

1 **Short title:** COP1 destabilizes RGL2 to promote seed germination.

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3 **Article Title:**

4 **COP1 promotes seed germination by destabilizing RGA-LIKE2**
5 **(RGL2) in Arabidopsis**

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17 **Author Contributions** B.-D.L., and N.-C.P. designed the research and conceived the study.
18 B.-D.L., Y.Y., E.C., S.-H.K., M.G.-L., and V.R. performed experiments. B.-D.L., E.C., V.R.,
19 S.F., and N.-C.P. discussed the results and redesigned experiments, B.-D.L., S.F. and N.-
20 C.P wrote the paper.

21

22 **One sentence summary:** COP1 positively regulates germination in Arabidopsis seeds by
23 directly ubiquitinating and promoting the degradation of RGL2, a key repressor of seed
24 germination.

25

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32

33 **Abstract**

34 Under favorable moisture, temperature and light conditions, gibberellin (GA) biosynthesis is
35 induced and triggers seed germination. A major mechanism by which GA promotes seed
36 germination is by promoting the degradation of the DELLA protein RGL2, a major repressor
37 of germination in Arabidopsis seeds. Analysis of seed germination phenotypes of
38 *constitutively photomorphogenic 1 (cop1)* mutants and complemented *COP1-OX/cop1-4*
39 lines in response to GA and paclobutrazol (PAC) suggested a positive role for COP1 in seed
40 germination and a relation with GA signaling. *cop1-4* mutant seeds showed PAC
41 hypersensitivity, but transformation with a *COP1* overexpression construct rendered them
42 PAC insensitive, with a phenotype similar to that of *rgl2* mutant (*rgl2-SK54*) seeds.
43 Furthermore, *cop1-4 rgl2-SK54* double mutants showed a PAC-insensitive germination
44 phenotype like that of *rgl2-SK54*, identifying COP1 as an upstream negative regulator of
45 RGL2. COP1 interacts directly with RGL2 and *in vivo* this interaction is strongly enhanced by
46 SPA1. COP1 directly ubiquitinates RGL2 to promote its degradation. Moreover, GA
47 stabilizes COP1 with consequent RGL2 destabilization. By uncovering this COP1-RGL2
48 regulatory module, we reveal a novel mechanism whereby COP1 positively regulates seed
49 germination and controls the expression of germination-promoting genes.

50

51 Introduction

52 In its seed form, a new plant generation can be dispersed and then wait to develop and
 53 grow once favorable environmental conditions, such as optimal moisture, light, and
 54 temperature, exist (Bewley, 1997). Favorable conditions trigger changes in the
 55 phytohormone levels of mature seeds, including a decrease in abscisic acid (ABA) and an
 56 increase in gibberellins (GA), which together, inhibit dormancy and promote seed
 57 germination (Steber *et al.*, 1998; Holdsworth *et al.*, 2008). Seed imbibition induces GA
 58 biosynthesis and triggers germination. Arabidopsis (*Arabidopsis thaliana*) mutants with
 59 impaired GA biosynthesis (e.g., *ga1-3* and *ga2*) fail to germinate in the absence of
 60 exogenous GA, underlining its importance for germination (Koornneef & van der Veen,
 61 1980).

62 GA is perceived by the receptor protein GA-INSENSITIVE DWARF 1 (GID1), which then
 63 changes conformation and binds to DELLA proteins, central repressors in the GA signaling
 64 pathway (Ueguchi-Tanaka *et al.*, 2005; Murase *et al.*, 2008; Shimada *et al.*, 2008). The
 65 formation of the GID1-GA-DELLA complex triggers GA-mediated DELLA degradation by the
 66 F-box protein SLEEPY1 (SLY1; SCF^{SLY1} complex) and its homolog SNEEZY1/SLY2 through
 67 ubiquitin-dependent proteolysis, and induces the expression of GA-responsive genes
 68 (McGinnis *et al.*, 2003; Dill *et al.*, 2004; Fu *et al.*, 2004; Ariizumi *et al.*, 2011). Thus, GA lifts
 69 DELLA repression of its downstream targets, triggering GA-mediated responses (Sun *et al.*,
 70 2010).

71 In Arabidopsis, five DELLA repressors, such as GA INSENSITIVE (GAI), REPRESSOR
 72 OF *ga1-3* (RGA), RGA-LIKE 1 (RGL1), RGL2, and RGL3, play overlapping yet distinct roles
 73 in many developmental processes, such as seed germination, stem elongation, and
 74 transition to flowering (Peng *et al.*, 1997; Dill & Sun 2001; Lee *et al.*, 2002; Wen & Chang,
 75 2002; Cao *et al.*, 2005). GAI and RGA are both involved in seed germination (Oh *et al.*,
 76 2007), but RGL2 is the major regulator of GA-mediated seed germination among the DELLA
 77 proteins. Under GA-deficient conditions, such as in the *ga1-3* mutant background or under
 78 paclobutrazol (PAC) treatment, only *rgl2* mutation, but not *rgl1* or *rgl3*, rescued seed
 79 germination rates under light conditions (Lee *et al.*, 2002; Tyler *et al.*, 2004; Cao *et al.*,
 80 2005).

81 The repressive role of RGL2 in seed germination operates through different molecular
 82 mechanisms that integrate GA, ABA, and light signals. RGL2 associates with transcription
 83 factors to regulate gene expression and control germination (Piskurewicz *et al.*, 2008; Liu *et al.*,
 84 2016; Ravindran *et al.*, 2017; Yang *et al.*, 2019; Sanchez-Montesino *et al.*, 2019).

85 Furthermore, RGL2 represses the expression of the *GA-STIMULATED ARABIDOPSIS6*
86 (*GASA6*), which is an integrator of glucose, GA and ABA signals in seed germination (Zhong
87 *et al.*, 2015). *GASA6* locates mainly in cell wall and regulates cell wall loosening to favor cell
88 elongation on embryonic axis during seed germination through EXPANSIN 1 (*EXPA1*)
89 (*Zhong et al.*, 2015).

90 Light impact on seed germination relies in part on phytochrome B (phyB)
91 activation/inactivation by red/far-red light. Once activated, phyB promotes the degradation of
92 PHYTOCHROME-INTERACTING FACTOR 1 (*PIF1*), a strong repressor of light-mediated
93 seed germination (Oh *et al.*, 2004; Oh *et al.*, 2006). Thus, *pif1* mutants display high
94 germination rates in the dark (Oh *et al.*, 2004). *PIF1* regulate germination in many ways, by
95 directly binds to the promoters of the DELLA repressors *GAI* and *RGA* to activate their
96 expression and repress GA signaling (Oh *et al.*, 2007); by directly repressing genes involved
97 in GA biosynthesis and activating GA catabolism (Kim *et al.*, 2016; Oh *et al.*, 2007; Oh *et al.*,
98 2009); by acting cooperatively with *ABI3* in the dark to activate *SOMNUS* (*SOM*), a key
99 negative regulator of seed germination; and by directly binding to the *ABI5* promoter (Park *et*
100 *al.*, 2011; Kim *et al.*, 2008). Thus, stabilization of *PIF1* in the dark is a key to halting seed
101 germination, as it mediates the repression and activation of the GA and ABA cascades,
102 respectively (Oh *et al.*, 2006, 2007; Kim *et al.*, 2008).

103 Previous reports have shown that additional regulators of light signaling are involved in
104 the control of seed germination. Among them is CONSTITUTIVELY
105 PHOTOMORPHOGENIC 1 (*COP1*), a master regulator of photomorphogenesis. *COP1*,
106 together with the E2 ubiquitin variant *COP10*, DE-ETIOLATED 1 (*DET1*), and *COP9*
107 SIGNALOSOME (*CSN*) proteins, is a member of the *COP/DET/FUSCA* family, whose strong
108 mutants produce dark-purple-pigmented seeds and exhibit seedling-lethal phenotypes
109 (McNellis *et al.*, 1994). *COP1*, in association with SPA proteins, acts as part of a substrate
110 adaptor module within CULLIN4 (*CUL4*)-based E3 ubiquitin ligases. The formation of
111 *CUL4*^{*COP1-SPA*} complexes mediates the targeted degradation of many positive regulators of
112 photomorphogenesis in darkness, as well as of other regulators of circadian clock and
113 photoperiodic flowering (Lau & Deng, 2012). In the case of seed germination, the *CUL4*^{*COP1-*}
114 ^{*SPA*} E3 ubiquitin ligase is necessary for the light-induced degradation of *PIF1* in Arabidopsis.
115 Accordingly, *cop1* and *spaQ* mutants display reduced seed germination in response to light,
116 consistent with the higher abundance of *PIF1* in these mutants compared to the wild-type
117 (Zhu *et al.*, 2015). Strikingly, in the dark, *PIF1* acts as a cofactor of *CUL4*^{*COP1-SPA*} E3
118 ubiquitin ligase, enhancing its function to synergistically degrade *HY5* and repress
119 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 post-germination seedling development (Yadukrish *et al.*, 2020a; Yadukrish *et al.*, 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 (Fig. 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 (Fig. 1B) (Wei *et al.*, 1994) germinated normally, like wild-type seeds, under normal conditions (mock), indicating that the *fusca* phenotypes are not intrinsically associated with germination defects (Fig. 1C). In the presence of 10 μ M gibberellic acid (GA_3), the germination rate of *cop1-5* seeds was greatly enhanced compared to that of mock-treated seeds (Fig. 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 Methods; Fig. 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 paclobutrazol (PAC) (Fig. 2). We found that the germination of *cop1-4* seeds was slightly, but significantly, delayed compared to that of wild-type seeds under normal conditions (mock treatment) but was fully restored in the presence of GA , (Fig. 2A,B). Moreover, compared to wild-type seeds, the faster germination rate of *COP1-OX/cop1-4* seeds at 1.5 d after incubation (DAI) at 22°C in long day conditions (Fig. 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 (Fig. 2B,C), the germination of *cop1-4* mutant seeds was impaired, whereas *COP1-OX/cop1-4* seeds germinated much faster and in higher percentage than wild-type 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

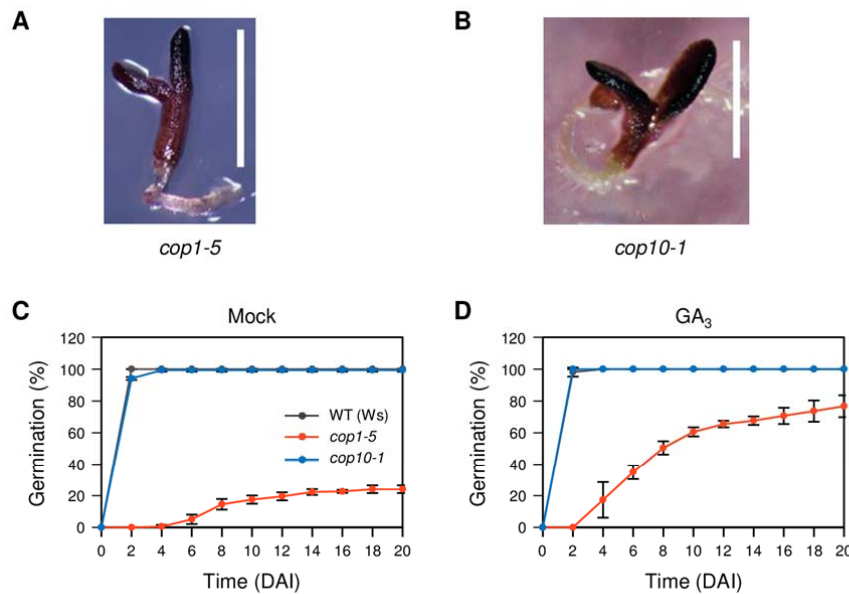


Figure 1. Germination rate of *cop1-5* seeds is dramatically enhanced in the presence of GA. (A, B) The seedling-lethal phenotypes of *cop1-5* (8 DAI) (A) and *cop10-1* (4 DAI) (B) mutants. (C, D) Germination rates of wild-type (WT; Ws ecotype), *cop1-5*, and *cop10-1* seeds on MS medium (mock) (C) or on the same medium containing 10 μM GA₃ (D). Germination rates were determined by counting the seeds with protruding radicles over 20 days. The mean and SD were obtained from three biological repeats (~100 seeds/repeat). The experiments were repeated five times with similar results. DAI, day(s) after incubation to 22°C under long days; GA, gibberellin.

During seed imbibition, GA upregulates the expression of downstream genes that induce cell wall remodeling needed for germination, expansins (EXPAs) and xyloglucan endotransglucosylases/endohydrolases (XTHs) (Weitbrecht *et al.*, 2011; Shi *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 qRT-PCR in *cop1-4* and *COP1-OX/cop1-4* seeds, as compared to wild-type seeds, at 3 DAI under normal conditions and in the presence of PAC (Fig. 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 upregulated 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 Fig. 1.

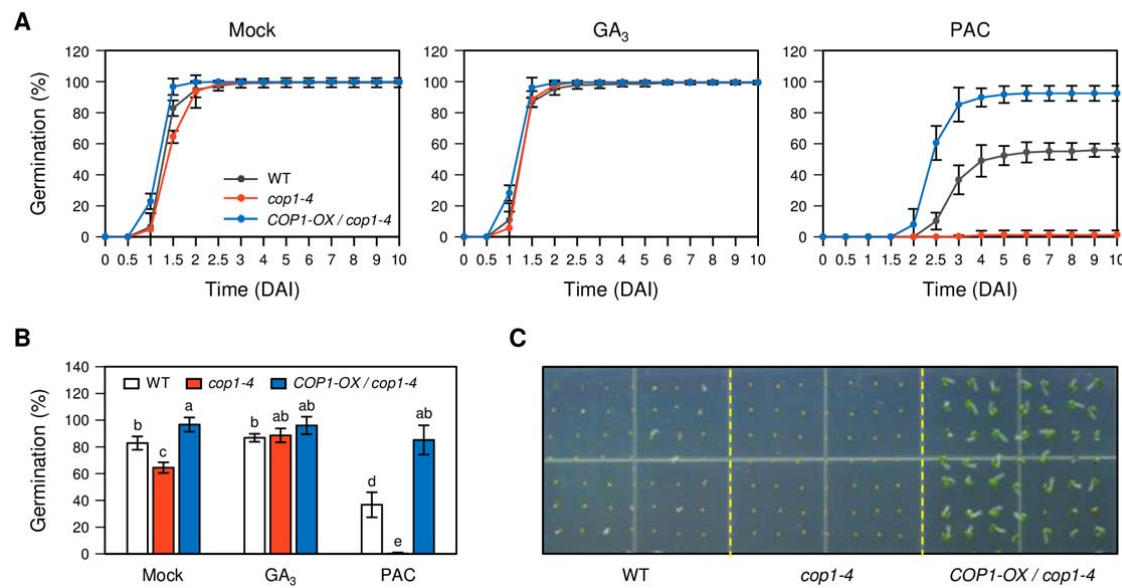


Figure 2. COP1 acts as a positive regulator in seed germination.

(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 GA₃ 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 independent repeats (~100 seeds/repeat). 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. DAI, day(s) after incubation to 22°C under LD; GA, gibberellin; PAC, paclobutrazol.

184 Light signaling regulators PIF1 and HY5 destabilized by COP1 do not explain the PAC 185 hypersensitivity of *cop1* mutant seeds.

186 Previous studies have shown that COP1 mediates PIF1 destabilization upon dark-to-light
187 transition to allow the establishment of the photomorphogenic developmental program (Zhu
188 *et al.* 2015). PIF1 also acts as a negative regulator of seed germination by promoting the
189 expression of *DELLA*, *DOF AFFECTING GERMINATION 1* (*DAG1*), and *SOM* (Oh *et al.*,
190 2007; Oh *et al.*, 2009; Kim *et al.*, 2008; Gabriele *et al.*, 2010; Seo *et al.*, 2009). However, a
191 relation between COP1 and PIF1 in seed germination was never found. In addition, HY5, a
192 positive regulator of photomorphogenesis that is destabilized by COP1 in darkness
193 (Osterlund *et al.*, 2000), is also involved in the ABA signaling pathway, where it negatively
194 regulates seed germination (Chen *et al.*, 2008; Yang *et al.*, 2018). Because these studies do
195 not discard a role of HY5 in GA-mediated seed germination, it could be speculated that HY5
196 destabilization by COP1 may be a mechanism to promote seed germination. To examine
197 whether *HY5* and *PIF1* play a role in GA-mediated seed germination, we analyzed the
198 germination rates of *pif1-1* and *hy5-215* single mutants and introgressed in a *cop1-4*

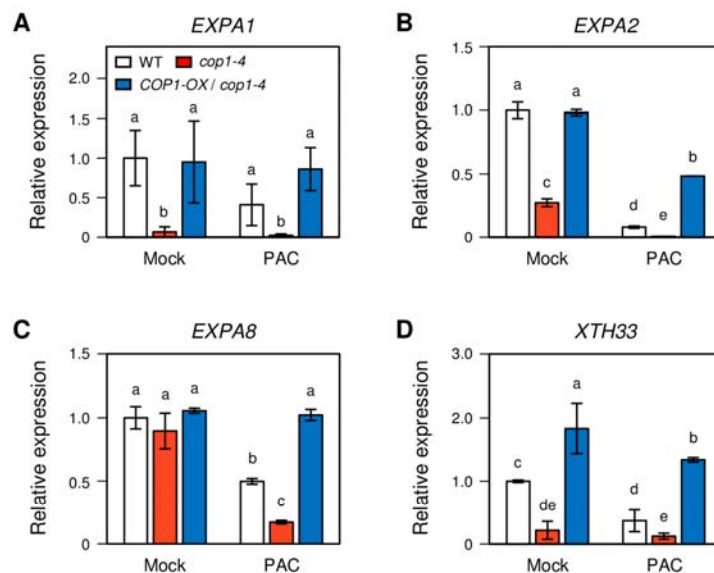


Figure 3. Expression profiles of germination-associated genes in *cop1-4* and *COP1-OX/cop1-4* seeds. (A-D) Expression levels of germination-associated genes, *EXPA1*, *EXPA2*, *EXPA8*, and *XTH33*, in *cop1-4* and *COP1-OX/cop1-4* seeds relative to those in WT seeds. After stratification, seeds were transferred to 22°C for germination and grown in MS phytoagar (mock) or in the presence of 10 μ M PAC until 3 DAI, and analyzed by qRT-PCR. The expression level of each gene was normalized to that of *ACTIN2* (*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. DAI, day(s) after incubation to 22°C under LD; PAC, paclobutrazol.

background under normal conditions and in the presence of PAC (Fig. **S2 and S3**). Seeds of *pif1-1* and *pif1-1 cop1-4* mutants, when exposed to light, behaved similarly to wild-type 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* (Fig. **S2**). The analysis of *hy5-215* and *cop1-4 hy5-215* germination shows that *hy5* germinates slightly early in PAC regarding the WT and the same is true for *cop1-4 hy5-215* regarding *cop1-4* background, showing that in PAC *hy5* mutation contributes to a slight partial suppression of the *cop1-4* germination defects (Fig. **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

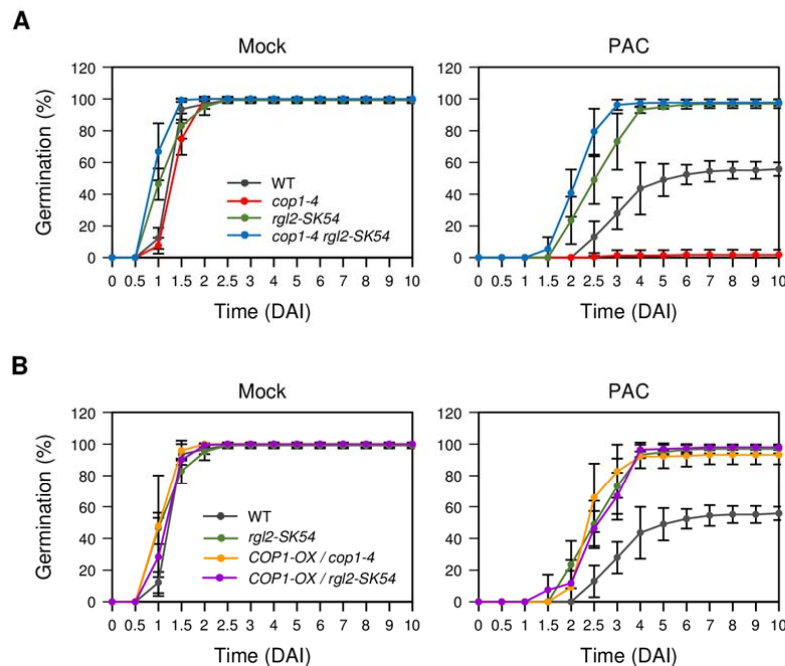


Figure 4. COP1 acts as an upstream regulator of RGL2 in seed germination.

(A) Germination rates of WT (Col-0), *cop1-4*, *rgl2-SK54*, and *cop1-4 rgl2-SK54* seeds on MS phytoagar medium (mock). (B) Germination rates of WT (Col-0), *rgl2-SK54*, *COP1-OX/cop1-4*, and *COP1-OX/rgl2-SK54* seeds in MS phytoagar medium containing 10 μ M PAC. After 3 days 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. DAI, day(s) after incubation to 22°C under LD; PAC, paclobutrazol.

five proteins, encoded by *GAI*, *RGA*, *RGA-LIKE1* (*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 (Fig. 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 wild-type and *cop1-4* seeds (Fig. 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* (Fig. 4A, right).

COP1-OX/cop1-4 seeds germinated much faster and in higher percentage than wild-type 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 (Fig. **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 (Lee *et al.* 2017; Stacey *et al.*, 1999). 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 (Fig. **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 target degradation, suggesting that the role of COP1 in seed germination requires a fully active nuclear E3 ubiquitin ligase activity.

COP1 interacts with and destabilizes RGL2

We then investigated whether COP1 regulates RGL2 indirectly at the transcriptional level or directly at the post-translational level. For this, we analyzed the expression levels of *RGL2* and *COP1* by qRT-PCR in *cop1-4* and *rgl2-SK54* seeds at 3 DAI, respectively, and compared them to those in wild-type 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 wild-type (Fig. **S6A,B**). These results indicated that COP1 does not regulate *RGL2* at the transcriptional level.

Next, to examine whether COP1 interacts with and destabilizes RGL2 post-translationally, we first tested the physical interaction between the two proteins. Y2H assays revealed that, whereas full-length COP1 did not interact with RGL2, a truncated version of COP1 containing the RING domain could directly interact with RGL2 (Fig. **5A**). Since all COP1 fragments and RGL2 are being expressed in yeast (Fig. **S7**), it seems that the full-length 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 *E. coli* (Fig. **5B**). *In vitro* purified MBP-COP1 could pull-down GST-RGL2 fusions, thus supporting a direct interaction between the full-length 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 (Fig. **5C**).

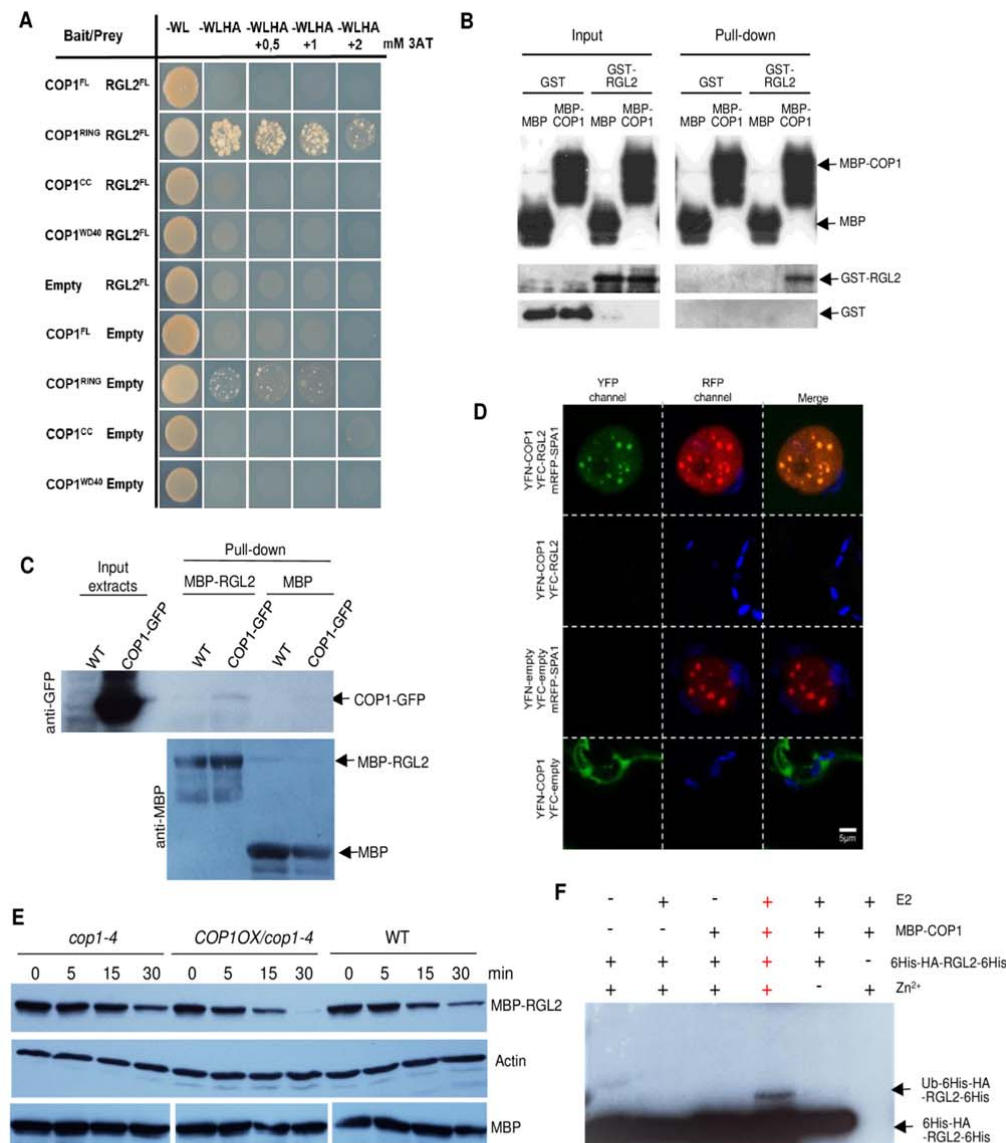


Figure 5. COP1 interacts with and destabilizes RGL2.

(A) Interaction of COP1 and RGL2 in Y2H assays. Full-length (FL) COP1, as well as three of its individual domains (RING, coiled-coil (CC), and WD40-repeat (WD40)), were used as baits and full-length RGL2 as prey. Selection for interaction was performed in selective media containing increasing concentrations of 3-Amino-1,2,4-Triazol (3-AT). (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. GST-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. As a control for equivalent extract amounts actin is shown. MBP when expressed alone remained stable. An anti-MBP antibody was used for fusion protein-detection. Protein levels at each time point are shown relative to those in the input sample (time 0) and normalized to the corresponding actin loading, and set as 1. 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 MBP-COP1 without Zn²⁺ as a negative control), rice E2 Rad6 (E2), and yeast E1 (E1; Boston Biochem). Ubiquitinated RGL2 was detected using an anti-HA antibody.

fluorescent complementation (BiFC) assays in *N. benthamiana* leaves. Contrary to the *in vitro* results, the co-expression of truncated YFN-COP1 and truncated YFC-RGL2 constructs showed no YFP (Yellow Fluorescent Protein) reconstitution signal. Based on the recent results by Blanco-Touriñan et al., (2020) 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 (Fig. 5D).

To examine whether COP1 destabilizes RGL2 *in vivo*, we examined the changes in RGL2 levels in the *cop1-4* and *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 wild-type, *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 slowly in *cop1-4* extracts than in wild-type whereas MBP alone remained stable (Fig. 5E). Furthermore, *in vitro* ubiquitination assays showed that RGL2 (6His-HA-RGL2-6His fusion) was directly ubiquitinated by MBP-COP1 (Fig. 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 (Zhong et al., 2015; Stamm et al., 2012; Rombolá-Caldentey et al., 2014; Yan et al., 2014). To further support a 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* (Fig. 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 cancelled 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

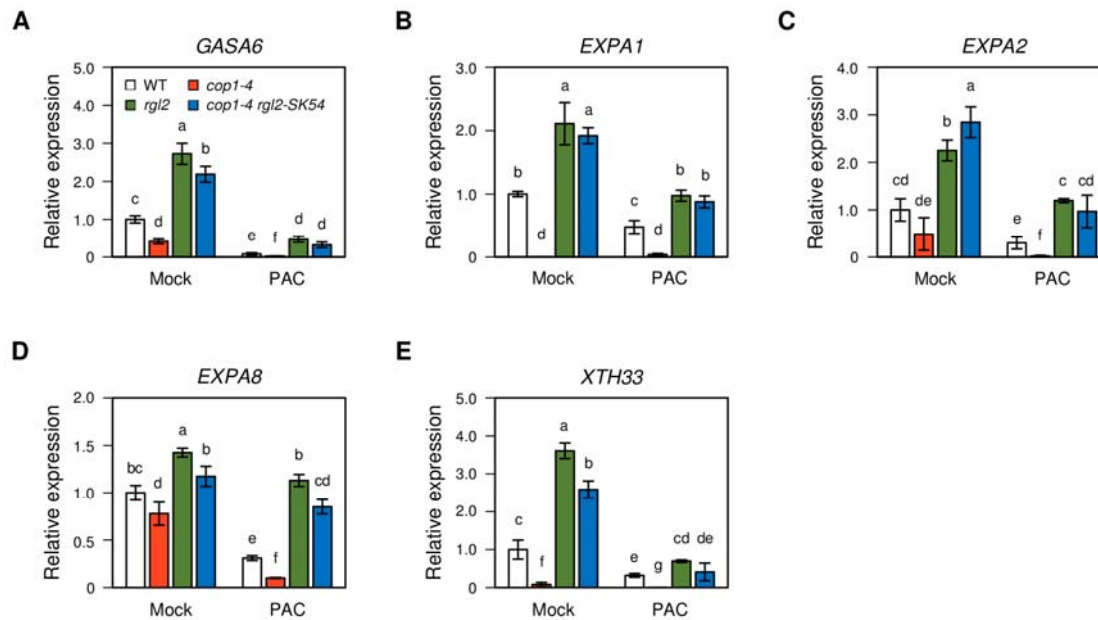


Figure 6. Effects of PAC on the expression of germination-associated genes in imbibed *cop1-4*, *rgl2-SK54*, and *cop1-4 rgl2-SK54* seeds.

Altered expression of five *RGL2*-downregulated germination-associated genes, *GASA6*, *EXPA1*, *EXPA2*, *EXPA8*, and *XTH33*, in imbibed WT (Col-0), *cop1-4*, *rgl2*, and *cop1-4 rgl2-SK54* seeds in the presence or absence of 10 μ M PAC at 3 DAI. The expression level of each gene obtained by qRT-PCR was normalized to that of *ACTIN2* (*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 ($P < 0.05$). DAI, day(s) after incubation to 22°C under LD; PAC, paclobutrazol.

298 germination.

299

300 GA increases COP1 stability in the imbibed seeds

301 In imbibed seeds, increased GA biosynthesis is one of the most important triggers for the
302 induction of seed germination (Steber *et al.*, 1998). The increase in endogenous GA
303 concentration decreases the stability of RGL2 (Tyler *et al.*, 2004). Thus, we wondered
304 whether GA or PAC have an impact on COP1 accumulation in imbibed seeds that could lead
305 to changes in RGL2 stability. To examine the effects of GA and PAC on COP1
306 accumulation, we measured both *COP1* mRNA and *COP1* protein levels in germinating
307 seeds and during the onset of post-germinative seedling establishment after treatments with
308 GA or PAC for 48 h (Fig. 7A–C). The results showed that *COP1* protein levels were
309 increased by GA treatment 12 hours after imbibition and slightly decreased by PAC
310 treatment (Fig. 7A–C). The differences in *COP1* protein accumulation in response to GA and

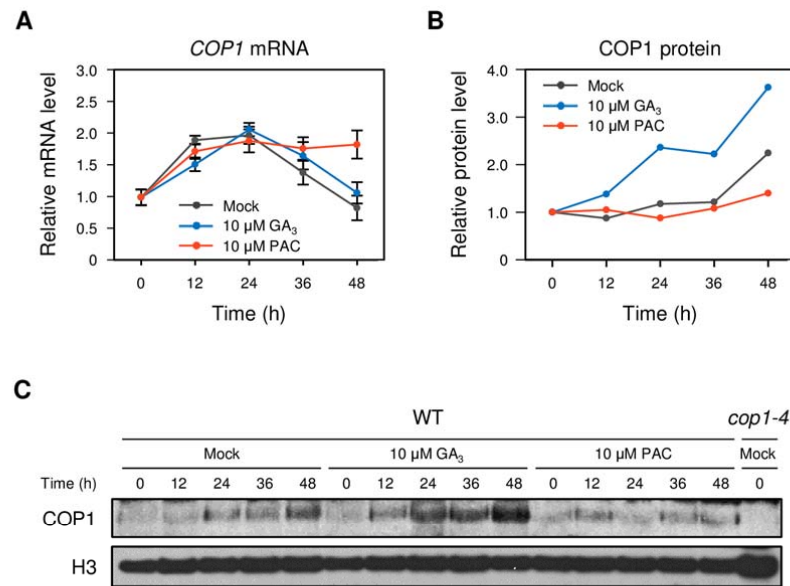


Figure 7. COP1 stability is enhanced by GA and decreased by PAC.

(A) Relative expression levels of *COP1* in seeds stratified for 3 days at 4°C on MS medium 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 *ACT1* (*ACT2*). The expression levels of *COP1* are shown relative to the expression at time 0 under mock treatment, which is set as 1. In qRT-PCR analysis, the mean and SD were obtained from three biological repeats (~30 seeds/repeat). (B, 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 and SD were obtained from three biological repeats (~100 seeds/repeat). GA, gibberellin; PAC, paclobutrazol.

PAC did not correlate with the variation pattern of *COP1* mRNA expression under these conditions, indicating that GA and PAC have post-translational effects on *COP1* abundance (Fig. 7A–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.

320 Discussion

321 Seeds are equipped with molecular sensors to monitor surrounding environmental conditions
 322 and determine whether they are favorable for plant establishment (Seo *et al.*, 2009). Here
 323 we describe a new regulatory module in which COP1 positively regulates seed germination
 324 by interfering with a component of the GA signaling pathway, the DELLA family member
 325 RGL2, which is a well-known repressor of seed germination. We showed that *cop1-4*
 326 mutants are strongly sensitive to PAC, while *COP1-OX/cop1-4* seeds are strongly insensitive
 327 to this GA biosynthesis inhibitor (Fig. 2), mimicking the phenotype of the *rgl2-SK54* mutant
 328 (Fig. 4, Fig. S4). In addition, COP1 promotes the degradation of RGL2 (Fig. 5). Supporting
 329 these findings, genetic analysis showed that *rgl2* is epistatic to *cop1*, as the double mutants
 330 show complete suppression of the *cop1-4* PAC hypersensitivity (Fig. 4A) and of its defects
 331 on the activation of genes encoding cell wall proteins involved in cell loosening during
 332 germination (Fig. 6). Furthermore, GA enhances COP1 protein accumulation upon imbibition
 333 (Fig. 7), evidencing the GA's broad role as a primary regulator in seed germination.

334

335 COP1 regulates seed germination through RGL2

336 The CUL4^{COP1-SPA} E3 ubiquitin ligase is well described as a repressor of light signaling,
 337 targeting for degradation photomorphogenesis-promoting factors (Lau & Deng, 2012; Zhu *et al.*,
 338 *et al.*, 2015). Our data show that *cop1* alleles display defects in seed germination that are
 339 unrelated to the fusca phenotypes as *cop10* fusca mutants do not display them. COP10 and
 340 DET1 belong to the same E3 ubiquitin ligase and it has been previously found that *det1*
 341 fusca mutant seeds germinate better than WT in normal conditions and are hypersensitive to
 342 ABA (Fernando and Schroeder, 2015). In the case of *cop1* mutant seeds, the defects can
 343 partially complemented by GA application and, likewise, can be exacerbated by the
 344 presence of PAC, suggesting that COP1 plays a role in seed germination by interacting with
 345 the GA signaling pathway (Fig. 1). CUL4^{COP1-SPA1} complexes play a positive role in seed
 346 germination by promoting the rapid degradation of PIF1 under red and far-red light
 347 conditions (Zhu *et al.*, 2015). Thus, by being degraded by COP1, PIF1 acts in light-mediated
 348 seed germination independently of GA, making unlikely that the COP1–PIF1 module could
 349 be responsible for GA-related germination defects. In addition, HY5, a well-described COP1
 350 target in photomorphogenesis, is known to repress seed germination by activating *ABI5*
 351 gene expression in response to ABA and salt stress (Chen *et al.*, 2008; Yu *et al.*, 2016).
 352 Thus, HY5 role in seed germination seems to be unrelated with GA signaling. This is
 353 consistent with our observation that *pif1* and *hy5* seeds germinate similarly to wild-type

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) (Fig. **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 (Fig. **4**), suggesting that RGL2 acts downstream of COP1 in seed germination and might be a direct target of COP1 ubiquitin ligase activity, which would represent a novel 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 (Fig. **5**). Thus, COP1-mediated degradation of RGL2 might occur in parallel with the canonical SLY1- and GA-dependent degradation. Blanco-Touriñan *et al.* (2020) 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.* (2020) 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.*, 2020). Moreover, we report that an active COP1 protein with full capacity to dimerize and enter the nucleus is essential to maintaining wild-type germination levels in the presence of PAC (Fig. **S5**).

The CSN complex allows the activation of cullin-based E3 ubiquitin ligases by maintaining proper cycles of neddylation (an ubiquitin-like modification) and deneddylation of cullins. Previous studies have shown that CSN mutants have poor germination and

hyperdormancy phenotypes (Wei & 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 ubiquitin ligases (Jin *et al.*, 2018). However, it cannot be ruled out that *csn* mutations also impair the activity of CUL4^{COP1-SPA} complexes towards RGL2. Indeed, it has been recently shown that CUL4^{CDD} complexes mediate COP1 degradation 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 & Deng, 2003; Franciosini *et al.*, 2015)

Our results uncovered a novel 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; Arizumi & Steber, 2007; Penfield *et al.*, 2006). Indeed, *sly1* loss-of-dormancy germination correlates better with endogenous levels of ABI5 and seems to depend on ABA biosynthesis (Piskurevich *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-EXPA1* 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*. Thus, 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 (Figs. 6, 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 (Fig. 6; Stamm *et al.*, 2012).

Notably, GA promotes COP1 protein accumulation during of seed germination and at the onset of seedling establishment (Fig. 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 for the ABA-mediated inhibition of post-germinative seedling establishment (Lau & Deng, 2012; Yadukrishnan *et al.*, 2020). 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 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*; *SpeI* restriction enzyme digestion) and PCR (*rgl2-SK54*) primers (Table S1). For the 35S:COP1-GFP (COP1-OX) constructs, the full-length COP1 cDNA PCR amplified from Col-0 cDNA, cloned into the pDONR221 vector (Invitrogen) and subsequently into the pMDC85 plasmid (Curtis & Grossniklaus, 2003). Through

Agrobacterium tumefaciens (GV3101)-mediated transformation in *cop1-4* or *rgl2-SK54* mutants by the floral-dip method (Clough & Bent, 1998) *COP1-OX/cop1-4* or *COP1-OX/rgl2-SK54* transgenic plants were obtained. The *COP1* mutant variants, i.e. 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 one month before the experiments) were used to measure germination rates. Seeds were surface sterilized with a solution containing 70% ethanol and 0.1% Triton X-100, for 20 min, and washed with 100% ethanol for three times. After being air-dried on sterile 3M filter paper, seeds were seeded on MS phytoagar medium (mock) or on the same medium supplemented with 10 μ M GA₃ or 10 μ M PAC. For stratification, seeds were kept at 4°C in darkness for 72 h. Germination experiments were initiated with the transferred to a growth chamber at a constant 22°C temperature under cool white fluorescent light (100 μ mol m⁻² s⁻¹) and long days (LD; 16-h light/day) and referred as days after incubation (DAI). To measure germination rates, germinated seeds were scored upon radicle emergence. For each experiment, three to five biological replicates of pools of about 100 seeds were used. Among each replicate seeds were collected from plants grown simultaneously under same conditions.

Yeast two-hybrid (Y2H) assays

Y2H assays were performed using the Matchmaker GAL4 two-hybrid system (Clontech). The full-length and/or partial (RING; aa 1–104, CC; aa 121–213, WD-40 repeat; aa 371–675) cDNAs of *COP1* were obtained by RT-PCR from wild-type (Col) plants (Yu *et al.*, 2008) and cloned into the pGBK vector (as baits), and the full-length *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) and an anti-myc (kindly provided by Xing Wang Deng) antibodies were used to check the expression of AD and BD fusion proteins.

Bimolecular fluorescence complementation (BiFC) assays

The full-length 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-Touriñán *et al.*, 2020). All the

clones were transformed into *Agrobacterium 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 μ M MG132 the day previous to the observation. The p19 protein was used to suppress gene silencing. The empty vectors were used as negative controls. Fluorescence was visualized in epidermal cells of leaves after 3 d of infiltration using a TCS SP8 Leica Microsystems confocal laser microscope.

Pull-down assays

For semi-*in vivo* pull-down assays, the full-length 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 μ g MBP-tagged protein fusion or MBP protein alone, bound to amylose resin for 1 hr at 4°C with rotation, washed three times with 1 ml of extraction buffer, eluted and denatured in sample buffer before immunoblot analysis.

For *in vitro* pull-down assays, the full-length coding sequence of RGL2 was cloned into the pGEX-4T-1 vector (Pharmacia) 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, Korea) according to the manufacturer's instruction. MBP and MBP-COP1 fusion protein were induced in BL21-CodonPlus (Stratagene) *E. coli* strain (Saijo et al., 2003) and purified using amylose resin beads (ELPIS Biotech, Korea). For *in vitro* pull-down assays, 2 μ g 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 three 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, 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 μ M GA₃ or 10 μ M PAC, and harvested at each time point.

Total crude extracts were prepared using extraction buffer (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 Na₂VO₃, 2 mM NaF and 50 µM MG132), separated by SDS-PAGE, and then immunoblotted with anti-COP1, anti-Myc (Santa Cruz Biotechnology, USA), and anti-GFP (Santa Cruz Biotechnology, USA) 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 (pH7.5), 100 mM NaCl, 10 mM MgCl₂, 5 mM DTT, and 5 mM ATP] from the wild-type (Col-0), *cop1-4*, and *COP1-OX/cop1-4* seedlings, previously grown at 22°C in the dark for 6 days. The reaction was stopped by adding Laemmli buffer at the respective times.

Reverse transcription and quantitative real-time PCR (qRT-PCR)

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). Expression levels of germination-associated genes were measured by qRT-PCR analysis using LightCycler 480 SYBR Green I Master mix (Roche) in a LightCycler 480 Real-Time PCR System (Roche, Basel, Switzerland). Expression levels were normalized by *ACTIN2* (*ACT2*). The gene-specific primer sets are shown in 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 E1 (Boston Biochem), 50 ng rice 6xHis-Rad6 (E2), 10 µg unlabeled ubiquitin (Boston Biochem), and 2 µg MBP-COP1 (previously incubated with 20 µM ZnCl₂) in 30 µL of reaction buffer (50 mM Tris pH 7.5, 5 mM MgCl₂, 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 (1:1000; Roche) antibody.

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.

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Supplemental Data

Supplementary Figure 1. 35S:*COP1-GFP* (*COP1-OX*) complements *cop1-4*.

Supplementary Figure 2. Epistasis analysis of *PIF1* and *COP1* in GA dependent seed germination.

Supplementary Figure 3. Epistasis analysis of *HY5* and *COP1* in GA dependent seed germination

Supplementary Figure 4. PAC-insensitive phenotype in *rgl2-SK54* mutant seeds.

Supplementary Figure 5. *COP1* dimerization and nuclear localization are essential for PAC-insensitive germination in *COP1-OX/cop1-4* plants.

Supplementary Figure 6. Expression of *RGL2* and *COP1* in *cop1-4* and *rgl2-SK54* mutants, respectively.

Supplementary Figure 7. Expression of AD and BD protein fusions in Y2H experiments shown in Fig. 5A.

Supplementary Table 1. Primers used in this study.

583

584

585 Figure Legends

586 **Figure 1.** Germination rate of *cop1-5* seeds is dramatically enhanced in the presence of GA.

587 (A, B) The seedling-lethal phenotypes of *cop1-5* (8 DAI) (A) and *cop10-1* (4 DAI) (B)
588 mutants. (C, D) Germination rates of wild-type (WT; Ws ecotype), *cop1-5*, and *cop10-1*
589 seeds on MS medium (mock) (C) or on the same medium containing 10 μ M GA₃ (D).
590 Germination rates were determined by counting the seeds with protruding radicles over 20
591 days. The mean and SD were obtained from three biological repeats (~100 seeds/repeat).
592 The experiments were repeated five times with similar results. DAI, day(s) after incubation
593 to 22°C under long days; GA, gibberellin.

594

595 **Figure 2.** COP1 acts as a positive regulator in seed germination.

596 (A) Germination rates of WT (Col-0 ecotype), *cop1-4*, and *COP1-OX/cop1-4* seeds on MS
597 phytoagar medium (mock) or on the same medium containing 10 μ M GA₃ or 10 μ M PAC
598 from 0 to 10 DAI. (B) Germination scored at 1.5 DAI of the same lines and treatments as in
599 (A). Germination rates were determined by counting the seeds with protruding radicles. The
600 mean and SD were obtained from three independent repeats (~100 seeds/repeat). Different
601 letters indicate significantly different values according to a one-way ANOVA and Duncan's
602 least significant range test ($P < 0.05$). (C) Germination phenotypes of WT, *cop1-4*, and
603 *COP1-OX/cop1-4* seeds on MS medium containing 10 μ M PAC at 3 DAI. The experiments
604 were repeated five times with similar results. DAI, day(s) after incubation to 22°C under LD;
605 GA, gibberellin; PAC, paclobutrazol.

606

607 **Figure 3.** Expression profiles of germination-associated genes in *cop1-4* and *COP1-*
608 *OX/cop1-4* seeds.

609 (A-D) Expression levels of germination-associated genes, *EXPA1*, *EXPA2*, *EXPA8*, and
610 *XTH33*, in *cop1-4* and *COP1-OX/cop1-4* seeds relative to those in WT seeds. After
611 stratification, seeds were transferred to 22°C for germination and grown in MS phytoagar
612 (mock) or in the presence of 10 μ M PAC until 3 DAI, and analyzed by qRT-PCR. The
613 expression level of each gene was normalized to that of *ACTIN2* (*ACT2*). Expression levels
614 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. DAI, day(s) after incubation to 22°C under LD; PAC, paclobutrazol.

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). (B) Germination rates of WT (Col-0), *rgl2-SK54*, *COP1-OX/cop1-4*, and *COP1-OX/rgl2-SK54* seeds in MS phytoagar medium containing 10 μM PAC. After 3 days 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. DAI, day(s) after incubation to 22°C under LD; PAC, paclobutrazol.

Figure 5. COP1 interacts with and destabilizes RGL2.

(A) Interaction of COP1 and RGL2 in Y2H assays. Full-length (FL) COP1, as well as three of its individual domains (RING, coiled-coil (CC), and WD40-repeat (WD40)), were used as baits and full-length RGL2 as prey. Selection for interaction was performed in selective media containing increasing concentrations of 3-Amino-1,2,4-Triazol (3-AT). (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. GST-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. As a control for equivalent extract amounts actin is shown. MBP when expressed alone remained stable. An anti-MBP antibody was used for fusion protein-detection. Protein levels at each time point are shown relative to those in the input sample (time 0) and normalized to the corresponding actin loading, and set as 1. 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 MBP-COP1 without Zn²⁺ as a negative control), rice E2 Rad6 (E2), and yeast E1 (E1; Boston Biochem). Ubiquitinated RGL2 was detected using an anti-HA antibody.

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*, *EXPA1*, *EXPA2*, *EXPA8*, and *XTH33*, 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 qRT-PCR was normalized to that of *ACTIN2* (*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 ($P < 0.05$). DAI, day(s) after incubation to 22°C under LD; PAC, paclobutrazol.

Figure 7. COP1 stability is enhanced by GA and decreased by PAC.

(A) Relative expression levels of *COP1* in seeds stratified for 3 days at 4°C on MS medium 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 qRT-PCR analysis, the mean and SD were obtained from three biological repeats (~30 seeds/repeat). (B, 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 and SD were obtained from three biological repeats (~100 seeds/repeat). GA, gibberellin; PAC, paclobutrazol.

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

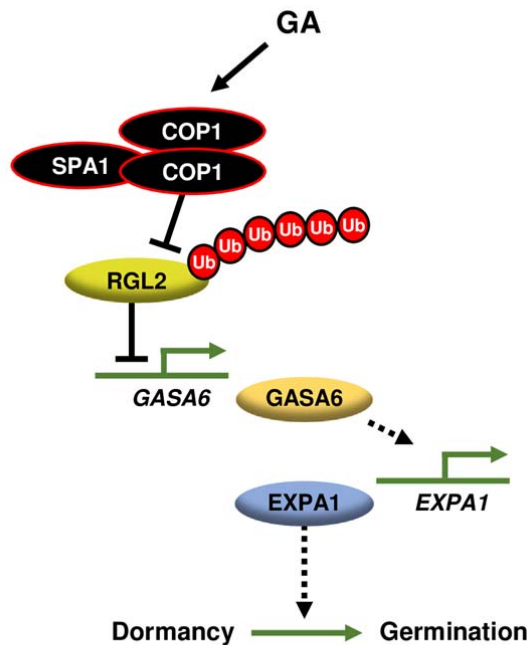


Figure 8. Model of the COP1–RGL2 regulatory module in seed germination.

Upon perception of favorable environmental cues, GA is synthesized in the seeds to induce germination. *COP1* is expressed, and *COP1* is stabilized by GA and interacts with *RGL2*, a negative regulator of the expression 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 non-canonical 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; GA, gibberellin; Ub, ubiquitin.

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Parsed Citations

- Ariizumi T, Hauvermale AL, Nelson SK, Hanada A, Yamaguchi S, Steber CM. 2013. Lifting DELLA repression of Arabidopsis seed germination by nonproteolytic gibberellin signaling. *Plant Physiology* 162(4): 2125.**
Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)
- Ariizumi T, Steber CM. 2007. Seed germination of GA-insensitive sleepy1 mutants does not require RGL2 protein disappearance in Arabidopsis. *Plant Cell* 19(3): 791-804.**
Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)
- Belda-Palazón B, Ruiz L, Martí E, Tárraga S, Tiburcio AF, Culiáñez F, Farràs R, Carrasco P, Ferrando A. 2012. Aminopropyltransferases involved in polyamine biosynthesis localize preferentially in the nucleus of plant cells. *PLoS One* 7(10):e46907.**
Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)
- Bewley JD. 1997. Seed germination and dormancy. *Plant Cell* 9(7): 1055.**
Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)
- Blanco-Touriñán N, Legris M, Minguet EG, Costigliolo-Rojas C, Nohales MA, Iniesto E, García-León M, Pacín M, Heucken N, Blomeier T, et al. 2020. COP1 destabilizes DELLA proteins in Arabidopsis. *PNAS* 117 (24): 13792-13799.**
Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)
- Cañibano E, Bourbousse C, García-León M, Garnelo Gómez B, Wolff L, García-Baudino C, Lozano-Durán R, Barneche F, Rubio V, Fonseca S. 2021. DET1-mediated COP1 regulation avoids HY5 activity over second-site targets to tune plant photomorphogenesis. *Molecular Plant*. 14(6): 963-982. doi: 10.1016/j.molp.2021.03.009.**
Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)
- Cao D, Hussain A, Cheng H, Peng J. 2005. Loss of function of four DELLA genes leads to light- and gibberellin-independent seed germination in Arabidopsis. *Planta* 223(1): 105-113.**
Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)
- Chen H, Zhang J, Neff MM, Hong S-W, Zhang H, Deng X-W, Xiong L. 2008. Integration of light and abscisic acid signaling during seed germination and early seedling development. *Proceedings of the National Academy of Sciences* 105(11): 4495-4500.**
Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)
- Clough SJ, Bent AF. 1998. Floral dip: a simplified method for Agrobacterium-mediated transformation of Arabidopsis thaliana. *Plant Journal* 16(6): 735-743.**
Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)
- Curtis MD, Grossniklaus U. 2003. A gateway cloning vector set for high-throughput functional analysis of genes in planta. *Plant Physiology* 133(2):462-9**
Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)
- Dill A, Sun T-P. 2001. Synergistic derepression of gibberellin signaling by removing RGA and GAI function in Arabidopsis thaliana. *Genetics* 159(2): 777-785.**
Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)
- Dill A, Thomas SG, Hu J, Steber CM, Sun TP. 2004. The Arabidopsis F-box protein SLEEPY1 targets gibberellin signaling repressors for gibberellin-induced degradation. *Plant Cell* 16(6): 1392-1405.**
Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)
- Dohmann EMN, Nill C, Schwechheimer C. 2010. DELLA proteins restrain germination and elongation growth in Arabidopsis thaliana COP9 signalosome mutants. *European journal of cell biology* 89(2-3): 163-168.**
Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)
- Earley KW, Haag JR, Pontes O, Opper K, Juehne T, Song K, Pikaard CS. 2006. Gateway-compatible vectors for plant functional genomics and proteomics. *Plant J* 45(4): 616-629.**
Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)
- Fernando VC, Schroeder DF. 2015. Genetic interactions between DET1 and intermediate genes in Arabidopsis ABA signalling. *Plant Science* 239:166-79.**
Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)
- Fonseca S, Solano R. 2013. Pull-down analysis of interactions among jasmonic acid core signaling proteins. In *Jasmonate Signaling*, pp. 159-171. Humana Press, Totowa, NJ.**
Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)
- Franciosi A, Moubayidin L, Du K, Matari Nahill H, Boccaccini A, Butera S, Vittorioso P, Sabatini S, Jenik Pablo D, Costantino P, et al. 2015. The COP9 SIGNALOSOME is required for postembryonic meristem maintenance in Arabidopsis thaliana. *Molecular Plant* 8(11): 1623-1634.**
Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)
- Fu X, Richards DE, Fleck B, Xie D, Burton N, Harberd NP. 2004. The Arabidopsis mutant sleepy1gar2-1 protein promotes plant growth by increasing the affinity of the SCFSLY1 E3 ubiquitin ligase for DELLA protein substrates. *Plant Cell* 16(6): 1406-1418.**

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Gabriele S, Rizza A, Martone J, Circelli P, Costantino P, Vittorioso P. 2010. The Dof protein DAG1 mediates PIL5 activity on seed germination by negatively regulating GA biosynthetic gene AtGA3ox1. Plant Journal 61(2): 312-323.

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Holdsworth MJ, Bentsink L, Soppe WJ. 2008. Molecular networks regulating Arabidopsis seed maturation, after-ripening, dormancy and germination. New Phytologist 179(1): 33-54.

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Jin D, Wu M, Li B, Bucker B, Keil P, Zhang S, Li J, Kang D, Liu J, Dong J, et al. 2018. The COP9 Signalosome regulates seed germination by facilitating protein degradation of RGL2 and ABI5. PLoS Genetics 14(2): e1007237.

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Koornneef M, van der Veen JH. 1980. Induction and analysis of gibberellin sensitive mutants in Arabidopsis thaliana (L.) heynh. Theoretical and Applied Genetics 58(6): 257-263.

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Lau OS, Deng XW. 2012. The photomorphogenic repressors COP1 and DET1: 20 years later. Trends in Plant Science 17(10): 584-593.

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Lee B-D, Kim MR, Kang M-Y, Cha J-Y, Han S-H, Nawkar GM, Sakuraba Y, Lee SY, Imaizumi T, McClung CR, et al. 2017. The F-box protein FKF1 inhibits dimerization of COP1 in the control of photoperiodic flowering. Nature Communications 8(1): 2259.

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Lee S, Cheng H, King KE, Wang W, He Y, Hussain A, Lo J, Harberd NP, Peng J. 2002. Gibberellin regulates Arabidopsis seed germination via RGL2, a GAI/RGA-like gene whose expression is up-regulated following imbibition. Genes and Development 16(5): 646-658.

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Liu X, Hu P, Huang M, Tang Y, Li Y, Li L, Hou X. 2016. The NF-YC-RGL2 module integrates GA and ABA signalling to regulate seed germination in Arabidopsis. Nature Communications 7: 12768.

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Maier A, Schrader A, Kokkelink L, Falke C, Welter B, Iniesto E, Rubio V, Uhrig JF, Hülskamp M, Hoecker U. 2013. Light and the E3 ubiquitin ligase COP1/SPA control the protein stability of the MYB transcription factors PAP1 and PAP2 involved in anthocyanin accumulation in Arabidopsis. Plant Journal 74(4): 638-651.

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

McGinnis KM, Thomas SG, Soule JD, Strader LC, Zale JM, Sun TP, Steber CM. 2003. The Arabidopsis SLEEPY1 gene encodes a putative F-box subunit of an SCF E3 ubiquitin ligase. Plant Cell 15(5): 1120-1130.

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

McNellis TW, von Arnim AG, Deng XW. 1994. Overexpression of Arabidopsis COP1 results in partial suppression of light-mediated development: evidence for a light-inactivable repressor of photomorphogenesis. Plant Cell 6(10): 1391-1400.

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Murase K, Hirano Y, Sun T-P, Hakoshima T. 2008. Gibberellin-induced DELLA recognition by the gibberellin receptor GID1. Nature 456(7221): 459.

Oh E, Kang H, Yamaguchi S, Park J, Lee D, Kamiya Y, Choi G. 2009. Genome-wide analysis of genes targeted by PHYTOCHROME INTERACTING FACTOR 3-LIKE5 during seed germination in Arabidopsis. Plant Cell 21(2): 403-419.

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Oh E, Kim J, Park E, Kim J-I, Kang C, Choi G. 2004. PIL5, a phytochrome-interacting basic helix-loop-helix protein, is a key negative regulator of seed germination in Arabidopsis thaliana. Plant Cell 16(11): 3045-3058.

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Oh E, Yamaguchi S, Hu J, Yusuke J, Jung B, Paik I, Lee H-S, Sun T-P, Kamiya Y, Choi G. 2007. PIL5, a phytochrome-interacting bHLH protein, regulates gibberellin responsiveness by binding directly to the GAI and RGA promoters in Arabidopsis seeds. Plant Cell 19(4): 1192-1208.

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Oh E, Yamaguchi S, Kamiya Y, Bae G, Chung W, Choi G. 2006. Light activates the degradation of PIL5 protein to promote seed germination through gibberellin in Arabidopsis. Plant Journal 47(1): 124-139.

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Osterlund MT, Hardtke CS, Wei N, Deng XW. 2000. Targeted destabilization of HY5 during light-regulated development of Arabidopsis. Nature 405(6785): 462-466.

Park J, Lee N, Kim W, Lim S, Choi G. 2011. ABI3 and PIL5 collaboratively activate the expression of SOMNUS by directly binding to its promoter in imbibed Arabidopsis seeds. Plant Cell 23(4): 1404-1415.

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Peng J, Carol P, Richards DE, King KE, Cowling RJ, Murphy GP, Harberd NP. 1997. The Arabidopsis GAI gene defines a signaling pathway that negatively regulates gibberellin responses. Genes and Development 11(23): 3194-3205.

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Penfield S, Li Y, Gilday AD, Graham S, Graham IA. 2006. Arabidopsis ABA INSENSITIVE4 regulates lipid mobilization in the embryo and reveals repression of seed germination by the endosperm. Plant Cell 18: 1887–1899

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Piskurewicz U, Jikumaru Y, Kinoshita N, Nambara E, Kamiya Y, Lopez-Molina L. 2008. The gibberellic acid signaling repressor RGL2 inhibits Arabidopsis seed germination by stimulating abscisic acid synthesis and ABI5 activity. Plant Cell 20(10): 2729-2745.

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Ravindran P, Verma V, Stamm P, Kumar PP. 2017. A Novel RGL2-DOF6 Complex Contributes to Primary Seed Dormancy in Arabidopsis thaliana by Regulating a GATA Transcription Factor. Molecular Plant 10(10): 1307-1320.

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Rombolá-Caldentey B, Rueda-Romero P, Iglesias-Fernández R, Carbonero P, Oñate-Sánchez L. 2014. Arabidopsis DELLA and two HD-ZIP transcription factors regulate GA signaling in the epidermis through the L1 box cis-element. Plant Cell 26(7):2905-19.

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Saijo Y, Sullivan JA, Wang H, Yang J, Shen Y, Rubio V, Ma L, Hoecker U, Deng XW. 2003. The COP1-SPA1 interaction defines a critical step in phytochrome A-mediated regulation of HY5 activity. Genes and Development 17:2642–2647.

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Sanchez-Montesino R, Bouza-Morcillo L, Marquez J, Ghita M, Duran-Nebreda S, Gomez L, Holdsworth MJ, Bassel G, Onate-Sanchez L. 2019. A regulatory module controlling GA-mediated endospermic expansion is critical for seed germination in Arabidopsis. Molecular Plant 12(1): 71-85.

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Seo M, Nambara E, Choi G, Yamaguchi S. 2009. Interaction of light and hormone signals in germinating seeds. Plant Molecular Biology 69(4): 463-472.

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Shi H, Zhong S, Mo X, Liu N, Nezames CD, Deng XW. 2013. HFR1 sequesters PIF1 to govern the transcriptional network underlying light-initiated seed germination in Arabidopsis. Plant Cell 25(10): 3770-3784.

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Shimada A, Ueguchi-Tanaka M, Nakatsu T, Nakajima M, Naoe Y, Ohmiya H, Kato H, Matsuoka M. 2008. Structural basis for gibberellin recognition by its receptor GID1. Nature 456(7221): 520-523.

Stacey MG, Hicks SN, Von Arnim AG. 1999. Discrete domains mediate the light-responsive nuclear and cytoplasmic localization of Arabidopsis COP1. Plant Cell 11(3): 349-363.

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Stamm P, Ravindran P, Mohanty B, Tan EL, Yu H, Kumar PP. 2012. Insights into the molecular mechanism of RGL2-mediated inhibition of seed germination in Arabidopsis thaliana. BMC Plant Biology 12: 179-179.

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Steber CM, Cooney SE, McCourt P. 1998. Isolation of the GA-response mutant sly1 as a suppressor of ABI1-1 in Arabidopsis thaliana. Genetics 149(2): 509-521.

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Sun T-P. 2010. Gibberellin-GID1-DELLA: A pivotal regulatory module for plant growth and development. Plant Physiology 154(2): 567.

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Tyler L, Thomas SG, Hu J, Dill A, Alonso JM, Ecker JR, Sun T-P. 2004. DELLA proteins and gibberellin-regulated seed germination and floral development in Arabidopsis. Plant Physiology 135(2): 1008.

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Ueguchi-Tanaka M, Ashikari M, Nakajima M, Itoh H, Katoh E, Kobayashi M, Chow TY, Hsing YI, Kitano H, Yamaguchi I, et al. 2005. GIBBERELLIN INSENSITIVE DWARF1 encodes a soluble receptor for gibberellin. Nature 437(7059): 693-698.

Voinnet O, Rivas S, Mestre P, Baulcombe D. 2003. An enhanced transient expression system in plants based on suppression of gene silencing by the p19 protein of tomato bushy stunt virus. Plant Journal 33(5):949-56

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Wei N, Chamovitz DA, Deng XW. 1994. Arabidopsis COP9 is a component of a novel signaling complex mediating light control of development. Cell 78:117–124.

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Wei N, Deng XW. 2003. The COP9 signalosome. Annual Review of Cell and Developmental Biology 19:261-86.

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Weitbrecht K, Müller K, Leubner-Metzger G. 2011. First off the mark: early seed germination. Journal of Experimental Botany 62(10): 1-14.

3289-3309.

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Wen CK, Chang C. 2002. Arabidopsis RGL1 encodes a negative regulator of gibberellin responses. Plant Cell 14(1):87-100.

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Xu X, Paik I, Zhu L, Bu Q, Huang X, Deng XW, Huq E. 2014. PHYTOCHROME INTERACTING FACTOR1 enhances the E3 ligase activity of CONSTITUTIVE PHOTOMORPHOGENIC1 to synergistically repress photomorphogenesis in Arabidopsis. Plant Cell 26(5): 1992.

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Yadukrishnan P, Rahul PV, Datta S. 2020. HY5 suppresses, rather than promotes, abscisic acid-mediated inhibition of postgermination seedling development. Plant Physiology 184(2): 574–578.

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Yadukrishnan P, Rahul PV, Ravindran N, Bursch K, Johansson H, Datta, S. 2020. CONSTITUTIVELY PHOTOMORPHOGENIC1 promotes ABA-mediated inhibition of post-germination seedling establishment. Plant Journal, 103: 481-496.

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Yan A, Wu M, Yan L, Hu R, Ali I, Gan Y. 2014. AtEXP2 is involved in seed germination and abiotic stress response in Arabidopsis. PLoS One 9: e85208.

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Yu JW, Rubio V, Lee NY, Bai S, Lee SY, Kim SS, Liu L, Zhang Y, Irigoyen ML, Sullivan JA, et al. 2008. COP1 and ELF3 control circadian function and photoperiodic flowering by regulating GI stability. Molecular Cell 32(5): 617-630.

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Yu Y, Wang J, Shi H, Gu J, Dong J, Deng XW, Huang R. 2016. Salt stress and ethylene antagonistically regulate nucleocytoplasmic partitioning of COP1 to control seed germination. Plant Physiology 170(4): 2340-2350.

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Zhong C, Xu H, Ye S, Wang S, Li L, Zhang S, Wang X. 2015. Gibberellic acid-stimulated Arabidopsis6 serves as an integrator of gibberellin, abscisic acid, and glucose signaling during seed germination in Arabidopsis. Plant Physiology 169(3): 2288-2303.

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Zhu L, Bu Q, Xu X, Paik I, Huang X, Hoecker U, Deng XW, Huq E. 2015. CUL4 forms an E3 ligase with COP1 and SPA to promote light-induced degradation of PIF1. Nature Communications 6: 7245.

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)