COP1 destabilizes DELLA proteins in *Arabidopsis*

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DELLA transcriptional regulators are central components in the control of plant growth responses to the environment. This control is considered to be mediated by changes in the metabolism of the hormones gibberellins (GAs), which promote the degradation of DELLAs. However, here we show that warm temperature or shade reduced the stability of a GA-insensitive DELLA allele in *Arabidopsis thaliana*. Furthermore, the degradation of DELLA induced by the warmth preceded changes in GA levels and depended on the E3 ubiquitin ligase CONSTITUTIVELY PHOTOMORPHOGENIC1 (COP1). COP1 enhanced the degradation of normal and GA-insensitive DELLA alleles when coexpressed in *Nicotiana benthamiana*. DELLA proteins physically interacted with COP1 in yeast, mammalian, and plant cells. This interaction was enhanced by the COP1 complex partner SUPRESSOR OF *phyA-105* 1 (SPA1). The level of ubiquitination of DELLA was enhanced by COP1 and COP1 ubiquitinated DELLA proteins in vitro. We propose that DEL-LAs are destabilized not only by the canonical GA-dependent pathway but also by COP1 and that this control is relevant for growth responses to shade and warm temperature.

shade avoidance | thermomorphogenesis | environment | gibberellin | growth

A plant can adopt markedly different morphologies depending on the environment it has to cope with. This plastic behavior relies on highly interconnected signaling pathways, which offer multiple points of control (1). Light and temperature are among the most influential variables of the environment in plant life. For instance, light cues from neighboring vegetation as well as elevated ambient temperature (e.g., 28 °C) enhance the growth of the hypocotyl (among other responses), respectively, to avoid shade (2) and enhance cooling (3).

Several features place DELLA proteins as central elements in environmental responses (4). First, DELLAs are nuclear-localized proteins that interact with multiple transcription factors and modulate their activity (5). Second, they are negative elements in the gibberellin (GA) signaling pathway and their stability is severely diminished upon recognition of their N-terminal domain by the GA-activated GIBBERELLIN INSENSITIVE1 (GID1) receptor, which recruits the SCF$^{SLY1/GID2}$ complex to promote their ubiquitination-dependent degradation by the proteasome (6). Third, GA metabolism is regulated by the environment; for instance, shade and warm temperature induce GA accumulation (3, 7).

DELLA levels increase during seedling deetiolation or cold exposure and promote transcriptional changes associated with photomorphogenesis or with the adaptation to low temperatures,

respectively (8–10). On the contrary, they decrease during the night and in response to shade inflicted by neighbor plants or to warm ambient temperature, allowing the promotion of hypocotyl and/or petiole elongation by transcription factors such as PHYTOCHROME INTERACTING FACTOR4 (11–14). Interestingly, the role of DELLAs in all these processes is the opposite to that of CONSTITUTIVELY PHOTOMORPHOGENIC1 (COP1), another central regulator of light and temperature responses. COP1 is an E3 ubiquitin ligase that promotes proteasome-dependent degradation of a number of transcription factors involved in light and temperature signaling. COP1 becomes inactivated by light perceived by phytochromes and cryptochromes and by low-to-moderate temperature (4 °C to 23 °C) (15–20) and requires the activity of the SUPRESSOR OF *phyA-105* proteins (SPA1 to 4 in *Arabidopsis*) to be active in vivo (21). Here we show the direct physical interaction between DELLAs and COP1/SPA1 complex and propose a mechanism of

Significance

DELLA proteins are plant-specific transcriptional regulators that act as signaling hubs at the interface between the environment and the transcriptional networks that control growth. The growth-promoting hormone gibberellin destabilizes DELLAs. Here we describe an alternative pathway to destabilize these proteins. We show that DELLAs are substrate of COP1, an E3 ubiquitin ligase that increases its activity to promote growth in response to shade or warmth. Our results show that COP1, and not changes in gibberellin levels, mediates the rapid destabilization of DELLAs in response to environmental cues.
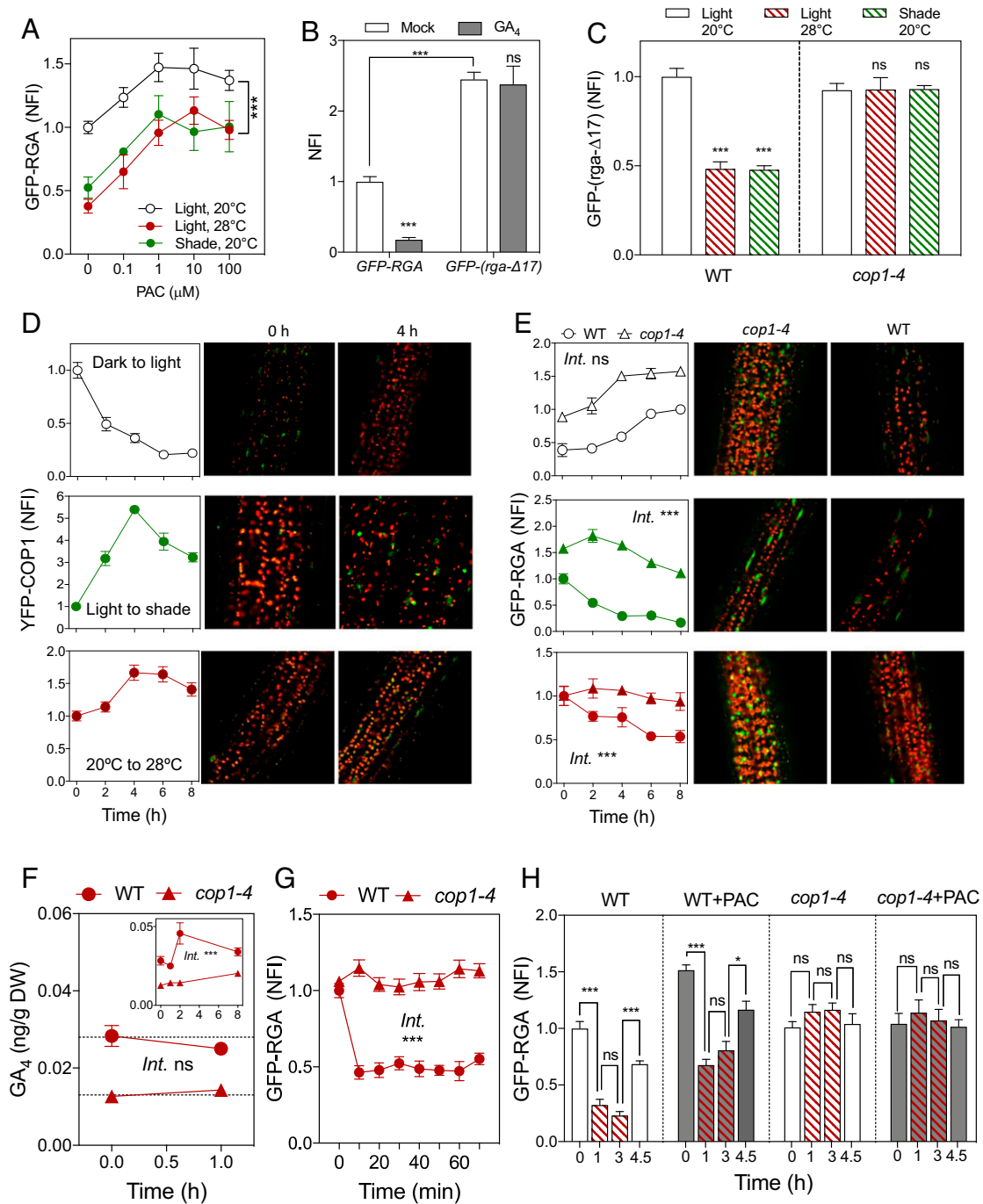
Fig. 1. COP1 regulates RGA levels in *Arabidopsis* hypocotyls in response to shade and warmth. (*A*) GFP-RGA levels respond to shade and warmth in the absence of GA synthesis. We incubated the seedlings with the indicated doses of PAC for 8 h, before exposure to shade or 28 °C for 4 h. (*B*) GFP-(rga-Δ17) is insensitive to GA-induced degradation. Seedlings of *pRGA:GFP-RGA* and *pRGA:GFP-(rga-Δ17)* lines were mock treated or treated with 3 μM GA$_4$ for 4 h. (*C*) Shade or warmth reduces GFP-(rga-Δ17) levels in a COP1-dependent-manner. Seedlings were exposed to the treatment for 3 d. (*D* and *E*) Dynamics of nuclear accumulation of YFP-COP1 (*D*) in the wild type and of GFP-RGA (*E*) in the wild type (circles) and *cop1-4* mutants (triangles) after the transfer from darkness to light (white symbols), light to shade (green symbols), and 20 °C to 28 °C (red symbols). (*F*) Time course of GA$_4$ levels in wild-type and *cop1-4* seedlings before (time = 0 h) and after transfer to 28 °C (*Inset* shows an extended time course). (*G*) Time course of nuclear levels of GFP-RGA in wild-type and *cop1-4* seedlings before (time = 0 h) and after transfer to 28 °C. (*H*) The reduction of GFP-RGA levels in response to 28 °C is reversible, unaffected by a saturating dose of PAC and dependent on COP1. Seedlings of *pRGA:GFP-RGA* in the wild-type and *cop1-4* backgrounds were returned to 20 °C after 3 h-treatment of 28 °C. We incubated the seedlings with PAC for 8 h before exposure to 28 °C. Confocal data (*A*, *E*, *G*, and *H*) show the normalized fluorescence intensity (NFI) in nuclei (NFI = 1 in the wild-type seedling control, and in *B* in the wild-type RGA control). For representative images, see *D* and *E* (8-h time point) and *SI Appendix*, Fig. S2 *A* and *B*. Confocal microscopy data are means and SE of 5 to 10 (*A*), 6 to 9 (*B*), 6 to 14 (*C*), 18 (*D* and *E*), 6 to 13 (*G*), and 18 (*H*) seedlings (a minimum of 10 and up to 50 nuclei were averaged per seedling replicate). GA$_4$ data are means and SE of three independent biological replicates. In *A*, *C*, and *G* we indicate the significance of the differences with the control condition (*B* also shows the difference between genotypes) in Student's *t* test or ANOVA followed by Bonferroni tests. In *E–G* we indicate the significance of the term accounting for the interaction (Int.) between condition (light, temperature) and genotype (wild type, *cop1*) in multiple regression analysis. In *H*, we indicate the significance of the comparison with the preceding bar in ANOVA followed by Bonferroni tests. *$P < 0.05$, ***$P < 0.005$; ns, nonsignificant.

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regulation of DELLA stability different from the canonical GA signaling pathway.

Results

Warm Temperature or Shade Decreases the Abundance of a GA-Insensitive DELLA Protein. Warm temperatures (28 °C hereafter) or shade decrease the abundance of the DELLA protein REPRESSOR OF *ga1-3* (RGA) (Fig. 1*A* and *SI Appendix*, Fig. S1*A*) (11, 14). Two observations indicate that changes in GA cannot fully account for these reductions. First, increasing doses of the GA-inhibitor paclobutrazol

Fig. 2. COP1 destabilizes DELLAs. (*A*) The reduction of GFP-RGA levels by warm temperature or shade requires the 26S proteasome and COP1. Confocal data show the NFI in nuclei (NFI = 1 in the wild-type seedling control). NFI data are means and SE of 6 to 9 seedlings (10 to 30 nuclei were averaged per seedling replicate). Asterisks indicate that the difference is statistically significant (Student's *t* test, ******P < 0.01 and *******P < 0.001; ns, nonsignificant). (*B* and *C*) COP1 destabilizes RGA (*B*) and the GA-insensitive rga-Δ17 (*C*) in *N. benthamiana* leaves. HA-RGA and HA-(rga-Δ17) were transiently expressed alone or with Flag-COP1 in leaves of *N. benthamiana*. For MG132 treatments, leaves were infiltrated with a solution of 25 μM of the inhibitor 8 h before sampling. HA-GFP was used as control to demonstrate the specificity of COP1 action. Blots show data from three individual infiltrated leaves per mixture. Plots show HA-RGA and HA-(rga-Δ17) normalized against HA-GFP. Data are means and SE of three leaves from one experiment, repeated twice with similar results. Asterisks indicate that the difference is statistically significant (Student's *t* test, *****P < 0.05 and ******P < 0.01; ns, nonsignificant).

(PAC) elevated RGA nuclear abundance observed by confocal microscopy in a *pRGA:GFP-RGA* line (22), but the reductions caused by shade or warmth persisted even under saturating levels of the inhibitor (Fig. 1*A* and *SI Appendix*, Fig. S2*A*). Second, warm temperature or shade reduced the levels of rga-Δ17, a mutant version of RGA that is fully insensitive to GA, in the *pRGA:GFP-(rga-Δ17)* line (Fig. 1 *B* and *C* and *SI Appendix*, Figs. S1*A* and S2*B*) (23). Changes in *RGA* transcript levels do not mediate the altered RGA abundance in response to shade (14) or warm temperature (*SI Appendix*, Fig. S1*B*). Importantly, treatment with the inhibitor of the 26S proteasome MG132 fully impaired changes in RGA abundance (Fig. 2*A*). Altogether, these results suggest the existence of a noncanonical pathway of DELLA degradation.

COP1 Affects RGA Levels in Response to Shade and Warmth. RGA levels are elevated in *cop1-4* seedlings (24). Compared to light at moderate temperature, darkness, or 2 to 8 h of shade or warm temperature increased the nuclear abundance of COP1 (15, 25, 26) in a *35S:YFP-COP1 cop1-4* line (27), while reducing RGA levels (9, 11, 14) (Fig. 1 *D* and *E*). The light-induced increase in RGA showed wild-type kinetics in the *cop1-4* seedlings (note parallel curves), suggesting that this change is driven by a COP1-independent light-induced down-regulation of GA biosynthesis (8, 9, 28, 29). Conversely, *cop1-4* seedlings grown in the light at moderate temperature (20 °C) and transferred either to shade at the same temperature or to light at 28 °C, showed a weaker decrease in GFP-RGA (Fig. 1*E*).

Changes in RGA Abundance Precede Changes in GA. GA$_4$ levels were unaffected by transferring the seedlings from 20 °C to 28 °C for 1 h (Fig. 1*F*), while 10 min of warm temperature were enough to induce significant nuclear accumulation of COP1 (*SI Appendix*, Fig. S2*C*) and decrease GFP-RGA levels in a COP1-dependent manner (Fig. 1*G*). These results indicate that rapid warmth-induced degradation of RGA requires COP1 and precedes changes in GA.

Relative Contribution of Each Pathway. GA levels did increase after 2 h of warm temperature (Fig. 1 *F*, *Inset*). However, two observations indicate a negligible contribution of these changes in GA levels to the reduced GFP-RGA abundance in response to warmth. First, we observed no significant decreases in GFP-RGA between 1 and 3 h at 28 °C (i.e., concomitantly with the increase in GA) (Fig. 1*H*), despite the fact that GFP-RGA responds to exogenously applied GA in less than 15 min (22). Second, application of a saturating dose of PAC to block GA synthesis significantly increased GFP-RGA levels, but resulted in a parallel kinetics in response to warm temperature (Fig. 1*H*). GFP-RGA levels increased rapidly after returning the seedlings from 28 °C to 20 °C, a response also observed in the presence of PAC (Fig. 1*H*). *cop1-4* showed reduced levels of GA$_4$ but retained some GA$_4$ response to temperature (*P* < 0.05, Fig. 1 *F*, *Inset*), which may have contributed to residual GFP-RGA degradation observed in this mutant beyond 4 h of shade or warmth (*P* < 0.05, Fig. 1*E*). Similarly, the *cop1* mutation lowers GA levels in the pea without eliminating its response to light (30). Taken together, these observations indicate that changes in GA have no major direct contribution to the rapid changes in RGA abundance, because when GA levels are elevated, COP1 has already induced RGA decay. However, the canonical GA pathway would make an indirect contribution to the rapid changes, setting basal RGA levels within a range where the system becomes sensitive to COP1. In fact, although shade and warmth did reduce GFP-(rga-Δ17) levels (Fig. 1*C* and *SI Appendix*, Fig. S2*B*), these effects were not rapid (*SI Appendix*, Fig. S2 *D* and *E*). Similarly, warm temperature does not provoke rapid changes in RGA levels in the GA-deficient mutant *ga1* (11).
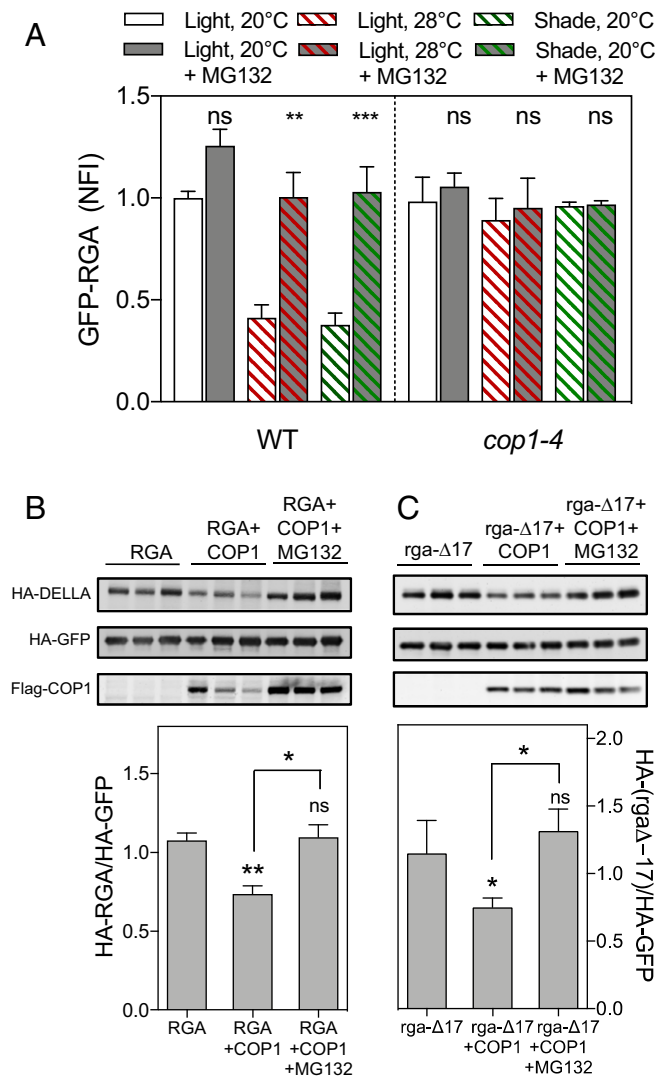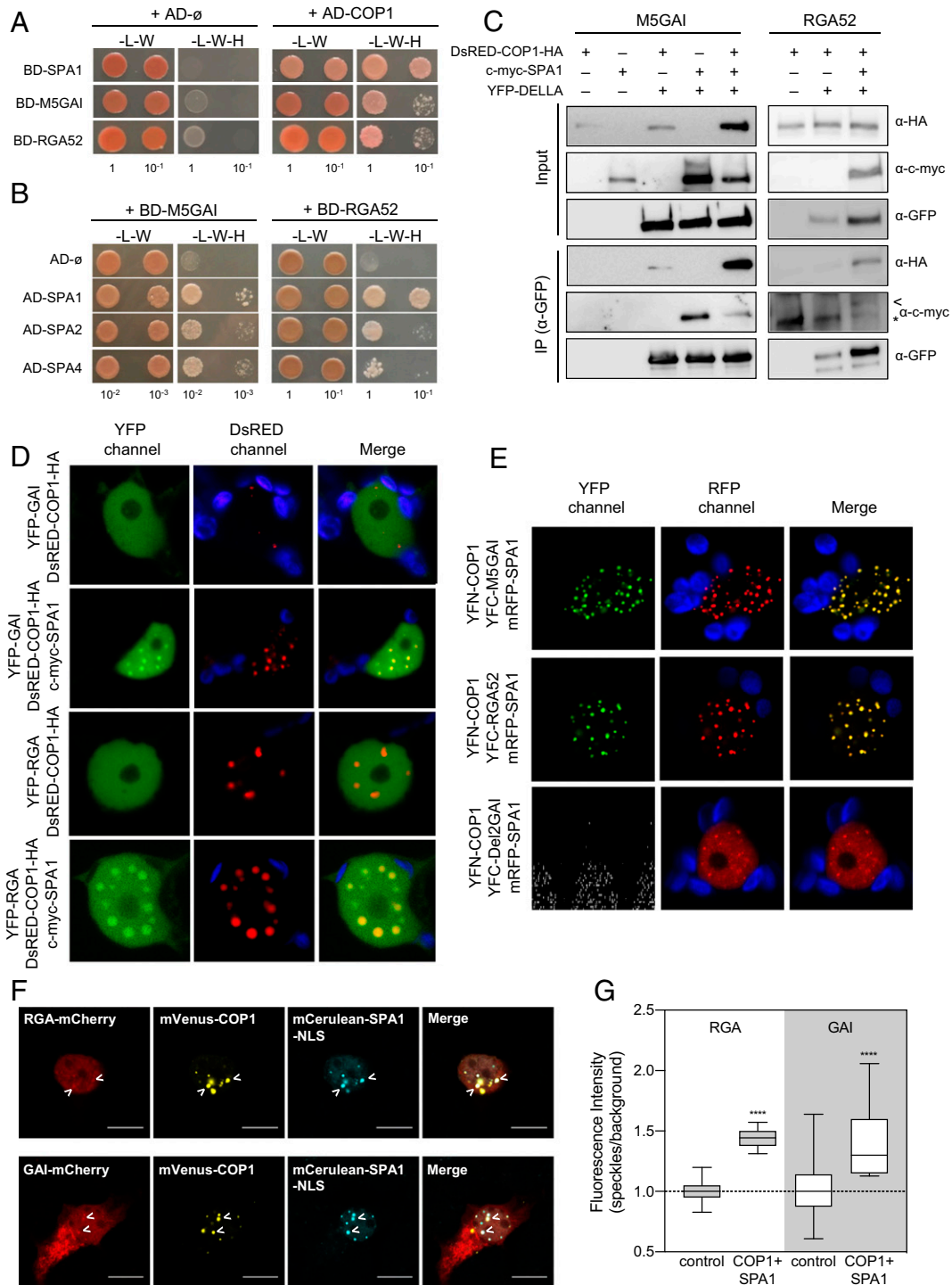
Fig. 3. COP1 physically interacts with DELLA proteins. (*A* and *B*) Y2H assays showing the interaction between N-terminal deleted versions of GAI and RGA with COP1 (*A*) and SPAs (*B*). L, leucine; W, tryptophan; H, histidine. Numbers indicate the dilutions used in the drop assay. (*C*) Coimmunoprecipitation assays showing interactions in planta. YFP-M5GAI and YFP-RGA52 were transiently expressed in leaves of *N. benthamiana* together with DsRED-COP1-HA, c-myc-SPA1, or both. Proteins were immunoprecipitated with anti-GFP antibody-coated paramagnetic beads. Leaves expressing DsRED-COP1-HA or c-myc-SPA1 alone were used as negative controls. The arrowhead and asterisk mark the coimmunoprecipitated c-myc-SPA1 and a nonspecific band, respectively. (*D*) YFP-GAI and YFP-RGA colocalize with DsRED-COP1-HA in nuclear bodies in the presence of c-myc-SPA1. Fusion proteins were transiently expressed in leaves of *N. benthamiana* and observed by confocal microscopy. One representative nucleus is shown. (*E*) BiFC assay showing that COP1 and SPA1 form a complex with M5GAI or RGA52 in nuclear bodies. The indicated proteins were expressed in leaves of *N. benthamiana* and observed by confocal microscopy. One representative nucleus is shown. (*F*) Representative HEK-293T cells cotransfected with mVenus-COP1, NLS-mCerulean-SPA1, and either GAI-mCherry or RGA-mCherry. (Scale bar, 10 μm.) The arrowheads point to two representative speckles co-occupied by DELLAs, SPA1, and COP1. (*G*) Fluorescence from GAI-mCherry or RGA-mCherry accumulates in the regions corresponding to speckles formed by COP1 and SPA1 in cells coexpressing mVenus-COP1 and mCerulean-SPA1-NLS. Data are mean from 10 to 13 transfected cells. The boxes extend from the first to the third quartile around the median, while whiskers go down to the smallest value and up to the largest. Asterisks indicate that the difference is statistically significant with the control condition (Student's *t* test, ****$P < 0.0001$).

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COP1 Promotes Degradation of a GA-Insensitive DELLA Protein. The fact that warm temperature or shade failed to reduce the nuclear abundance of RGA or rga-Δ17 in *cop1-4* mutants (Figs. 1 *C*, *E*, *G*, and *H*, and (2*A*) and that these changes are dependent on the 26S proteasome (Fig. 2*A*), suggests that COP1 promotes DELLA degradation. We first tested this possibility in transient expression assays in *Nicotiana benthamiana* leaves. Coexpression of COP1 caused 26S proteasome-dependent decrease of HA-RGA and HA-(rga-Δ17) in leaves of long-day-grown *N. benthamiana* plants, while it had no impact on levels of the unrelated protein HA-GFP (Fig. 2 *B* and *C*). Warm temperature decreased HA-(rga-Δ17) in a COP1-mediated manner (*SI Appendix*, Fig. S3). This suggests that COP1 mediates the destabilization of RGA by noncanonical mechanisms.

COP1 Interacts Physically with GAI and RGA in Yeast. To explore if COP1 mediates RGA degradation by noncanonical mechanisms, we first investigated whether COP1 physically interacts with DELLA proteins. We performed yeast two-hybrid (Y2H) assays between COP1 and the two DELLAs with a major role in light- and temperature-dependent growth, RGA and GIBBERELLIC ACID INSENSITIVE (GAI) (11, 12, 31). To avoid the reported strong autoactivation of full-length DELLAs in yeast, we used previously established variants with deletions of the N terminus named M5GAI and RGA52 (13, 32). COP1 was able to interact with both (Fig. 3*A*). SUPPRESSOR OF *phyA-105* 1 (SPA1) and other SPA proteins involved in a functional complex with COP1 (21, 33) were also able to interact with GAI and RGA in Y2H assays (Fig. 3*B*).

COP1 Interacts with GAI and RGA In Planta. To investigate whether the interaction between DELLAs and COP1 also occurs in plant cells, we first performed coimmunoprecipitation assays in leaves of *N. benthamiana* coexpressing DsRED-COP1-HA and YFP-M5GAI or YFP-RGA52. While DsRED-COP1-HA was pulled down by anti-GFP antibodies from leaf extracts coexpressing YFP-M5GAI, and the interaction appeared to be enhanced in the presence of c-myc-SPA1, the DsRED-COP1-HA and YFP-RGA52 interaction was only observed when the three proteins were coexpressed (Fig. 3*C*). c-myc-SPA1 was also specifically coimmunoprecipitated with YFP-M5GAI (Fig. 3*C*). These results suggest that SPA1 enhances the interaction between COP1 and DELLA proteins. Consistent with this idea, we observed relocalization of YFP-GAI, YFP-RGA, and RGA52-YFP to nuclear bodies co-occupied by DsRED-COP1-HA in the presence of c-myc-SPA1 (Fig. 3*D* and *SI Appendix*, Fig. S4*A*).

COP1-SPA1 Forms a Ternary Complex with DELLA. The formation of a ternary complex was evidenced by bimolecular fluorescence complementation (BiFC) assays in leaves of *N. benthamiana*, in which the colocalization of signals from mRFP-SPA1 and the reconstituted YFP, due to the interaction between YFC-DELLAs and YFN-COP1, was evident in nuclear bodies (Fig. 3*E* and *SI Appendix*, Fig. S4*B*). Similarly, YFP signal in nuclear bodies was observed by coexpressing c-myc-SPA1 (*SI Appendix*, Fig. S4*C*). However, no YFP fluorescence was detected in the absence of SPA1 or when YFC was fused to Del2GAI, a truncated version of GAI that does not interact with SPA1 (Fig. 3*E* and *SI Appendix*, Fig. S4 *B–E*). As expected, mRFP-SPA1 was recruited to nuclear bodies when coexpressed with YFN-COP1 (Fig. 3*E* and *SI Appendix*, Fig. S4*B*) (34).

To quantify the interaction between GAI or RGA and the COP1-SPA1 complex we expressed these proteins tagged to fluorescent reporters in mammalian cells. This orthogonal system allows the performance of such studies with the components of interest, in the absence of other plant proteins that might interfere with the evaluation. The fluorescence from DELLA-mCherry fusions in the cytosol and nucleus was relatively homogeneous when

either GAI or RGA was expressed alone (Fig. 3*F*; note the ratio of fluorescence between different nuclear regions close to 1 in Fig. 3*G*). However, the ratio between DELLA fluorescence inside/outside the speckle-like structures formed in the nucleus by the COP1-SPA1 complex was above 1 (Fig. 3 *F* and *G* and *SI Appendix*, Fig. S5), suggesting that the COP1-SPA1 complex drags RGA and GAI to the speckles by physical interaction. Taken together, these observations demonstrate that the COP1-SPA1 complex interacts with DELLA proteins.

COP1 Ubiquitinates GAI and RGA In Vitro. In vivo levels of ubiquitinated GFP-RGA were enhanced by overexpression of COP1 (*SI Appendix*, Fig. S6). To test whether this is the result of the direct interaction between COP1 and DELLAs, we performed an in vitro ubiquitination assay using recombinant MBP-COP1 and 6xHis-M5GAI or 6xHis-RGA52. A slow-migrating band corresponding to the size of Ub-6xHis-M5GAI or Ub-6xHis-RGA52 was observed only when MBP-COP1 and the E2 enzyme were included in the assays (Fig. 4 *A* and *B*). The delayed band did not appear, however, when Zn^{2+} ions, which are required for the proper arrangement of the RING domain of E3 ubiquitin ligases like COP1 (35), were excluded from the reaction mixtures (Fig. 4 *A* and *B*). To confirm that the slow migration of 6xHis-M5GAI and 6xHis-RGA52 is due to ubiquitination, we repeated the assay for 6xHis-M5GAI in the presence of HA-tagged ubiquitin. We detected low-migrating bands in the immunoblot with anti-GAI antibody when free ubiquitin was included in the assay, which were further upshifted when we used the HA-tagged version of ubiquitin instead (Fig. 4*C*). This result indicates that M5GAI and RGA52 are targets of the E3 ubiquitin ligase activity of COP1 in vitro.
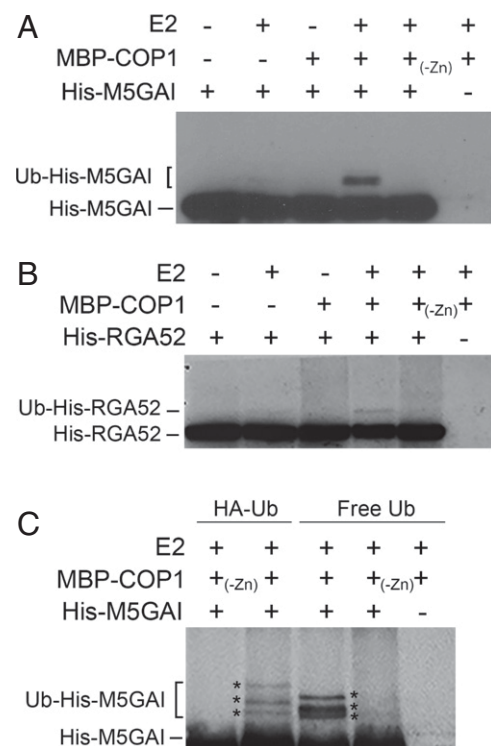
Fig. 4. COP1 ubiquitinates GAI and RGA. (*A* and *B*) The 6xHis-M5GAI (*A*) and 6xHis-RGA52 (*B*) ubiquitination assay using recombinant MBP-COP1, rice E2, and unmodified ubiquitin. (*C*) The 6xHis-M5GAI ubiquitination assay using unmodified and HA-tagged ubiquitin. Modified and unmodified 6xHis-M5GAI and 6xHis-RGA52 were detected with anti-GAI and anti-6xHis antibodies, respectively.

COP1 Controls Hypocotyl Elongation in a DELLA-Dependent Manner.
The growth phenotypes caused by GA deficiency or *cop1* mutations in the dark (31, 36), in response to light cues from neighbors (14, 26) or to warm temperature (11, 15, 37), are very similar. To determine the physiological relevance of the regulation of DELLA levels by COP1, we studied how mutations at *COP1* and *DELLA* genes and GA treatments impact on the hypocotyl growth rate of dark-grown seedlings transferred to light at 20 °C (deetiolation) as well as light-grown seedlings transferred to shaded or warm environments. Noteworthy, the patterns differed between the first case, where DELLA levels build up, and the other two cases, where DELLA levels decrease (Fig. 1*E*). In fact, during deetiolation, growth in the presence of 5 μM GA$_4$ promoted the rate of hypocotyl elongation in seedlings transferred to the light but not in seedlings that remained in the dark, suggesting that endogenous GA levels are not limiting in darkness (Fig. 5). As expected, the *cop1* mutants showed reduced growth in darkness; however, they retained a significant growth response to light. This response was only marginally enhanced by adding GA$_4$ or by the *gai-td1* and *rga-29* (24) mutations of *DELLA* genes. In other words, during deetiolation, the rapid inactivation of COP1 does not appear to be rate limiting for the RGA accumulation (Fig. 1*E*) or the growth inhibition (Fig. 5) responses. Addition of 5 μM GA$_4$ promoted growth in light-grown seedlings transferred to shade or to warm temperature (Fig. 5), suggesting that GA signaling is limiting under those conditions. The *cop1-4* and *cop1-6* mutants failed to respond to shade or warm temperatures but the responses were restored both by the application of GA$_4$ and by the presence of mutations

of both *DELLA* genes. This indicates that the responses were limited by the elevated levels of DELLAs in *cop1* and reducing the DELLA pool either genetically or by the GA treatment was enough to rescue the *cop1* phenotype.

Discussion

The results presented here establish a functional link between DELLA proteins and COP1, two of the major hubs in the control of plant architecture. The growth of the hypocotyl of *Arabidopsis* shifted from light at moderate temperatures to either warm or shade conditions requires COP1 only if DELLA proteins are present (Fig. 5). These environmental cues reduce phyB activity (2, 38, 39) and enhance COP1 nuclear abundance (Fig. 1*D*), while reducing the levels of RGA in a COP1-dependent manner (Fig. 1*E*). COP1 does not simply reduce DELLA protein abundance by increasing GA levels. First, COP1 migrates to the nucleus and mediates RGA degradation in response to warm temperature well before increasing GA levels (Fig. 1 *F*–*H* and *SI Appendix*, Fig. S2*C*). Similarly, simulated shade takes more than 4 h to modify GA levels (40) while already causing large COP1-mediated effects on RGA at 2 h (Fig. 1*E*). Second, warm temperature or shade reduces the abundance of RGA in the presence of saturating levels of a GA synthesis inhibitor (Fig. 1*A*). Third, warm temperature or shade reduces the abundance of the mutant protein rga-Δ17, which cannot be recognized by GID1 (41) and is fully insensitive to GA (Fig. 1 *B* and *C* and *SI Appendix*, Fig. S2). The latter effects require COP1, providing evidence for a branch of COP1 action on DELLA that does not involve activating the canonical GA/GID1 pathway.
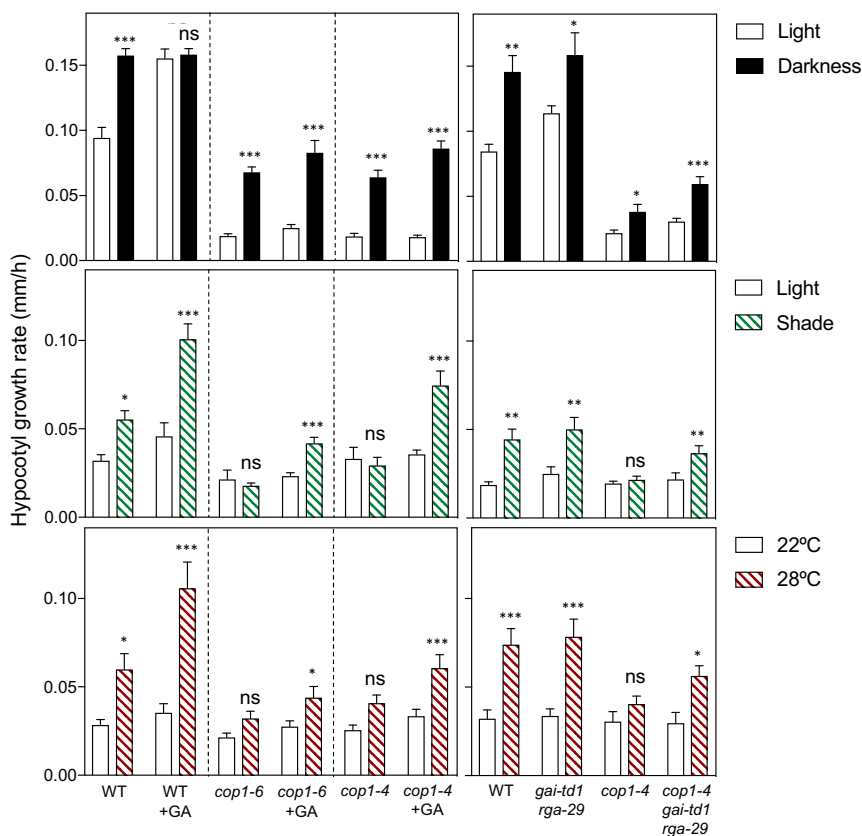

Fig. 5. COP1 regulates the rate of hypocotyl elongation in response to shade and warm temperature in a DELLA-dependent manner. Bars indicate the hypocotyl growth rate of seedlings of the indicated genotypes during deetiolation or after transfer to shade or 28 °C measured over a period of 9 h. Where indicated, seedlings were germinated and grown in the presence of 5 μM GA$_4$. Values correspond to the mean and SE of 8 (light treatments) or 24 (temperature treatments) replicate boxes; 10 seedlings were averaged per replicate box. Asterisks indicate that the difference is statistically significant with the control condition (Student's *t* test, *$P < 0.05$, **$P < 0.01$, ***$P < 0.005$; ns, nonsignificant).

COP1 effects on RGA and rga-Δ17 depend on the 26S proteasome (Fig. 2). Taking into account the well-established role of COP1 in E3-ligase complexes that ubiquitinate and target to proteasomal degradation proteins involved in environmental signaling (42), the simplest interpretation of the above observations is that COP1 directly regulates DELLA protein stability. Different results lend support to this hypothesis. First, RGA and GAI interact with COP1 and its complex partner SPA1 in yeast (Fig. 3 *A* and *B*) and in planta (Fig. 3*C*). Second, COP1, SPA1, and GAI or RGA form a tertiary complex in mammalian and plant cells, and this complex is present in nuclear bodies (Fig. 3 *D–G* and *SI Appendix*, Figs. S4 *A–C* and S5). Third, COP1 ubiquitinates RGA and GAI in vitro (Fig. 4) and the levels of ubiquitinated RGA in vivo are enhanced by COP1 (*SI Appendix*, Fig. S6).

Tight regulation of abundance is a common feature of proteins that act as signaling hubs in mammals (43) and in yeast (44). Posttranslational modifications (45–47) and interaction with other transcriptional regulators (48, 49) modulate DELLA activity. However, the mechanism reported here is unique. In contrast to previously reported modes of regulation of DELLA abundance, which converge to control its stability via GA/GID1, the rapid COP1-mediated regulation occurs by a mechanism that acts in parallel to the canonical GA/GID1 pathway.

COP1 might represent an ancient regulatory mechanism of control of DELLA levels, preceding the acquisition of the GA/GID1 system because the GA/GID1 system appears in lycophytes (50), while orthologs of COP1 and DELLA proteins are already present in the genome of the liverwort *Marchantia polymorpha* (51). However, in *Arabidopsis*, these two pathways appear to operate in concert. Blocking GA synthesis with PAC reveals that the canonical pathway makes a negligible direct contribution to the rapid changes in RGA abundance in response to warmth or shade (Fig. 1 *A* and *H*), simply because by the time GA levels increase, the COP1 pathway has already acted (Fig. 1 *F* and *G* and *SI Appendix*, Fig. S2C). However, the GA pathway has a large effect in a developmental time scale, as demonstrated by the massive accumulation of RGA in the GA-deficient mutant *ga1* (11, 22) or the GA-insensitive version rga-Δ17 (Fig. 1*B*) (23). Although rga-Δ17 retains the COP1-mediated

response (Fig. 1*C* and *SI Appendix*, Fig. S2B), this effect is no longer rapid (*SI Appendix*, Fig. S2 *D* and *E*), consistently with the lack of rapid changes in RGA levels in response to warmth in the *ga1* mutant (11). This indicates that the GA pathway sets the sensitivity to the COP1 pathway. Coexistence of the COP1- and GA-dependent regulation would provide the advantage of a faster and tunable adjustment to the suddenly fluctuating cues of the natural environment.

Materials and Methods

Detailed description of the plant materials and growth conditions, and methods used for protein–protein interaction assays, protein localization, and in vitro ubiquitination can be found at *SI Appendix, Methods*.

Data Availability. All data discussed in the paper are available in the main text and *SI Appendix*. Materials used in the paper are available upon request from the corresponding authors.

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