in the *sgt1b* mutant (Zhang et al., 2015). To the best of our knowledge, this observation was not validated with additional experiments. Potential targets of HSP90, SGT1b, or other co-chaperones and a role for HOP in the GA pathway have also not previously been described. In this work, we provide multiple lines of evidence showing that HOP proteins contribute to the GA pathway, probably by assisting in SNE folding, thus leading to reduced accumulation of this F-box protein in the *hop* mutants. These data suggest that, in contrast to other proteins that spontaneously acquire their native conformations or whose folding is assisted only by main chaperones, SNE (like COI1 and TIR1) undergoes quite elaborate folding that requires the assistance of co-chaperones, in particular of HOPs. In addition, the present work shows that reduced accumulation of SNE in the *hop* mutants leads to multiple alterations associated with the GA

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pathway. This work, therefore, highlights the relevance of proper SNE accumulation for the GA network.

HOP interaction with SNE and its contribution to SNE accumulation is quite interesting and unexpected because it was previously speculated that HOP (like SGT1b) may modulate accumulation of F-box LRR proteins (Muñoz et al., 2022), a hypothesis that was valid for COI1 and TIR1, but not for SNE. Indeed, aside from the F-box domain, there are notable differences between SNE and TIR1 and COI1. SNE is a small protein of 157 amino acids (aa), whereas TIR1 and COI1 comprise 594 and 592 aa, respectively. In addition, the two latter proteins contain multiple LRR domains and support in the same molecule the functions of F-box proteins and auxin and JA co-receptors, respectively. However, SNE lacks LRR domains and acts solely, to the best of our knowledge, as an F-box protein in the GA pathway, as the GA receptor function is performed by GID1. Because the F-box domain and the role in E3 ligases are common to all three proteins, our findings suggest a role for HOP in assisting in the folding/accumulation of the F-box domain of important signaling E3 ligases. In addition, these results underscore the importance of proper folding/accumulation of F-box proteins in the auxin, JA, and GA signaling pathways.

HOP proteins seem to show a degree of specificity for SNE

In *Arabidopsis*, the three members of the HOP family (HOP1, HOP2, and HOP3) share high homology and a similar domain structure (Toribio et al., 2020). Furthermore, all members interact *in vivo* with HSP90 (Fernandez-Bautista et al., 2018). Nevertheless, despite these analogies, the genes also show unique characteristics. Indeed, they display different expression patterns, with *HOP3* being the only member that is highly induced under heat (Fernandez-Bautista et al., 2018). Furthermore, some HOP members play a more important role than others under specific conditions (Toribio et al., 2020; Zhang et al., 2022). For instance, HOP3 was specifically involved in the alleviation of ER stress and was shown to play a predominant role in the JA pathway (Fernandez-Bautista et al., 2017; Muñoz et al., 2021). In this work, we show that although all members of the *Arabidopsis* HOP family play a role in GA signaling during the early stages of germination, HOP2 and HOP3 seem to have a more predominant role at subsequent stages. These two proteins, in contrast to HOP1, are more efficiently co-purified with SNE *in vivo*, which further reveals that HOP family members show a certain specificity in the GA pathway.

This specialization is also observed at the level of target selection. Although all HOP members are able to interact with SNE and SLY1 (with lesser strength) in the yeast two-hybrid system, HOP2 and HOP3, in contrast to HOP1, strongly interact with SNE but not with SLY1 *in vivo*. Even though some HOP-dependent regulation of SLY1 cannot be fully discarded, the previous data, together with the predominant interaction of HSP90 and HSP70 with SNE rather than SLY1, suggest that SNE is likely to be the main target of HOP function. The preference of HOP for SNE over SLY1 is also consistent with the mild germination phenotype of the *hop1 hop2 hop3* mutant, which is highly reminiscent of that observed in *sne* mutants but much less severe than that expected for mutants affected in SLY1 function (Ariizumi and Steber, 2007).

Role of HOP in the folding/accumulation of SNE

Based on the conserved function of HOP in eukaryotes, SNE interaction with HOP and HSP90 strongly suggests that HOP contributes to SNE folding/stability through the HS70-HSP90 cycle, which further impinges on SNE accumulation. Consistent with this scenario, we observed a decrease in SNE abundance and greater accumulation of RGA protein in the *hop* mutants. It is well known that SNE, in addition to RGA, also modulates the degradation of the DELLA protein GAI (Ariizumi et al., 2011), making it possible that levels of GAI protein are also increased in the *hop1 hop2 hop3* mutant, contributing to its GA-related phenotype.

Interestingly, despite SNE accumulation being compromised in the *hop1 hop2 hop3* mutant, it is not fully abolished. This suggests at least two possibilities. On the one hand, HOPs may be required for a specific step in SNE folding. In an extreme scenario, this would cause all SNE molecules to have a folding defect that nevertheless would not completely abolish SNE function. While this situation seems possible, we favor an alternative hypothesis. For this, it is worth mentioning that in humans, it has been demonstrated that HOP is not strictly required for GR folding but that HOP significantly increases the overall ratio of GR's native conformation (Morishima et al., 2000). In such an alternative scenario, fully folded SNE proteins would coexist with partially folded SNE molecules in the absence of HOPs, explaining the mild GA sensitivity–associated phenotypes observed in the *hop* mutants. In addition, this alternative also explains why overexpression of SNE rescues the *hop* mutant phenotype: SNE overexpression may increase the levels of SNE functional protein, allowing functional SNE to reach the minimal threshold necessary to restore the GA response (Figure 7).

We have previously demonstrated that HOP proteins are involved in the accumulation/stability of the JA and auxin-associated F-box proteins TIR1 and COI1, and in this work, we expand this understanding to the GA-related F-box protein SNE, positioning this family of co-chaperones as master regulators of hormonal signaling in plants. The *Arabidopsis* genome includes nearly 700 F-box proteins (Zhang et al., 2019). Whether HOPs assist in the folding of other F-box proteins or whether other co-chaperones, such as SGT1b, are also involved in assisting with SNE folding are open questions that require further investigation. Nevertheless, this work reveals the role of HOPs in the GA pathway, underscoring the importance of protein folding and proper accumulation of relevant components of the GA network.

METHODS

Materials, constructs, and growth conditions

Arabidopsis thaliana ecotype Columbia-0 (Col-0) was used as the wild-type genetic background control. *Arabidopsis* T-DNA insertion mutants *hop1-1* (GK-420A10.15), *hop2-1* (GK-399G03.03), and *hop3-1* (Salk_00794) were previously characterized in Fernandez-Bautista et al. (2017, 2018). *sne* (CS103608) was acquired from the Arabidopsis Biological Resource Center (ABRC). The *hop1 hop2 hop3* and *rga-28 gai-td1* mutants were previously described in Fernandez-Bautista et al. (2018) and Plackett et al. (2014), respectively.

The clones *DKLAT4G24210* and *DKLAT5G48170* expressing, under the control of the *35S* promoter, either *SLY1* or *SNE* fused to

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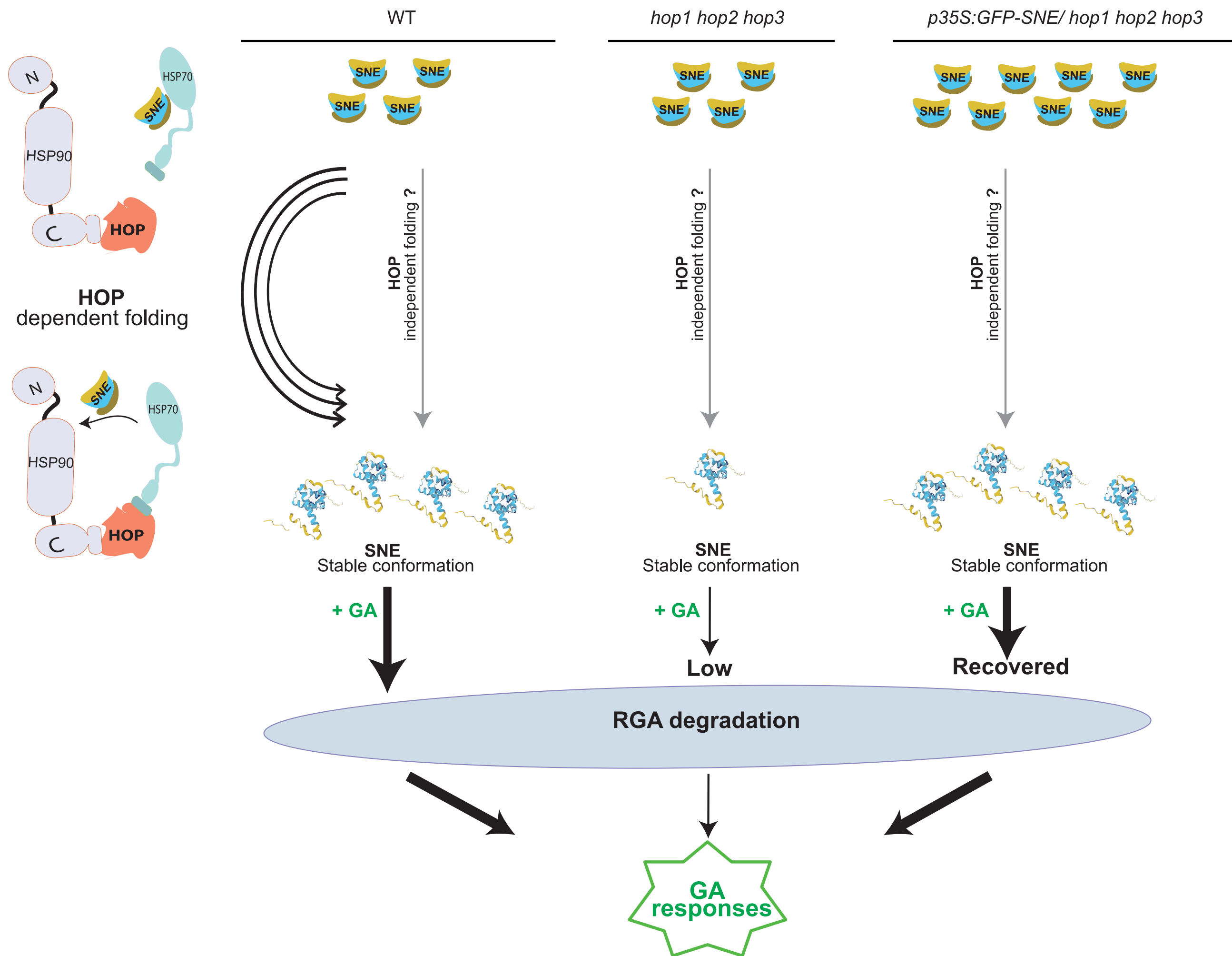

Figure 7. Model of the role of HOP in GA signaling.
Under control conditions, HOP proteins would facilitate the stability and therefore the accumulation of SNE molecules by assisting in their folding through the HSP70-HSP90 cycle. Nevertheless, it is also possible that some of the SNE molecules could either fold spontaneously or be assisted in their folding by other co-chaperones. In the *hop1 hop2 hop3* mutant, although the HOP-dependent mechanism would be blocked, reducing the accumulation of SNE, some molecules of SNE could, through a HOP-independent mechanism or through residual activity of HOP in the *hop1 hop2 hop3* mutant, still achieve an active conformation that would explain the reduction, but not the complete absence, of SNE proteins in the mutant background. This would also explain why the wild-type (WT) phenotype is recovered in the *hop1 hop2 hop3* mutant when overall SNE levels are enhanced by overexpression of SNE, since more molecules are allowed to be folded. The accumulation of SNE in the different conditions would affect the efficiency of RGA and, probably of GAI degradation, which would lead to reduced GA signaling in the *hop1 hop2 hop3* mutant.

9xMyc-6xHis-2xIgG Binding Domain (IgGBD) were obtained from the ABRC. These clones were used to express the proteins in *N. benthamiana* or as templates for *SLY1* and *SNE* coding sequence (CDS) amplification and cloning into *pDONR* vectors. Cloning of the *HOP1*, *HOP2*, and *HOP3* CDSs into *pDONR* vectors was described previously (Fernandez-Bautista et al., 2017, 2018). The constructs *p35S-SLY1-HA*, *p35S-SNE-HA*, *GAL4-BD-SLY1*, and *GAL4-BD-SNE* were obtained by cloning the *SLY1* or *SNE* coding sequence in frame with the HA epitope or the *GAL4-BD* by recombination of the corresponding *pDONR* plasmids with *pGWB14* (Nakagawa et al., 2007) and *pDEST-GBKT7* (Rossignol et al., 2007), respectively. The constructs *p35S-HA-HOP1*, *p35S-HA-HOP2*, *p35S-FLAG-HOP2*, *GAL4-AD-HOP1*, and *GAL4-AD-HOP2* were obtained by cloning the *HOP1* or *HOP2* CDS in frame with the HA or FLAG epitopes or with the *GAL4-AD* by recombination of the corresponding *pDONR* plasmids with *pGWB15, pGWB12* (Nakagawa et al., 2007) and *pDEST-GADT7* (Rossignol et al., 2007), respectively. The constructs *p35S:HA-HOP3* and *p35S:HSP90.1-HA* were described previously (Fernandez-Bautista et al., 2017; Muñoz et al., 2022).

The *pRGA:GFP-RGA* line (Silverstone et al., 2001) in the Col-0 background was obtained from Dr. Salomé Prat (CRAG, Spain). This line was crossed with the *hop2-1* mutant to obtain lines expressing *pRGA:GFP-RGA* in the *hop2-1* background. The construct *p35S:GFP-SNE* was obtained by cloning the *SNE* CDS in frame with the *GFP* gene by recombination of the corresponding *pDONR* plasmid with the binary vector *pGWB6* (Nakagawa et al., 2007). This latter construct was transformed into both Col-0 and the *hop1 hop2 hop3* mutant by the floral dip method (Clough and Bent, 1998). In both cases, homozygous lines for the reporter constructs and mutants were used for further experiments.

Unless otherwise stated, seeds were surface-sterilized and grown at 22°C using a 16-h light photoperiod. For growth on plates, Murashige and Skoog (MS) medium supplemented with 1% (w/v) sucrose was used.

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Bolting assays

Twenty plants from each genotype were grown on soil at 22°C in long-day conditions, i.e., a 16 h/8 h light/dark regimen. The number of days required by each plant for bolting initiation (observed as a rapid elongation of the stem) was scored and quantified. GA treatments were carried out as described in Yang et al. (2020). In brief, 21-day-old plants were sprayed twice a week with a 0.02% (v/v) Silwet-77 solution with or without 100 μM GA_3 until bolting initiation was observed. Three independent biological replicates were analyzed and used for calculations and statistical analyses, applying the statistical tests described in the figure legends.

Salt stress experiments

For salt stress experiments carried out in hydroponic conditions, seeds were directly stratified for 48 h at 4°C in the dark in Araponic boxes (Araponics NV, Liege, Belgium). The plants were then grown for 2 weeks in a 1.8-L plastic pot filled with a complete nutrient solution including the following: 1.0 mM $Ca(NO_3)_2$, 0.5 mM $MgSO_4$, 0.5 mM H_3PO_4, 50 μM Fe-EDTA, 50 μM $CaCl_2$, 25 μM H_3BO_3, 2 μM $ZnSO_4$, 2 μM $MnSO_4$, 0.5 μM $CuSO_4$, 0.5 μM molybdic acid, 1 mM KCl, and 2.5 mM MES. The pH was brought to 6.0 by addition of $Ca(OH)_2$. After this time, the seedlings were transferred to fresh hydroponic medium containing 0, 20, or 50 mM NaCl and were allowed to grow for 2 more weeks.

For germination experiments under salt stress, stored seeds were directly plated without prior stratification on 0.6% (w/v) plant agar in the presence or absence of 150 mM NaCl or of 150 mM NaCl plus 50 μM gibberellin. Seeds were germinated as described above, and germination rate was scored at different time points. Three independent biological replicates were analyzed and used for calculations and statistical analyses, applying the statistical tests described in the figure legends.

Analysis of GA sensitivity

Stored seeds (50 seeds per genotype and condition) were plated on 0.6% (w/v) plant agar in the absence or presence of 10 μM paclobutrazol (PAC), different concentrations of gibberellin GA_3 (0, 1, 5, 10, or 50 μM) or PAC (10 μM) plus GA_3 (5 μM), as described in the figure legends. Seeds were directly germinated (without prior stratification) at 20°C under a 16-h light photoperiod, and germination rate was scored at different time points. In each case, three independent biological replicates were analyzed and used for calculations and statistical analyses, applying the statistical tests described in the figure legends.

Yeast two-hybrid analyses

Yeast transformation into AH109 and interaction analyses were carried out as described in (Castellano and Sablowski, 2008).

Co-immunoprecipitations in *N. benthamiana* leaves

Co-immunoprecipitation studies were carried out as described in Muñoz and Castellano (2018) with minor modifications highlighted in the figure legends. After immunoprecipitation, the specific epitopes incorporated in the fusion proteins were detected by western blotting using anti-HA (Roche), anti-FLAG (Sigma), anti-Myc (Merck), or anti-HSP70 (Agrisera). These experiments were repeated three times with similar results.

qRT-PCR analysis

qRT-PCRs were performed as described in Echevarria-Zomeno et al. (2015) using *PP2A* (*AT1G13320*) for normalization. Each experiment was conducted as three technical replicates of three biological replicates, which were used for statistical analyses with the statistical tests described in the figure legends. Primer sequences are listed in Supplemental Table 1.

Analyses of RGA and SNE accumulation in different genetic backgrounds

For analysis of ectopic RGA and SNE accumulation, approximately 40 seedlings of the respective lines (*pRGA*:*GFP-RGA* or *p35S*:*GFP-SLY2*) in Col-0 or in the corresponding *hop* mutant backgrounds were grown for 7 days. Protein was extracted in a buffer containing 50 mM Tris-HCl (pH 7.5), 150 mM NaCl, 5% (v/v) glycerol, 0.1% (v/v) Triton X-100, 0.5 mM phenyl-methanesulfonyl fluoride, and protease inhibitor cocktail (Sigma). Protein was quantified by the Bradford method, and equal amounts of protein were loaded in a gel and analyzed by western blotting using anti-GFP antibody (Roche).

For analysis of endogenous RGA levels, 40 seedlings of Col-0 or the *hop1 hop2 hop3* mutant were grown for 3 days. Protein extraction was carried out as before, and proteins were analyzed by western blotting using anti-RGA antibody (Agrisera). Actin accumulation (loading control) was analyzed using the anti-Actin antibody (Sigma).

Analyses of hypocotyl elongation at control and warm temperatures

Arabidopsis seedlings from the different genotypes were grown side by side in a vertical position for 4 days under short day conditions at 20°C. After this time, the plates were maintained at 20°C or transferred to 29°C for an additional 5 or 6 days as described in the figure legends. For the combination of GA treatments with these experiments, 4-day-old seedlings grown in short days at 20°C were transferred onto fresh MS media in the presence or absence of 50 μM GA, and replicate plates were transferred to either 20 or 29°C. Hypocotyl growth after the transfer was measured using ImageJ (https://imagej.nih.gov/ij/index.html). For each experiment, 30 seedlings per genotype and condition were assayed. Statistically significant differences of n = 3 independent biological replicates were calculated, applying the statistical tests described in the figure legends.

DATA AVAILABILITY

The data that support the findings of this study are available from the corresponding author upon reasonable request.

SUPPLEMENTAL INFORMATION

Supplemental information is available at *Plant Communications Online*.

FUNDING

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AUTHOR CONTRIBUTIONS

S.M. and A.M. participated in the design of the experiments and contributed to writing the article. S.M. carried out most of the experimentation with the help of A.M. and L.F.C., who carried out the analyses of SLY1 and SNE interactions with HSP90 and HSP70 and bolting, respectively. M.M.C. planned the research, participated in the design, supervised the experiments, and wrote the article with contributions from all authors.

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