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Research Article

CONSTITUTIVE PHOTOMORPHOGENIC 1 promotes seed germination by destabilizing RGA-LIKE 2 in Arabidopsis

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Abstract

Under favorable moisture, temperature, and light conditions, gibberellin (GA) biosynthesis is induced and triggers seed germination. A major mechanism by which GA promotes seed germination is by promoting the degradation of the DELLA protein RGA-LIKE 2 (RGL2), a major repressor of germination in Arabidopsis (*Arabidopsis thaliana*) seeds. Analysis of seed germination phenotypes of *constitutive photomorphogenic 1* (*cop1*) mutants and complemented *COP1-OX/cop1-4* lines in response to GA and paclobutrazol (PAC) suggested a positive role for COP1 in seed germination and a relation with GA signaling. *cop1-4* mutant seeds showed PAC hypersensitivity, but transformation with a *COP1* overexpression construct rendered them PAC insensitive, with a phenotype similar to that of *rgl2* mutant (*rgl2-SK54*) seeds. Furthermore, *cop1-4 rgl2-SK54* double mutants showed a PAC-insensitive germination phenotype like that of *rgl2-SK54*, identifying COP1 as an upstream negative regulator of RGL2. COP1 interacted directly with RGL2, and in vivo this interaction was strongly enhanced by SUPPRESSOR OF PHYA-105 1. COP1 directly ubiquitinated RGL2 to promote its degradation. Moreover, GA stabilized COP1 with consequent RGL2 destabilization. By uncovering this COP1–RGL2 regulatory module, we reveal a mechanism whereby COP1 positively regulates seed germination and controls the expression of germination-promoting genes.

Introduction

In its seed form, a new plant generation can be dispersed and then wait to develop and grow once favorable environmental conditions, such as optimal moisture, light, and temperature, exist (Bewley, 1997). Favorable conditions trigger changes in the phytohormone levels of mature seeds, including a decrease in abscisic acid (ABA) and an increase in gibberellins (GAs), which together, inhibit dormancy and promote seed germination (Steber et al., 1998; Holdsworth et al., 2008). Seed imbibition induces GA biosynthesis and

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triggers germination. Arabidopsis (*Arabidopsis thaliana*) mutants with impaired GA biosynthesis (e.g. *ga1-3* and *ga2*) fail to germinate in the absence of exogenous GA, underlining its importance for germination (Koornneef and van der Veen, 1980).

GA is perceived by the receptor protein GA-INSENSITIVE DWARF1 (GID1), which then changes conformation and binds to DELLA proteins, central repressors in the GA signaling pathway (Ueguchi-Tanaka et al., 2005; Murase et al., 2008; Shimada et al., 2008). The formation of the GID1-GA-DELLA complex triggers GA-mediated DELLA degradation by the F-box protein SLEEPY1 (SLY1; SCF^{SLY1} complex) and its homolog SNEEZY1/SLY2 through ubiquitin (Ub)-dependent proteolysis, and induces the expression of GA-responsive genes (McGinnis et al., 2003; Dill et al., 2004; Fu et al., 2004; Ariizumi et al., 2011). Thus, GA lifts DELLA repression of its downstream targets, triggering GA-mediated responses (Sun, 2010).

In Arabidopsis, five DELLA repressors, such as GA INSENSITIVE (GAI), REPRESSOR OF *ga1-3* (RGA), RGA-LIKE 1 (RGL1), RGL2, and RGL3, play overlapping yet distinct roles in many developmental processes, such as seed germination, stem elongation, and transition to flowering (Peng et al., 1997; Dill and Sun, 2001; Lee et al., 2002; Wen and Chang, 2002; Cao et al., 2005). GAI and RGA are both involved in seed germination (Oh et al., 2007), but RGL2 is the major regulator of GA-mediated seed germination among the DELLA proteins. Under GA-deficient conditions, such as in the *ga1-3* mutant background or under paclobutrazol (PAC) treatment, only *rgl2* mutation, but not *rgl1* or *rgl3*, rescued seed germination rates under light conditions (Lee et al., 2002; Tyler et al., 2004; Cao et al., 2005).

The repressive role of RGL2 in seed germination operates through different molecular mechanisms that integrate GA, ABA, and light signals. RGL2 associated with transcription factors to regulate gene expression and control germination (Piskurewicz et al., 2008; Liu et al., 2016; Ravindran et al., 2017; Sanchez-Montesino et al., 2019; Yang et al., 2020). Furthermore, RGL2 represses the expression of the *GA-STIMULATED ARABIDOPSIS6* (*GASA6*), which is an integrator of glucose, GA, and ABA signals in seed germination (Zhong et al., 2015). GASA6 locates mainly in cell wall and regulates cell wall loosening to favor cell elongation on embryonic axis during seed germination through EXPANSIN 1 (EXPA1) (Zhong et al., 2015).

Light impact on seed germination relies in part on phytochrome B (phyB) activation/inactivation by red/far-red light. Once activated, phyB promotes the degradation of PHYTOCHROME-INTERACTING FACTOR 1 (PIF1), a strong repressor of light-mediated seed germination (Oh et al., 2004, 2006). Thus, *pif1* mutants display high germination rates in the dark (Oh et al., 2004). PIF1 regulates germination in many ways, by directly binding to the promoters of the DELLA repressors *GAI* and *RGA* to activate their expression and repress GA signaling (Oh et al., 2007); by directly repressing genes involved in GA biosynthesis and activating GA catabolism (Oh et al., 2007, 2009; Kim et al., 2016); by acting cooperatively with ABSCISIC ACID INSENSITIVE3 (ABI3) in the dark to activate *SOMNUS* (*SOM*), a key negative regulator of seed germination; and by directly binding to the *ABI5* promoter (Kim et al., 2008; Park et al., 2011). Thus, stabilization of PIF1 in the dark is a key to halting seed germination, as it mediates the repression and activation of the GA and ABA cascades, respectively (Oh et al., 2006, 2007; Kim et al., 2008).

Previous reports have shown that additional regulators of light signaling are involved in the control of seed germination. Among them is CONSTITUTIVE PHOTOMORPHOGENIC 1 (COP1), a master regulator of photomorphogenesis. COP1, together with the E2 Ub variant COP10, DE-ETIOLATED 1 (DET1), and COP9 SIGNALOSOME (CSN) proteins, is a member of the COP/DET/FUSCA family, whose strong mutants produce dark purple-pigmented seeds and exhibit seedling-lethal phenotypes (McNellis et al., 1994). COP1, in association with SUPPRESSOR OF PHYA-105 (SPA) proteins, acts as part of a substrate adaptor module within CULLIN4 (CUL4)-based E3 Ub ligases. The formation of $CUL4^{COP1\text{-}SPA}$ complexes mediates the targeted degradation of many positive regulators of photomorphogenesis in darkness, as well as of other regulators of circadian clock and photoperiodic flowering (Lau and Deng, 2012). In the case of seed germination, the $CUL4^{COP1\text{–}SPA}$ E3 Ub ligase is necessary for the light-induced degradation of PIF1 in Arabidopsis. Accordingly, *cop1* and *spaQ* mutants display reduced seed germination in response to light, consistent with the higher abundance of PIF1 in these mutants compared to the wild-type (WT; Zhu et al., 2015). Strikingly, in the dark, PIF1 acts as a cofactor of $CUL4^{COP1\text{–}SPA}$ E3 Ub ligase, enhancing its function to synergistically degrade ELONGATED HYPOCOTYL 5 (HY5) and repress photomorphogenesis, being then degraded in the light (Xu et al., 2014; Zhu et al., 2015). Despite its role as a positive regulator of photomorphogenesis, HY5 binds to the *ABI5* promoter and is required for *ABI5* expression in developing seeds, positively controlling seed maturation and dormancy (Chen et al., 2008). Both COP1 and HY5 proteins have been recently shown to be involved in ABA-mediated inhibition of postgermination seedling development (Yadukrish et al., 2020a, 2020b).

Here, we provide evidence that COP1 acts as a positive regulator of seed germination by limiting the accumulation of the DELLA protein RGL2. In germination analysis, the *cop1-4* weak mutant showed a PAC-hypersensitive germination phenotype, but the *COP1*-overexpressing plants exhibited PAC insensitivity in an RGL2-dependent manner. COP1 was stabilized by GA and directly interacted with and ubiquitinated RGL2 to promote its degradation, releasing the expression of downstream regulators of seed germination (such as *GASA6* and *EXPA1*). Taken together, our data suggest that COP1- and GA-mediated seed germination converges on RGL2 regulation. This finding contributes to the

understanding of the regulatory role of COP1 in the germination of Arabidopsis seeds.

Results

COP1 is a regulator of seed germination

COP1 is a member of the *COP/DET/FUS* gene family, whose strong mutants produce dark purple-pigmented (fusca) seeds and exhibit a seedling-lethal phenotype (McNellis et al., 1994). We found that strong mutants of *COP1*, especially the *cop1-5* seedling-lethal mutant caused by a T-DNA insertion (Figure 1A), exhibited extremely delayed or failed seed germination (McNellis et al., 1994). To determine whether fusca phenotypes were in general associated to seed germination defects we tested the germination of another fusca mutant, *cop10-1*. COP10 belongs to the CDDD complex that conforms an E3 ligase (Lau and Deng, 2012). The purple-colored seeds of the *cop10-1* seedling-lethal mutant (Figure 1B; Wei et al., 1994) germinated normally, like WT seeds, under normal conditions (mock), indicating that the fusca phenotypes are not intrinsically associated with germination defects (Figure 1C). In the presence of 10 μM gibberellic acid (GA_3), the germination rate of *cop1-5* seeds was greatly enhanced compared to that of mock-treated seeds (Figure 1D).

To further confirm the regulatory role of COP1 in seed germination, we generated *COP1-OX/cop1-4* transgenic plants by transforming *cop1-4* (*cop1* weak allele) mutant plants (McNellis et al., 1994) with a *35S::COP1-GFP* construct (see "Materials and methods"; Supplemental Figure S1). Next, we examined the germination rates of *cop1-4* and *COP1-OX/cop1-4* seeds in the presence of GA or the GA biosynthesis inhibitor PAC (Figure 2). We found that the germination of *cop1-4* seeds was slightly, but significantly, delayed compared to that of WT seeds under normal conditions (mock treatment) but was fully restored in the presence of GA (Figure 2, A and B). Moreover, compared with WT seeds, the faster germination rate of *COP1-OX/cop1-4* seeds at 1.5 d after incubation (DAI) at 22°C in long-day (LD) conditions (Figure 2B) indicated not only the full rescue of the *cop1-4* defect in delayed germination but also a positive effect of COP1 on seed germination. However, in the presence of PAC at 3 DAI (Figure 2, B and C), the germination of *cop1-4* mutant seeds was impaired, whereas *COP1-OX/cop1-4* seeds germinated much faster and in higher percentage than WT seeds, showing strong insensitivity to the negative effect of PAC on seed germination. Thus, COP1's effect on seed germination is stronger when GA levels are reduced. These results suggest that COP1 positively regulates seed germination, either by targeting a component of the GA signaling pathway involved in germination or by affecting a GA-independent pathway concurrently implicated in seed germination.

COP1 upregulates the expression of germination-associated genes in imbibed seeds

During seed imbibition, GA upregulates the expression of downstream genes that induce cell wall remodeling needed for germination, EXPAs, and xyloglucan endotransglucosylases/endohydrolases (XTHs) (Weitbrecht et al., 2011; Shi

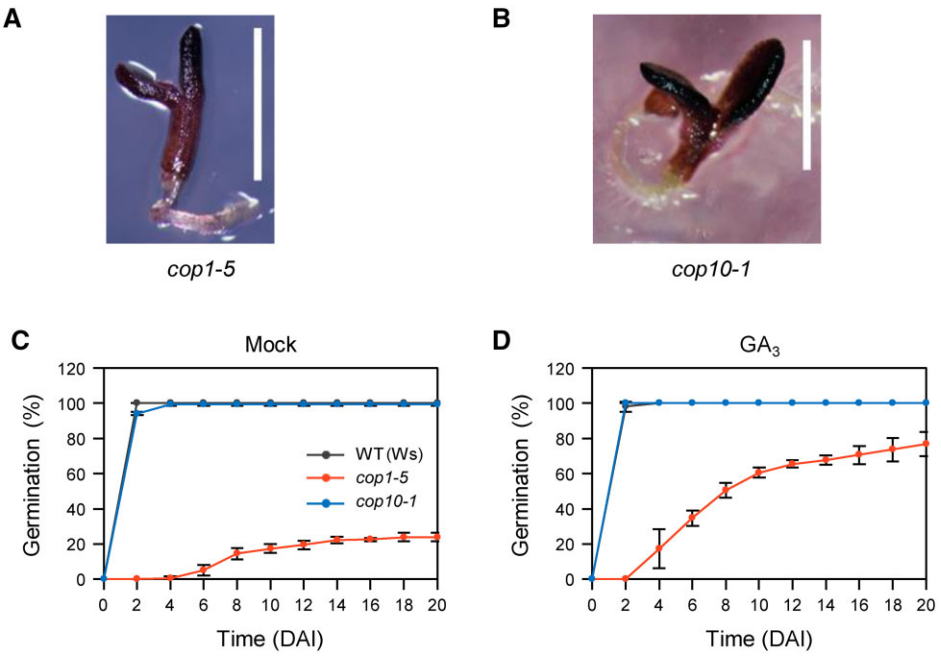

Figure 1 Germination rate of *cop1-5* seeds is dramatically enhanced in the presence of GA. A and B, The seedling-lethal phenotypes of *cop1-5* (8 DAI) (A) and *cop10-1* (4 DAI) (B) mutants. Scale bar represents 2 mm. C and D, Germination rates of WT (Ws ecotype), *cop1-5*, and *cop10-1* seeds on MS medium (mock) (C) or on the same medium containing 10 μM GA_3 (D). Germination rates were determined by counting the seeds with protruding radicles over 20 d. The mean and SD were obtained from three biological repeats (~100 seeds/repeat). The experiments were repeated five times with similar results.

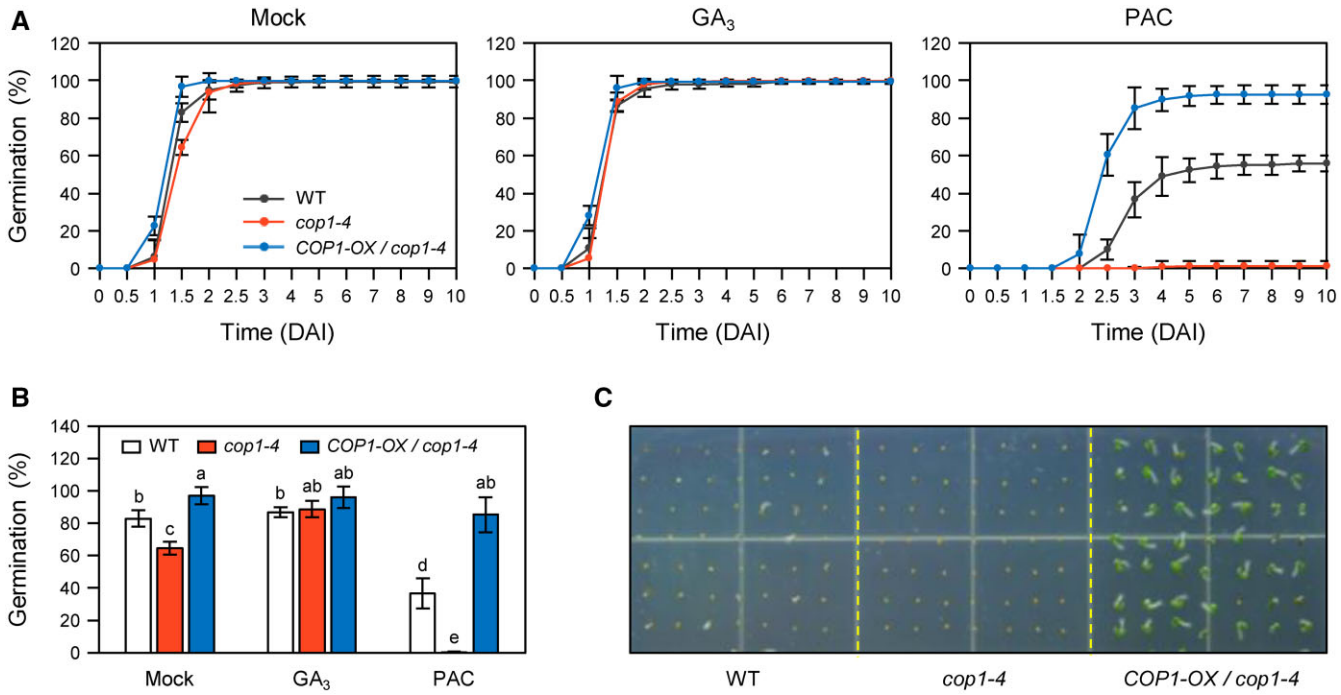

Figure 2 COP1 acts as a positive regulator of seed germination. A, Germination rates of WT (Col-0 ecotype), *cop1-4*, and *COP1-OX/cop1-4* seeds on MS phytoagar medium (mock) or on the same medium containing 10 μM GA_3 or 10 μM PAC from 0 to 10 DAI. B, Germination scored at 1.5 DAI of the same lines and treatments as in (A). Germination rates were determined by counting the seeds with protruding radicles. The mean and SD were obtained from three biological replicates in which around 100 seeds were used for germination counting. Different letters indicate significantly different values according to a one-way ANOVA and Duncan's least significant range test ($P < 0.05$). C, Germination phenotypes of WT, *cop1-4*, and *COP1-OX/cop1-4* seeds on MS medium containing 10 μM PAC at 3 DAI. The experiments were repeated five times with similar results.

et al., 2013). To examine the positive role of COP1 in seed germination at the transcriptional level, we analyzed the expression levels of four cell wall remodeling genes by reverse transcription-quantitative (RT-qPCR) in *cop1-4* and *COP1-OX/cop1-4* seeds, when compared with WT seeds, at 3 DAI under normal conditions and in the presence of PAC (Figure 3). We found that expression of *EXPA1*, *EXPA2*, and *XTH33* was downregulated in the *cop1-4* seeds and only *XTH33* was upregulated in *COP1*-OX/*cop1-4* seeds under normal conditions. In the presence of PAC, the expression of *EXPA1*, *EXPA2*, *EXPA8*, and *XTH33* was downregulated in *cop1-4* seeds and significantly upregulated (except for EXPA1- although the tendency is the same, differences are not significant) in *COP1-OX/cop1-4* seeds. Therefore, our results show that COP1 positively regulates the expression of genes involved in cell wall remodeling and that this regulatory role is evidenced upon inhibition of GA biosynthesis by PAC treatment, in accordance with the germination phenotypes observed in Figure 2.

Light signaling regulators PIF1 and HY5 destabilized by COP1 do not explain the PAC hypersensitivity of *cop1* mutant seeds

Previous studies have shown that COP1 mediates PIF1 destabilization upon dark-to-light transition to allow the establishment of the photomorphogenic developmental program (Zhu et al., 2015). PIF1 also acts as a negative regulator of seed germination by promoting the expression of *DELLA*, *DOF AFFECTING GERMINATION1*, and *SOM* (Oh et al., 2007, 2009; Kim et al., 2008; Seo et al., 2009; Gabriele et al., 2010). However, a relation between COP1 and PIF1 in seed germination was never found. In addition, HY5, a positive regulator of photomorphogenesis that is destabilized by COP1 in darkness (Osterlund et al., 2000), is also involved in the ABA signaling pathway, where it negatively regulates seed germination (Chen et al., 2008; Yang et al., 2018). Because these studies do not discard a role of HY5 in GA-mediated seed germination, it could be speculated that HY5 destabilization by COP1 may be a mechanism to promote seed germination. To examine whether HY5 and PIF1 play a role in GA-mediated seed germination, we analyzed the germination rates of *pif1-1* and *hy5-215* as single mutants and when introgressed in a *cop1-4* background under normal conditions and in the presence of PAC (Supplemental Figures S2 and S3). Seeds of *pif1-1* and *pif1-1 cop1-4* mutants, when exposed to light, behaved similarly to WT and *cop1-4* seeds respectively, in mock and under PAC treatment, suggesting that *PIF1* is not involved in GA-mediated seed germination neither acts in the same pathway than *COP1* (Supplemental Figure S2). The analysis of *hy5-215* and *cop1-4 hy5-215* germination shows that *hy5* germinates slightly early in PAC compared to the WT and the same is true for *cop1-4 hy5-*

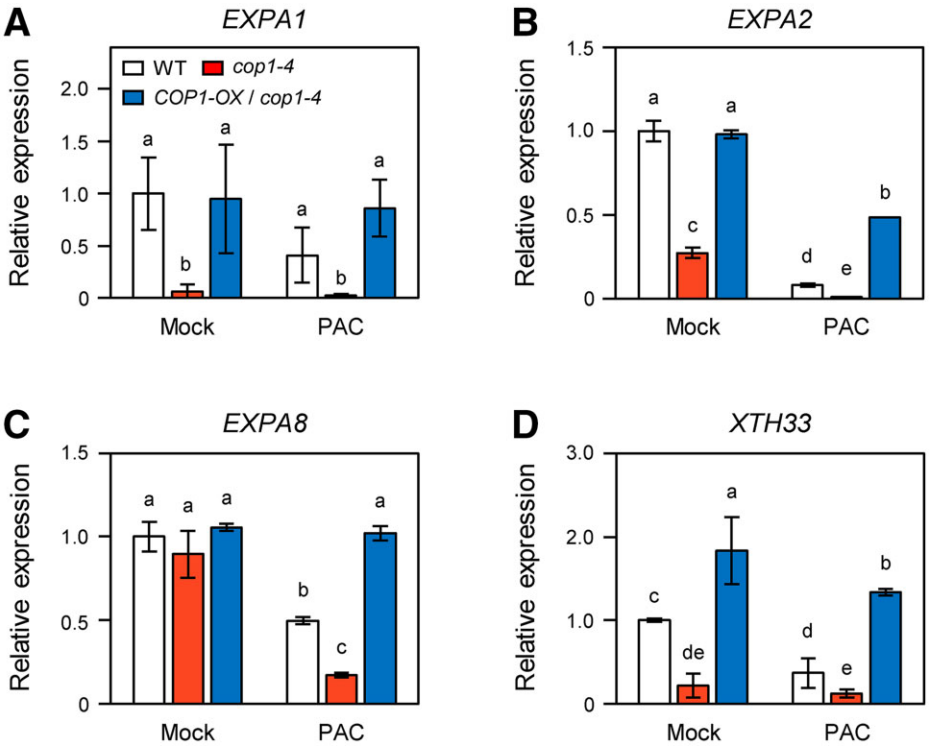

Figure 3 Expression profiles of germination-associated genes in *cop1-4* and *COP1-OX/cop1-4* seeds. A–D, Expression levels of germination-associated genes, *EXPA1* (A), *EXPA2* (B), *EXPA8* (C), and *XTH33* (D), in *cop1-4* and *COP1-OX/cop1-4* seeds relative to those in WT seeds. After stratification, seeds were transferred to 22°C for germination and grown in MS phytoagar (mock) or in the presence of 10 μM PAC until 3 DAI, and analyzed by RT-qPCR. The expression level of each gene was normalized to that of *ACT2*. Expression levels of each gene are shown relative to the expression of WT in the mock-treatment group, which is set as 1. The mean and SD were obtained from three biological repeats (~30 seeds/repeat). Different letters indicate significantly different values according to a one-way ANOVA and Duncan's least significant range test ($P < 0.05$). The experiments were repeated three times with similar results.

215 compared with *cop1-4*, showing that in PAC *hy5* mutation contributes to a slight partial suppression of the *cop1-4* germination defects (Supplemental Figure S3). Altogether, these results suggested that neither PIF1 nor HY5 can be responsible for the strong *cop1-4* germination defects observed in the presence of PAC.

RGL2, a negative regulator of GA-mediated seed germination, is epistatic to COP1

DELLA proteins are major negative regulators in the GA signaling pathway that comprise five proteins, encoded by *GAI*, *RGA*, *RGL1*, *RGL2*, and *RGL3* (Sun, 2010). All five DELLA proteins are stabilized by PAC as it represses GA biosynthesis. Among them, RGL2 is a key regulator of GA-mediated seed germination (Lee et al., 2002). When we examined germination rates of mutants of these five genes, only the *rgl2-SK54* mutant showed a PAC-insensitive phenotype, as previously reported (Supplemental Figure S4; Lee et al., 2002; Tyler et al., 2004). To determine the genetic interaction between *COP1* and *RGL2* in seed germination, we generated *cop1-4 rgl2-SK54* double mutants. In addition, we obtained *COP1-OX/rgl2-SK54* transgenic plants by transforming *35S::COP1-GFP* into the *rgl2-SK54* mutant. Under normal conditions, *rgl2-SK54* single- and *cop1-4 rgl2-SK54* double-mutant seeds germinated faster than WT and *cop1-4* seeds (Figure 4A, left). In the presence of PAC, however, *cop1-4 rgl2-SK54* seeds showed an almost PAC-insensitive phenotype, similar to that of *rgl2-SK54* seeds, indicating that the *rgl2* mutation suppresses the PAC-hypersensitive phenotype of *cop1-4* (Figure 4A, right).

COP1-OX/cop1-4 seeds germinated much faster and in higher percentage than WT seeds, and similarly to *rgl2-SK54* and *COP1-OX/rgl2-SK54* seeds, in the presence of PAC, suggesting that RGL2 acts downstream of COP1 and could be a COP1 target (Figure 4B, right). Following up on the idea that an active COP1 should be then necessary to degrade RGL2 and promote seed germination, we measured the germination rates of the *cop1-4* lines overexpressing *COP1* variants that failed to dimerize or enter the nucleus, thus compromising COP1 E3 ligase function (Stacey et al., 1999; Lee et al., 2017). $COP1^{WT}$-GFP and $COP1^{L105A}$-GFP fusions, which are able to form COP1 homodimers, fully complemented the *cop1-4* germination defects in the presence of PAC, whereas $COP1^{L170A}$-GFP, whose ability to form dimers is severely impaired, and $COP1^{cyt}$-GFP, which is retained in the cytoplasm, did not rescue the *cop1-4* germination defects (Supplemental Figure S5). Although we could not rule out the possibility that these effects might be due to collateral effects of *cop1* mutation, these results are consistent with the fact that COP1 dimerization is required for

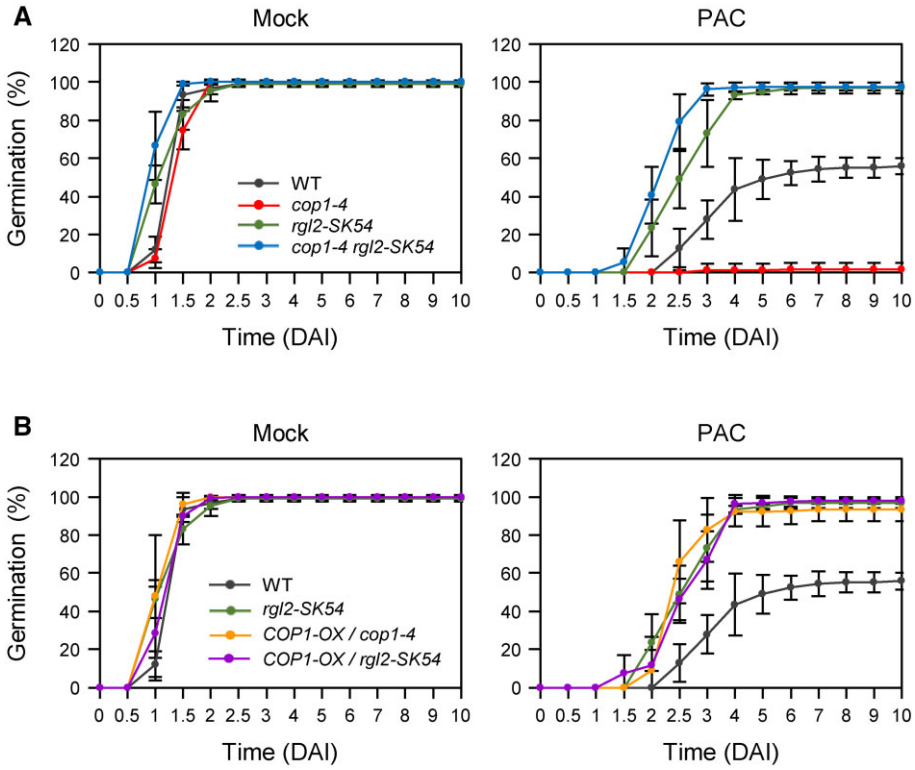

Figure 4 COP1 acts as an upstream regulator of RGL2 in seed germination. A, Germination rates of WT (Col-0), *cop1-4*, *rgl2-SK54*, and *cop1-4 rgl2-SK54* seeds on MS phytoagar medium (mock) or in in MS phytoagar medium containing 10 μM PAC. B, Germination rates of WT (Col-0), *rgl2-SK54*, *COP1-OX/cop1-4*, and *COP1-OX/rgl2-SK54* seeds in MS phytoagar medium containing or not 10 μM PAC. After 3 d of stratification, the seeds were transferred to 22°C and the germination rate was determined by counting the seeds with protruding radicles at the indicated time from 0 to 10 DAI. The mean and SD were obtained from three biological repeats (∼100 seeds/repeat). The experiments were repeated three times with similar results.

target degradation, suggesting that the role of COP1 in seed germination requires a fully active nuclear E3 Ub ligase activity.

COP1 interacts with and destabilizes RGL2

We then investigated whether COP1 regulates RGL2 indirectly at the transcriptional level or directly at the posttranslational level. For this, we analyzed the expression levels of *RGL2* and *COP1* by RT-qPCR in *cop1-4* and *rgl2-SK54* seeds at 3 DAI, respectively, and compared them with those in WT seeds. We found that the expression levels of *RGL2* in *cop1-4* and those of *COP1* in *rgl2-SK54* did not differ significantly from those of the WT (Supplemental Figure S6, A and B). These results indicated that COP1 does not regulate *RGL2* at the transcriptional level.

Next, to examine whether COP1 interacts with and destabilizes RGL2 posttranslationally, we first tested the physical interaction between the two proteins. Yeast two-hybrid (Y2H) assays revealed that, whereas full-length (FL) COP1 did not interact with RGL2, a truncated version of COP1 containing the RING domain could directly interact with RGL2 (Figure 5A). Since all COP1 fragments and RGL2 are being expressed in yeast (Supplemental Figure S7), it seems that the FL COP1 is less efficient in promoting a direct interaction than the COP1 RING domain alone in the yeast cells. To overcome this technical limitation, we performed in vitro pull-down assays with recombinant proteins expressed in *Escherichia coli* (Figure 5B). In vitro purified MBP-COP1 could pull-down GST–RGL2 fusions, thus supporting a direct interaction between the FL COP1 and RGL2. To further confirm this interaction we performed semi-in vivo pull-down assays in the opposite direction. In this experiment, MBP-RGL2 could pull-down COP1-GFP from Arabidopsis seedling extracts whereas MBP protein alone could not (Figure 5C).

In addition, to confirm whether this interaction occurs in vivo we performed bimolecular fluorescent complementation (BiFC) assays in *Nicotiana benthamiana* leaves. Contrary to the in vitro results, the co-expression of truncated YFN-COP1 and truncated YFC-RGL2 constructs showed no yellow fluorescent protein (YFP) reconstitution signal. Based on the recent results by Blanco-Touriñan et al. (2019) where the addition of SPA1 was necessary to visualize the interaction between COP1 and the DELLA proteins RGA and GAI, we tested the effect of mRFP-SPA1 addition to COP1-RGL2 BiFC assays. Co-expression with SPA1 fusion rendered a very strong YFP-reconstitution signal visible in nuclear speckles, suggesting that SPA1 protein is necessary for the in vivo efficient recognition of RGL2 by COP1 (Figure 5D).

To examine whether COP1 destabilizes RGL2 in vivo, we examined the changes in RGL2 levels in the *cop1-4* and

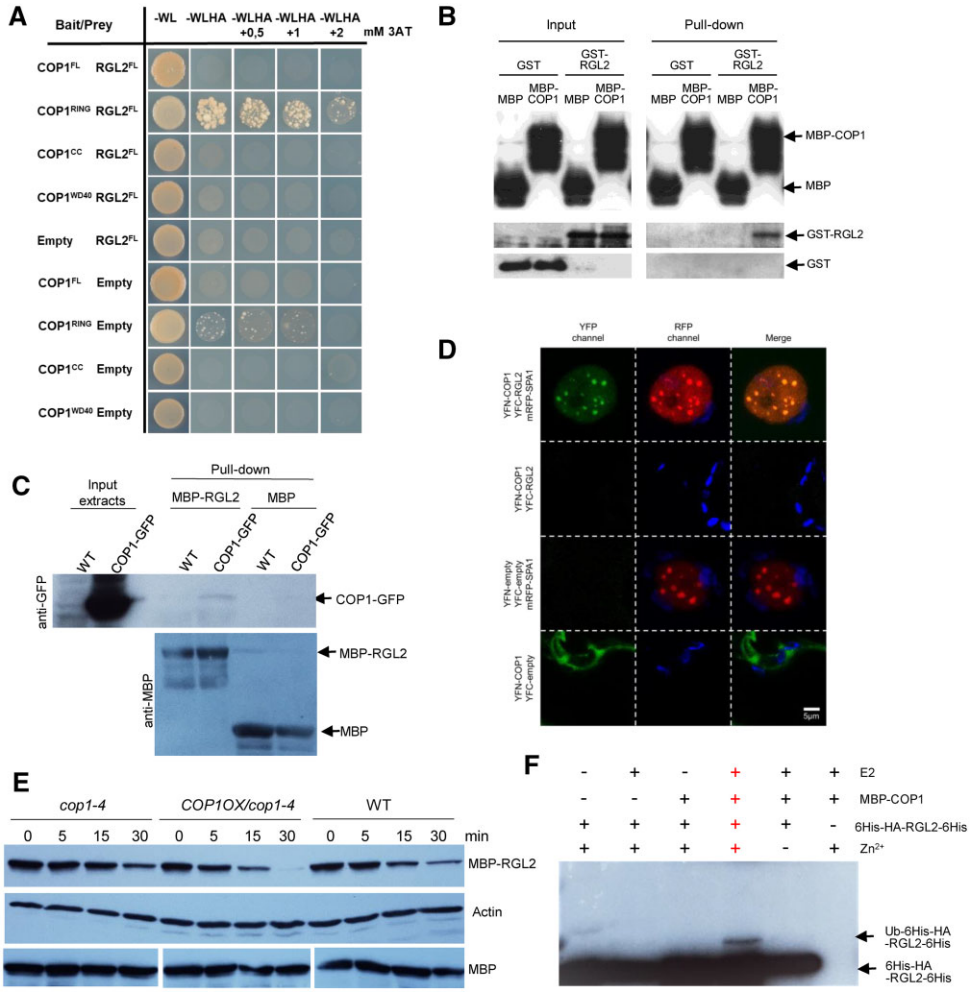

Figure 5 COP1 interacts with and destabilizes RGL2. A, Interaction of COP1 and RGL2 in Y2H assays. FL COP1, as well as three of its individual domains (RING, CC, and WD40-repeat [WD40]), were used as baits and FL RGL2 as prey. Selection for interaction was performed in selective media containing increasing concentrations of 3-Amino-1,2,4-Triazol. B, MBP-COP1 pulls-down GST-RGL2 in vitro. Fusion proteins were detected with an anti-MBP antibody and anti-GST antibody. C, Semi in vivo pull-down assays. MBP-RGL2 pulls down COP1-GFP from Arabidopsis protein extracts. D, BiFC assay showing that COP1 and RGL2 interact in the presence of SPA1. The indicated constructs were expressed in *N. benthamiana* leaves and observed by confocal microscopy. One representative nucleus is shown. E, Degradation of MBP-RGL2 fusion by soluble protein extracts from seedlings of different genotypes as determined with a cell-free degradation assay. MBP-RGL2 proteins were incubated for the indicated times (min) with total soluble protein extracted from 6-d-old WT (Col-0), *cop1-4*, or *COP1-OX/cop1-4* etiolated seedlings. Actin is shown as a control for equivalent extract amounts. MBP when expressed alone remained stable. An anti-MBP antibody was used for fusion protein-detection, and the three panels are from the same hybridization. The experiments were repeated three times with similar results. F, COP1 ubiquitinates RGL2 in vitro. RGL2 (6His-HA–RGL2-6His fusion) ubiquitination assays were performed using MBP-COP1 (or MBP-COP1 without Zn^{2+} as a negative control), rice E2 Rad6 (E2), and yeast E1 (E1; Boston Biochem; added to all the samples). Ubiquitinated RGL2 was detected using an anti-HA antibody.

COP1-OX/cop1-4 backgrounds using cell-free (or in vitro) degradation assays. To this end, we incubated MBP-RGL2 protein fusions with the total soluble protein extracts of WT, *cop1-4*, and *COP1-OX/cop1-4* grown in the dark and detected the changes in MBP-RGL2 levels over 30 min by immunoblot analysis using an anti-MBP antibody. MBP-RGL2 was degraded faster in *COP1-OX/cop1-4* extracts and more slowly in *cop1-4* extracts than in WT whereas MBP alone remained stable (Figure 5E). Furthermore, in vitro ubiquitination assays showed that RGL2 (6His-HA–RGL2-6His fusion) was directly ubiquitinated by MBP-COP1 (Figure 5F). These results indicate that COP1 directly interacts with and ubiquitinates RGL2 to induce its destabilization and suggest a molecular mechanism by which COP1 regulates RGL2 levels to induce seed germination.

COP1 is as a positive regulator of germination-promoting genes that act downstream of *RGL2*

To inhibit germination, RGL2 represses the expression of *GASA6*, *EXPA*, and *XTH* genes which promote seed germination (Stamm et al., 2012; Rombolá-Caldentey et al., 2014; Yan et al., 2014; Zhong et al., 2015). To further support a

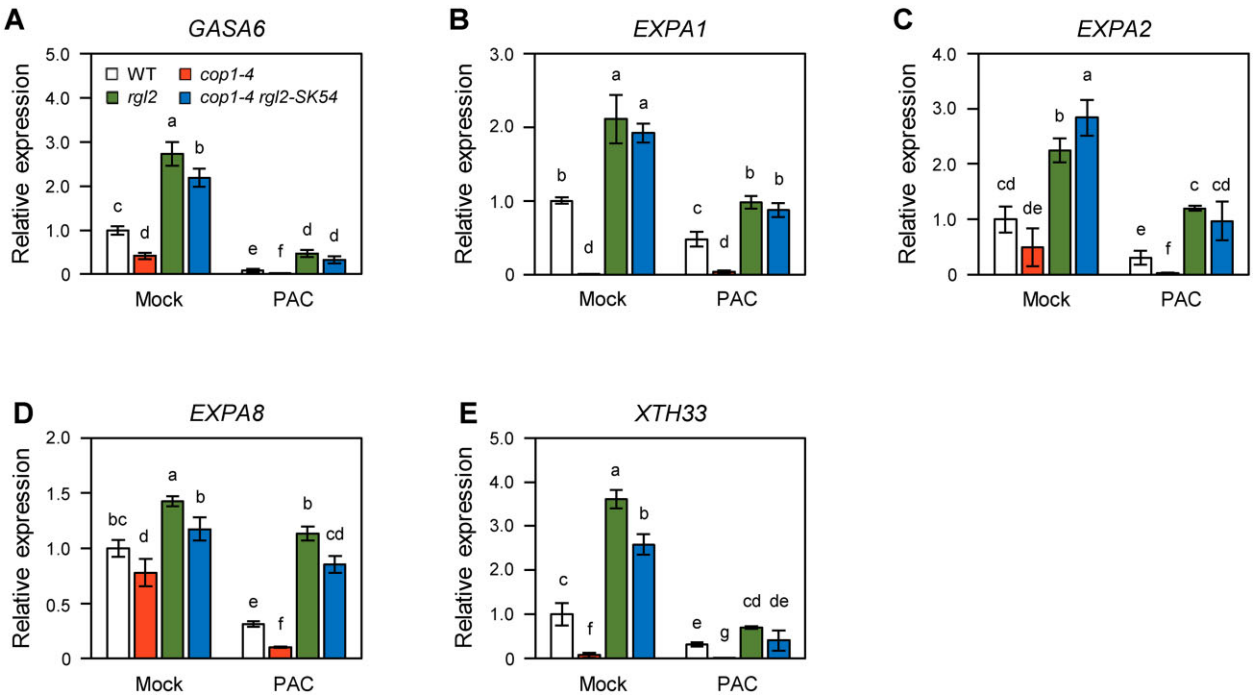

Figure 6 Effects of PAC on the expression of germination-associated genes in imbibed *cop1-4*, *rgl2-SK54*, and *cop1-4 rgl2-SK54* seeds. Altered expression of five *RGL2*-downregulated germination-associated genes, *GASA6* (A), *EXPA1* (B), *EXPA2* (C), *EXPA8* (D), and *XTH33* (E), in imbibed WT (Col-0), *cop1-4*, *rgl2*, and *cop1-4 rgl2-SK54* seeds in the presence or absence of 10 μM PAC at 3 DAI. The expression level of each gene obtained by RT-qPCR was normalized to that of *ACT2* and represented relatively to the expression levels in WT under normal conditions (mock), which is set as 1. The mean and SD were obtained from three biological samples (~30 seeds/repeat). Different letters indicate significantly different values according to a one-way ANOVA and Duncan's least significant range test ($P < 0.05$).

regulatory link between COP1 and RGL2, we investigated the effect of COP1 function on the expression of five germination-associated genes regulated by RGL2, including *GASA6*, *EXPA1*, *EXPA2*, *EXPA8*, and *XTH33* (Figure 6). In mock- and PAC-treatment conditions, these germination-associated genes were downregulated in *cop1-4* seeds and upregulated in *rgl2-SK54* seeds. In *cop1-4 rgl2-SK54* seeds, the expression levels of the germination-associated genes were quite similar to those in *rgl2-SK54* seeds, demonstrating that the negative effect of *cop1-4* mutation on their expression was canceled out by the *rgl2* mutation. These results show that COP1, through RGL2 destabilization, positively regulates the expression of *GASA6* and *EXPA1*, as well as other genes related to cell wall remodeling that are involved in seed germination.

GA increases COP1 stability in the imbibed seeds

In imbibed seeds, increased GA biosynthesis is one of the most important triggers for the induction of seed germination (Steber et al., 1998). The increase in endogenous GA concentration decreases the stability of RGL2 (Tyler et al., 2004). Thus, we wondered whether GA or PAC has an impact on COP1 accumulation in imbibed seeds that could lead to changes in RGL2 stability. To examine the effects of GA and PAC on COP1 accumulation, we measured both *COP1* mRNA and COP1 protein levels in germinating seeds and during the onset of postgerminative seedling establishment after treatments with GA or PAC for 48 h (Figure 7, A–C). The results showed that COP1 protein levels were increased by GA treatment 12 h after imbibition and slightly decreased by PAC treatment (Figure 7, B and C). The differences in COP1 protein accumulation in response to GA and PAC did not reflect the variation pattern of *COP1* mRNA expression under these conditions, indicating that GA and PAC have posttranslational effects on COP1 abundance (Figure 7, A–C). These results suggest that increased GA levels upon seed imbibition promote a sustained accumulation of COP1 at the onset of the seedling establishment process. These results suggest that by regulating COP1 levels, GA promotes RGL2 degradation allowing seeds to germinate and contributing to attain adequate COP1 levels, required for seedling establishment and further development under light/dark cycles.

Discussion

Seeds are equipped with molecular sensors to monitor surrounding environmental conditions and determine whether they are favorable for plant establishment (Seo et al., 2009). Here we describe a regulatory module in which COP1 positively regulates seed germination by interfering with a component of the GA signaling pathway, the DELLA family member RGL2, which is a well-known repressor of seed germination. We showed that *cop1-4* mutants are strongly sensitive to PAC, while *COP1-OX/cop1-4* seeds are strongly

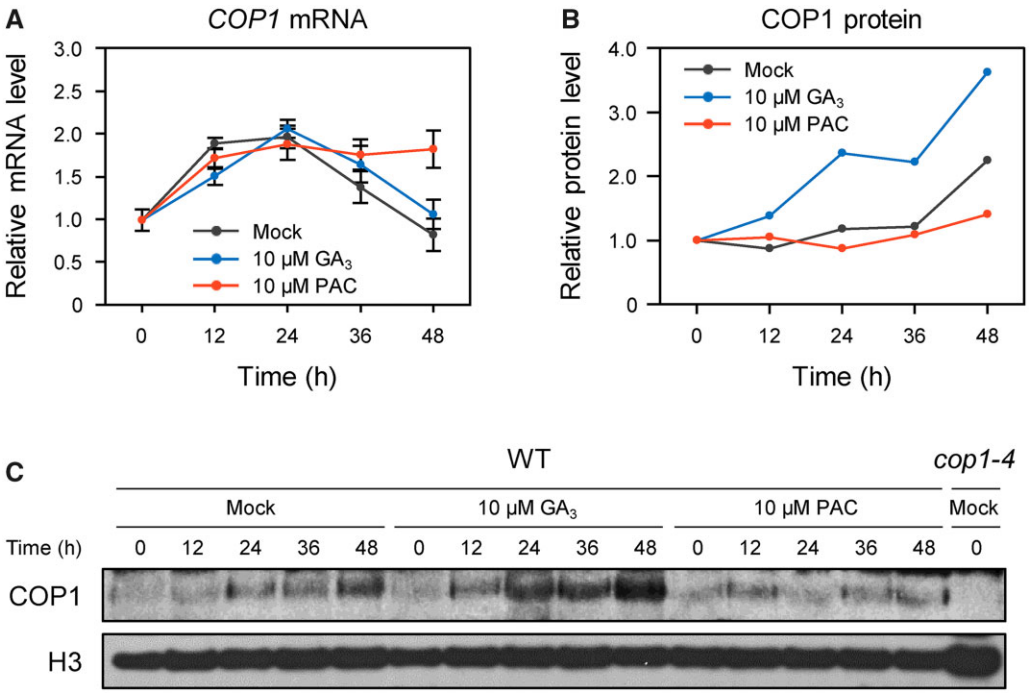

Figure 7 COP1 stability is enhanced by GA and decreased by PAC. A, Relative expression levels of *COP1* in seeds stratified for 3 d at 4°C on MS medium treated or not with 10 μM GA_3 or 10 μM PAC and then transferred to 22°C. Seeds were sampled at the indicated time points after transfer. Relative expression levels of *COP1* were normalized to those of *ACTIN2* (*ACT2*). The expression levels of *COP1* are shown relative to the expression at time 0 under mock treatment, which is set as 1. In RT-qPCR analysis, the mean and SD were obtained from three biological repeats (~30 seeds/repeat). B and C, COP1 stability is increased by GA treatment but decreased by PAC treatment. COP1 and H3 histone protein levels were detected by immunoblot analysis and relative band intensity measured by ImageJ. Protein levels of each COP1 band were normalized to the level of H3 in each lane. The protein levels for each COP1 are shown relative to the expression at time 0 under mock treatment, which is set as 1. The mean of three biological replicates (~100 seeds/repeat) was used.

insensitive to this GA biosynthesis inhibitor (Figure 2), mimicking the phenotype of the *rgl2-SK54* mutant (Figure 4 and Supplemental Figure S4). In addition, COP1 promotes the degradation of RGL2 (Figure 5). Supporting these findings, genetic analysis showed that *rgl2* is epistatic to *cop1*, as the double mutants show complete suppression of the *cop1-4* PAC hypersensitivity (Figure 4A) and of its defects on the activation of genes encoding cell wall proteins involved in cell loosening during germination (Figure 6). Furthermore, GA enhances COP1 protein accumulation upon imbibition (Figure 7), evidencing the GA's broad role as a primary regulator in seed germination.

COP1 regulates seed germination through RGL2

The $CUL4^{COP1\text{-}SPA}$ E3 Ub ligase is well described as a repressor of light signaling, targeting for degradation photomorphogenesis-promoting factors (Lau and Deng, 2012; Zhu et al., 2015). Our data show that *cop1* alleles display defects in seed germination that are unrelated to the fusca phenotypes as *cop10* fusca mutants do not display them. COP10 and DET1 belong to the same E3 Ub ligase and it has been previously found that *det1* fusca mutant seeds germinate better than WT in normal conditions and are hypersensitive to ABA (Fernando and Schroeder, 2015). In the case of *cop1* mutant seeds, the defects can be partially complemented by GA application and, likewise, can be exacerbated by the presence of PAC, suggesting that COP1 plays a role in seed germination by interacting with the GA signaling pathway (Figure 1). $CUL4^{COP1\text{-}SPA1}$ complexes play a positive role in seed germination by promoting the rapid degradation of PIF1 under red and far-red light conditions (Zhu et al., 2015). Thus, by being degraded by COP1, PIF1 acts in light-mediated seed germination independently of GA, making it unlikely that the COP1–PIF1 module could be responsible for GA-related germination defects. In addition, HY5, a well-described COP1 target in photomorphogenesis, is known to repress seed germination by activating *ABI5* gene expression in response to ABA and salt stress (Chen et al., 2008; Yu et al., 2016). Thus, HY5 role in seed germination seems to be unrelated with GA signaling. This is consistent with our observation that *pif1* and *hy5* seeds germinate similarly to WT seeds in the presence of PAC. Together with the fact that *pif1* has a null and *hy5* only a little suppressive effect on the *cop1* germination hypersensitivity to PAC, both transcription factors are unlikely to be involved in the GA-related seed germination response.

The best-described mutants showing PAC insensitivity during germination are *rgl2* mutants. Indeed, this characteristic is a specific signature of *rgl2* among other *della* mutants (Lee et al., 2002; Tyler et al., 2004; Cao et al., 2005;

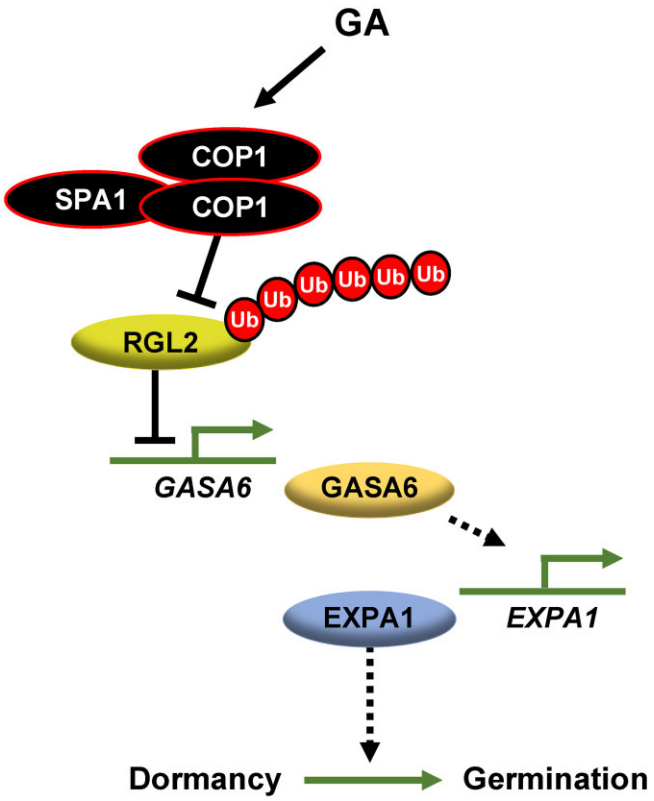

Figure 8 Model of the COP1–RGL2 regulatory module in seed germination. Upon perception of favorable environmental cues, GA is synthesized in the seeds to induce germination. COP1 is stabilized by GA and interacts with RGL2, a negative regulator of germination-associated genes such as *GASA6*, *EXPA* genes, and *XTH*. The COP1–RGL2 interaction destabilizes RGL2, and consequently germination-associated genes are induced in the imbibed seeds. SPA1 is required for the in vivo interaction. Our model defines a noncanonical pathway by which GA inhibits RGL2 repression of seed germination through the activity of COP1. Arrows signify positive effects; blocked line, negative effect; dashed line, indirect regulation.

Supplemental Figure S4). Moreover, among the five Arabidopsis DELLA proteins, RGL2 has been described as a primary player in seed germination. Upon GA perception by the GID1 receptor, GID-GA-DELLA complexes are degraded by SCF^{SLY1} in a GA-dependent manner, which is the canonical pathway for RGL2 degradation (McGinnis et al., 2003; Dill et al., 2004). According to our experiments, the *rgl2-SK54* mutation completely suppresses the germination defects of *cop1-4* seeds (Figure 4), suggesting that RGL2 acts downstream of COP1 in seed germination and might be a direct target of COP1 Ub ligase activity, which would represent a mechanism for the targeted degradation of RGL2.

COP1 destabilizes RGL2

We found that COP1 directly interacts with RGL2, in a mechanism involving SPA1, and mediates its ubiquitination to control RGL2 abundance (Figure 5). Thus, COP1-mediated degradation of RGL2 might occur in parallel with the canonical SLY1- and GA-dependent degradation. Blanco-Touriñan et al. (2019) recently reported that COP1–SPA1 complexes mediate the destabilization of two other DELLA proteins, GAI and RGA, to promote hypocotyl elongation in response to shade and temperature cues. These results are complementary to our findings, and suggest that the targeted degradation of DELLA proteins by COP1 goes far beyond germination and can be extended to other DELLA- and GA-regulated processes during plant development. A striking similarity between our results and those of Blanco-Touriñan et al. (2019) is that in vivo, COP1 requires the presence of SPA1 for interaction with GAI and RGA, though not for their ubiquitination (Blanco-Touriñan et al., 2019). Moreover, we report that an active COP1 protein with full capacity to dimerize and enter the nucleus is essential to maintaining WT germination levels in the presence of PAC (Supplemental Figure S5).

The CSN complex allows the activation of cullin-based E3 Ub ligases by maintaining proper cycles of neddylation (an Ub-like modification) and deneddylation of cullins. Previous studies have shown that CSN mutants have poor germination and hyperdormancy phenotypes (Wei and Deng, 2003; Dohmann et al., 2010; Franciosini et al., 2015; Jin et al., 2018). In the case of *csn1-10*, the hyperdormancy phenotype was totally dependent on the failure to degrade RGL2. This phenotypic defect might be due to altered neddylation states and activities of $SCF^{SLY1/2}$ E3 Ub ligases (Jin et al., 2018). However, it cannot be ruled out that *csn* mutations also impair the activity of $CUL4^{COP1\text{-}SPA}$ complexes toward RGL2. Indeed, it has been recently shown that $CRL4^{CDDD}$ complexes mediate COP1 destabilization in a process that requires functional CSN (Cañibano et al., 2021). Supporting this notion, the deneddylation/neddylation ratios of CUL1, CUL3, and CUL4 have all been found to be higher after seed imbibition, suggestive of increased activity of CSN and various CULLIN-associated complexes during germination (Wei and Deng, 2003; Franciosini et al., 2015).

Our results uncovered a regulatory mechanism that restricts RGL2 function, suggesting that different mechanisms besides the canonical targeted degradation of RGL2 by $SCF^{SLY1/2}$ act coordinately to govern seed germination. These mechanisms likely include GA-independent processes. In fact, the release from dormancy of *sly1* hyperdormant seeds is independent of the accumulation of the RGL2, GAI, and RGA DELLA proteins (McGinnis et al., 2003; Dill et al., 2004; Penfield et al., 2006; Ariizumi and Steber, 2007). Indeed, *sly1* loss-of-dormancy germination relates better with endogenous levels of ABI5 and seems to depend on ABA biosynthesis (Piskurewicz et al., 2008). These results highlight the complexity of the mechanisms involved in seed germination and release from dormancy.

COP1 promotes the expression of cell wall modification genes and is induced by GA

RGL2 repression of seed germination depends on a number of transcription factors that end up connecting RGL2

function to structural genes that mechanically affect cell wall composition and the control of germination (Stamm et al., 2012; Rombolá-Caldentey et al., 2014; Yan et al., 2014; Sánchez-Montesino et al., 2019). For most target genes, their upstream regulatory mechanism is still unclear as is case for the *GASA6–EXP1A* regulatory module. GASA6 promotes cell elongation at the embryonic axis through the action of *EXPA1* by an unknown mechanism (Zhong et al., 2015). Since RGL2 represses *GASA6* and *EXPA1*, by repressing RGL2, COP1 can positively regulate the expression of these genes, supporting a role for COP1 in promoting embryonic axis cell elongation during seed germination (Figures 6 and 8). As shown by our analyses, COP1 promotes the expression of additional cell-wall-modifying genes that were previously reported to be targets of RGL2 in seed germination (Figure 6; Stamm et al., 2012).

Notably, GA promotes COP1 protein accumulation during seed germination and at the onset of seedling establishment (Figure 7). Though the mechanism behind this process requires further elucidation, it is clear that COP1 plays a major role in initial seedling development by promoting growth according to day/night cycles and circadian regulation contributing also to the ABA-mediated inhibition of postgerminative seedling establishment (Lau and Deng, 2012; Yadukrishnan et al., 2020a, 2020b). In this way, increased accumulation of COP1 in response to GA might prevent precocious photomorphogenesis after seed germination and might afterward be necessary to maintain an equilibrium between the regulation of growth by elongation and photomorphogenic development in initial seedling developmental stages.

Conclusions

Together, our data uncover a key role for COP1 in seed germination through promotion of the degradation of RGL2, a GA-regulated master repressor of seed germination. Therefore, COP1 contributes to the GA signaling pathway to promote seed germination and cell elongation, and thus is essential for initial seedling establishment. Further physiological and genetic studies will be key to fully understanding the GA–COP1 relations and fully integrating COP1 into the intricate network of seed germination regulatory components.

Materials and methods

Plant materials and growth conditions

The Arabidopsis (*A. thaliana*) mutants used were of Columbia (Col) ecotype except for the *cop1-5* (Deng et al., 1992) and *cop10-1* (Wei et al., 1994) [Wassilewskija (Ws) ecotype] mutants, which are seedling lethal and were maintained as heterozygotes. The single mutants (Col ecotype) were *cop1-4* (a weak mutant allele; McNellis et al., 1994), *gai-t6* (Peng et al., 1997), *rga-28* (SALK_089146), *rgl1-SK62* (SALK_136162), *rgl2-SK54* (SALK_027654), *rgl3-3* (CS16355), *hy5-215* (Osterlund et al., 2000), and *pif1-1* (Oh et al., 2006). The double mutants used were *cop1-4 hy5-215* (Maier et al., 2013) and *pif1-1 cop1-4* (Xu et al., 2014). The *cop1-4 rgl2-SK54* double mutants were generated by crossing the single mutants and F2 genotyping with dCAPS (*cop1-4*; *Spe*I restriction enzyme digestion) and PCR (*rgl2-SK54*) primers (Supplemental Table S1). For the *35S:COP1-GFP* (*COP1-OX*) constructs, the FL *COP1* cDNA PCR amplified from Col-0 cDNA, cloned into the pDONR221 vector (Invitrogen, Waltham, MA, USA) and subsequently into the pMDC85 plasmid (Curtis and Grossniklaus, 2003) by using the Gateway cloning system (Invitrogen). Through *Agrobacterium tumefaciens* (GV3101)-mediated transformation in *cop1-4* or *rgl2-SK54* mutants by the floral-dip method (Clough and Bent, 1998) *COP1-OX/cop1-4* or *COP1-OX/rgl2-SK54* transgenic plants were obtained. The *COP1* mutant variants, that is the *35S:COP1*^{WT}*-GFP/cop1-4*, *35S:COP1*^{L105A}*-GFP/cop1-4*, *35S:COP1*^{L170A}*-GFP/cop1-4*, and *35S:COP1*^{cyt}*-GFP/cop1-4* transgenic plants, were previously described (Lee et al., 2017).

Germination rates

Fresh seeds (harvested within 1 month before the experiments) were used to measure germination rates. Seeds were surface sterilized with a solution containing 70% (v/v) ethanol and 0.1% (v/v) Triton X-100, for 20 min, and washed with 100% (v/v) ethanol for three times. After being air-dried on sterile 3M filter paper, seeds were seeded on MS (Murashige and Skoog) phytoagar medium (mock) or on the same medium supplemented with 10 μM GA_3 or 10 μM PAC. For stratification, seeds were kept at 4°C in darkness for 72 h. Germination experiments were initiated with the transfer to a growth chamber at a constant 22°C temperature under cool white fluorescent light (100 μmol m^{-2} s^{-1}) and LDs (16-h light/day) and referred to as DAI. To measure germination rates, germinated seeds were scored upon radicle emergence. For each experiment, three to five biological replicates were performed. In each replicate, about 100 seeds were used to score the radicle emergence. Among each replicate the seeds used were collected from plants grown simultaneously under the same conditions. Statistical analysis was performed by using one-way ANOVA and Duncan's test was used.

Y2H assays

Y2H assays were performed using the Matchmaker GAL4 two-hybrid system (Clontech, Mountain View, CA, USA). The FL and/or partial (RING; aa 1–104, coiled-coil [CC]; aa 121–213, WD-40 repeat; aa 371–675) cDNAs of *COP1* were obtained by RT-PCR from WT (Col) plants (Yu et al., 2008) and cloned into the pGBK vector (as baits), and the FL *RGL2* cDNA was cloned into the pGAD vector (as prey). Yeast (strain AH109) cotransformation was performed according to the Yeast Handbook (Clontech). An anti-HA (Roche, Basel, Switzerland) and an anti-myc (kindly provided by Xing Wang Deng) antibodies were used to check the expression of AD and BD fusion proteins.

BiFC assays

The FL cDNA of *RGL2* was gateway recombined to generate a YFC fusion into the BiFC plasmid sets (Belda-Palazón et al., 2012). The YFN–COP1 and mRFP–SPA1 constructs were kindly provided by David Alabadi (Blanco-Touriñan et al., 2019). All the clones were transformed into *A. tumefaciens* (GV301). Clones expressing fusion proteins as indicated were co-infiltrated into the abaxial leaf surface of 3-week-old *N. benthamiana* plants as described (Voinnet et al., 2003).The leaves were infiltrated with 50 μM MG132 the day previous to the observation. The p19 protein was used to suppress gene silencing. The empty vectors were used as negative controls. Samples were imaged 3 d later on a Leica TCS SP8 confocal microscope using a water-immersion objective lens (HC PL APO CS2 63 × /1.20). YFP was excited at 488 nm and RFP at 580 nm. Fluorescence from YFP was detected between 497 and 555 nm and fluorescence from RFP between 588 and 651 nm. Chloroplasts autofluorescence was detected between 659 and 735 nm.

Pull-down assays

For semi-in vivo pull-down assays, the FL RGL2 coding sequence was cloned into the pKM596 (a gift from David Waugh, Addgene plasmid # 8837) and the MBP recombinant protein fusions were expressed in the *E. coli* BL21 (DE3). Recombinant proteins were purified and pull-down assays were performed according to Fonseca and Solano (2013). MBP-tagged protein fusions were purified using amylose agarose beads. Equal amounts of seedling protein extracts were combined with 10 μg MBP-tagged protein fusion or MBP protein alone, bound to amylose resin for 1 h at 4°C with rotation, washed three times with 1 mL of extraction buffer, eluted and denatured in sample buffer before immunoblot analysis. Anti-GFP-HRP (Milteny Biotec) and anti-MBP (Abcam) were used.

For in vitro pull-down assays, the FL coding sequence of *RGL2* was cloned into the pGEX-4T-1 vector (Pharmacia New Jersey, NJ, USA) to generate a GST-RGL2 fusion protein, and transformed in the BL21-CodonPlus (Stratagene) *E. coli* strain. GST and GST-RGL2 were induced by IPTG, and purified using glutathione Sepharose resin beads (ELPIS Biotech, Daejeon, Korea) according to the manufacturer's instruction. MBP and MBP–COP1 fusion protein were induced in BL21-CodonPlus (Stratagene, San Diego, CA, USA) *E. coli* strain (Saijo et al., 2003) and purified using amylose resin beads (ELPIS Biotech, Korea). For in vitro pull-down assays, 2 μg of GST and GST–RGL2 proteins were incubated with immobilized MBP and MBP–COP1 proteins in binding buffer (50 mM Tris–HCl pH 8.0, 150 mM NaCl, and 1 mM EDTA) and incubated at 4°C for 2 h. After being washed 3 times with the binding buffer, the protein-retained beads were boiled in Laemmli buffer and immunoblotted using anti-GST and anti-MBP antibodies (Santa Cruz Biotechnology, Dallas, TX, USA).

Immunoblotting on seed extracts

To detect endogenous COP1 protein levels, an anti-COP1 polyclonal antibody was used (Lee et al., 2017). Fresh seeds (harvested within 1 month before use) were imbibed in distilled water with or without 10 μM GA_3 or 10 μM PAC, and harvested at each time point. Total crude extracts were prepared using extraction buffer containing 50 mM Tris–HCl pH 7.5, 4 M urea, 150 mM NaCl, 1 mM EDTA, and protease and phosphatase inhibitor mixtures (1 mM PMSF, 5 μg/mL leupeptin, 5 μg/mL aprotinin, 5 μg/mL pepstatin, 5 μg/mL antipain, 5 μg/mL chymostatin, 2 mM Na_2VO_3, 2 mM NaF, and 50 μM MG132), separated by SDS-PAGE, and then immunoblotted with anti-COP1, and anti-Histone 3 (Abcam) antibodies.

Cell-free degradation assays

MBP-tagged RGL2 proteins were prepared from BL21-CodonPlus *E. coli* cells (Stratagene) and purified using an amylose resin according to the manufacturer's instructions. For each reaction, 100 ng MBP-RGL2 or MBP proteins was incubated with 100 μg total soluble protein extract at 22°C in assay buffer (50 mM Tris–HCl [pH 7.5], 100 mM NaCl, 10 mM $MgCl_2$, 5 mM DTT, and 5 mM ATP) from the WT (Col-0), *cop1-4*, and *COP1*-OX/*cop1-4* seedlings, previously grown at 22°C in the dark for 6 d. The reaction was stopped by adding Laemmli buffer at the respective times.

RT-qPCR

Total RNA was extracted from seeds using the Fruit-mate (Takara, Japan) and MG RNAzol (Macrogen, South Korea) reagents according to the manufacturer's instructions. First-strand cDNA was synthesized from 2 μg total RNA using M-MLV reverse transcriptase with oligo-dT primer (Promega, Madison, WI, USA). Expression levels of germination-associated genes were measured by RT-qPCR analysis using LightCycler 480 SYBR Green I Master mix (Roche) in a LightCycler 480 Real-Time PCR System (Roche, Basal, Switzerland). Expression levels were normalized by *ACTIN2* (*ACT2*). The gene-specific primer sets are shown in Supplemental Table S1.

In vitro ubiquitination assays

Assays were performed as previously reported (Yu et al., 2008) with minor modifications. Ubiquitination reaction mixtures contained 50 ng yeast Ub-activating enzyme (E1; Boston Biochem, Cambridge, MA, USA), 50 ng rice 6xHis-Rad6 (E2, Ub conjugating enzyme), 10 μg unlabeled Ub (Boston Biochem), and 2 μg MBP-COP1 (previously incubated with 20 μM $ZnCl_2$) in 30 μL of reaction buffer (50 mM Tris pH 7.5, 5 mM $MgCl_2$, 2 mM ATP, and 0.5 mM DTT). As a substrate, 50 ng 6xHis-HA-RGL2-6His fusion was used per reaction. After 2 h incubation at 30°C, reactions were stopped by adding 30 μL of Laemmli buffer, and then half of each mixture (30 μL) was boiled for 5 min and separated by 7.5% SDS-PAGE. 6xHis-HA-RGL2-6His and its ubiquitinated conjugates were detected using anti-HA (1:1,000; Roche) antibody.

Accession numbers

Sequence data from this article can be found in the GenBank/EMBL and TAIR data libraries under the following accession numbers: *COP1*, At2g32950; *COP10*, At3g13550; *ACT2*, At3g18780; *GASA6*, At1g74670; *EXPA1*, At1g69530; *EXPA2*, At5g05290; *EXPA8*, At2g40610; *GAI*, At1g14920; *RGA*, At2g01570; *RGL1*, At1g66350; *RGL2*, At3g03450; *RGL3*, At5g17490; *XTH33*, At1g10550.

Supplemental data

The following materials are available in the online version of this article.

Supplemental Figure S1. *35S:COP1-GFP* (*COP1-OX*) complements *cop1-4*.

Supplemental Figure S2. Epistasis analysis of *PIF1* and *COP1* in GA-dependent seed germination.

Supplemental Figure S3. Epistasis analysis of *HY5* and *COP1* in GA-dependent seed germination.

Supplemental Figure S4. PAC-insensitive phenotype in *rgl2-SK54* mutant seeds.

Supplemental Figure S5. COP1 dimerization and nuclear localization are essential for PAC-insensitive germination in *COP1-OX*/*cop1-4* plants.

Supplemental Figure S6. Expression of *RGL2* and *COP1* in *cop1-4* and *rgl2-SK54* mutants, respectively.

Supplemental Figure S7. Expression of AD and BD protein fusions in Y2H experiments shown in Figure 5A.

Supplemental Table S1. Primers used in this study.

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Conflict of interest statement. The authors declare no conflict of interest.

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