PCP
PLANT & CELL PHYSIOLOGY

Loss of *Arabidopsis thaliana* Seed Dormancy is Associated with Increased Accumulation of the GID1 GA Hormone Receptors

Amber L. Hauvermale[1], Keiko M. Tuttle[2], Yumiko Takebayashi[3], Mitsunori Seo[3] and Camille M. Steber[1,2,4,*]

[1]Department of Crop and Soil Science, Washington State University, Pullman, WA 99164-6420, USA
[2]Molecular Plant Sciences Program, Washington State University, Pullman, WA 99164-6420, USA
[3]RIKEN, Center for Sustainable Resource Sciences, Yokohama, Kanagawa, Japan
[4]USDA-ARS, Wheat Genetics, Quality, Physiology, and Disease Research Unit, Pullman, WA, USA
*Corresponding author: E-mail, csteber@wsu.edu; Fax, (509)335-2553.
(Received December 4, 2014; Accepted June 2, 2015)

Regular Paper

Dormancy prevents seeds from germinating under favorable conditions until they have experienced dormancy-breaking conditions, such as after-ripening through a period of dry storage or cold imbibition. Abscisic acid (ABA) hormone signaling establishes and maintains seed dormancy, whereas gibberellin (GA) signaling stimulates germination. ABA levels decrease and GA levels increase with after-ripening and cold stratification. However, increasing GA sensitivity may also be critical to dormancy loss since increasing seed GA levels are detectable only with long periods of after-ripening and imbibition. After-ripening and cold stratification act additively to enhance GA hormone sensitivity in *ga1-3* seeds that cannot synthesize GA. Since the overexpression of the GA receptor *GID1* (*GIBBERELLIN-INSENSITIVE DWARF1*) enhanced this dormancy loss, and because *gid1a gid1b gid1c* triple mutants show decreased germination, the effects of dormancy-breaking treatments on *GID1* mRNA and protein accumulation were examined. Partial after-ripening resulted in increased *GID1b*, but not *GID1a* or *GID1c* mRNA levels. Cold imbibition stimulated the accumulation of all three *GID1* transcripts, but resulted in no increase in GA sensitivity during *ga1-3* seed germination unless seeds were also partially after-ripened. This is probably because after-ripening was needed to enhance GID1 protein accumulation, independently of transcript abundance. The rise in *GID1b* transcript with after-ripening was not associated with decreased ABA levels, suggesting there is ABA-independent *GID1b* regulation by after-ripening and the 26S proteasome. GA and the DELLA *RGL2* repressor of GA responses differentially regulated the three *GID1* transcripts. Moreover, DELLA *RGL2* appeared to switch between positive and negative regulation of *GID1* expression in response to dormancy-breaking treatments.

Keywords: After-ripening • DELLA • Dormancy • Gibberellin • GID1 • Seed.

Abbreviations: ANOVA, analysis of variance; ABA, abscisic acid; CUL1, CULLIN1; DELLA, Asp-Glu-Leu-Leu-Ala; ESI-LC-MS/MS, electrospray ionization liquid chromatography tandem mass spectrometry; GID1, *GIBBERELLIN INSENSITIVE DWARF1*; gibberellin A4 (GA^4); HA:GID1-OE, overexpression of HA-tagged GID1 using the constitutive 35S promoter; JA, jasmonic acid; JA-Ile, jasmonic acid isoleucine; MS, Murashige and Skoog; PIF, PHYTOCHROME INTERACTING FACTOR; RT–qPCR, reverse transcription–quantitative PCR; RGA, REPRESSOR OF GA1-3; RGL2, RGA-LIKE2; SLY1, SLEEPY1; WRI1, WRINKLED1; WT, wild type.

Introduction

The evolution of seeds and seed dormancy were critical to the success of land plants by providing a mechanism to ensure species dispersal and survival in changing environments. Dormant seeds cannot germinate under favorable environmental conditions, thus allowing time for dispersal, preventing germination out of season and ensuring survival of natural catastrophes as seeds in the soil (reviewed in Bewley and Black 2013). Seed germination begins with water uptake during imbibition and ends with radical emergence. Seeds acquire the ability to germinate through dormancy-breaking processes including after-ripening (a period of dry storage) and cold stratification (moist chilling or cold imbibition). Dormancy loss is associated with decreasing levels of the seed dormancy hormone ABA, and increasing levels of the germination-promoting hormone gibberellin (GA) (Ali-Rachedi et al. 2004, Yamauchi et al. 2004, Ariizumi et al. 2013). Because dormancy loss is also associated with increasing GA hormone sensitivity, this study explored the effects of dormancy-breaking treatments on the regulation of the Arabidopsis GA receptors *GIBBERELLIN INSENSITIVE DWARF1* (*GID1a*, *GID1b* and *GID1c*) during seed imbibition.

Classic studies postulated that seed dormancy and germination are controlled by the balance between the opposing effects of ABA and GA, based on data showing that the ratio of the ABA to GA signal correlates with the degree of seed dormancy (Koornneef et al. 1980, Koornneef et al. 1982, Karssen and Lacka 1986, Karssen et al. 1989). Environmental stimuli, such as changes in light and temperature, appear to alter germination potential through changes in ABA and GA levels or sensitivity (Derkx et al. 1994, Yamauchi et al. 2004,

Plant Cell Physiol. 56(9): 1773–1785 (2015) doi:10.1093/pcp/pcv084, Advance Access publication on 1 July 2015, available online at www.pcp.oxfordjournals.org

Oh et al. 2007, Seo et al. 2009). GA biosynthetic mutants of Arabidopsis and tomato, *ga1-1* and *gib-1*, germinate only when GA hormone is applied. The GA-rescued phenotypes of these mutants, including failure to germinate, dwarfism and infertility, indicated that GA is required for seed germination, stem elongation and fertility. The ABA biosynthetic mutant *aba1-1* rescued the germination of *ga1-1*, an allele of the GA biosynthesis enzyme ent-copalyl synthase (CPS; Sun and Kamiya, 1994), leading to the conclusions that ABA is required to induce seed dormancy, and that GA is not required for germination if seeds do not become dormant in the first place. Further characterization suggested that ABA and GA may have opposing effects on seed germination in different tissues and at different times during development. ABA induces seed dormancy during embryo maturation, and maintains dormancy in mature seeds (Karssen et al. 1983, Karssen and Lacka 1986, Karssen et al. 1989, Kushiro et al. 2004, Okamoto et al. 2006). GA stimulates nutrient reserve mobilization, loosening of barrier tissues in the seed coat, and embryo expansion at the time of germination (Groot and Karssen 1987, Steber et al. 1998, Piskurewicz et al. 2008, Lee et al. 2010). However, the role of GA in seed dormancy-breaking treatments such as after-ripening and cold stratification is less clear.

The relative roles of ABA and GA in controlling dormancy loss have been somewhat controversial. Dormancy loss by dry after-ripening and cold stratification were correlated with increased ABA turnover due to increased ABA 8′-hydroxylase expression (Ali-Rachedi et al. 2004, Kushiro et al. 2004, Millar et al. 2006, Okamoto et al. 2006, Barrero et al. 2009). However, reduced ABA signaling is not the sole determinant of dormancy release since transcriptome analysis showed that not all changes in gene expression with after-ripening were ABA dependent in Arabidopsis (Carrera et al. 2008). If GA acts during dormancy loss, then we would expect to see a rise in GA hormone levels. A rise in bioactive GA levels was detected during Arabidopsis cold stratification, but no increase was observed with after-ripening until seeds were imbibed for at least 12 h (Ogawa et al. 2003, Yamauchi et al. 2004, Ariizumi et al. 2013). A significant increase in GA precursors was detected with barley after-ripening, suggesting that there is increased potential to synthesize GA (Jacobsen et al. 2002). While it is difficult to detect increased GA levels with after-ripening, it is clear that GA is required for dormancy loss since after-ripening and cold imbibition do not rescue *ga1-1* germination, and since complete rescue of the *ga1-1* germination by *aba1-1* required some after-ripening (Karssen and Lacka 1986). Moreover, the *ga1-1* biosynthesis mutant does respond to dormancy loss, since it responds to both cold stratification and after-ripening with increased GA hormone sensitivity (Karssen and Lacka 1986, Karssen et al. 1989, Derkx and Karssen 1993). This increase in GA sensitivity may result from decreasing ABA hormone levels or from increased expression of GA signaling genes such as the GA receptors.

GA binding to the GID1 receptor stimulates GA responses through the targeted degradation of negative regulators named for the conserved protein motif DELLA (Asp-Glu-Leu-Leu-Ala) (Ueguchi-Tanaka et al. 2005, Ueguchi-Tanaka and Matsuoka 2010, Hauvermale et al. 2012). GA binding causes a conformational change in GID1, resulting in formation of a DELLA-binding surface (Murase et al. 2008, Shimada et al. 2008). The positive regulator of GA responses, SLEEPY1 (SLY1), is the F-box component of the SCF^{SLY1} E3 ubiquitin ligase that recognizes the GID1–GA–DELLA complex and ubiquitinates DELLA, thereby targeting it for destruction via the 26S proteasome. GA-stimulated DELLA destruction lifts DELLA repression of GA responses including seed germination (Tyler et al. 2004, Ariizumi and Steber 2007).

Mutations in GA signaling genes alter seed dormancy (reviewed by Finkelstein et al. 2008). The *sly1* mutant and *gid1a gid1b gid1c* multiple mutants result in either increased seed dormancy or the inability to germinate unless the seed coat is cut (Steber et al. 1998, Griffiths et al. 2006, Ariizumi and Steber 2007, Iuchi et al. 2007, Willige et al. 2007, Voegele et al. 2011). This increased seed dormancy is associated with increased accumulation of DELLA repressor proteins. Loss-of-function mutations in the five Arabidopsis DELLA genes act additively to rescue the germination of the GA biosynthesis mutant *ga1-3* by relieving DELLA repression of seed germination (Tyler et al. 2004, Cao et al. 2005, Piskurewicz et al. 2008). Mutations in DELLA *RGA-LIKE2* (*RGL2*) have the strongest effect, sufficient to rescue *ga1-3* germination in the light, indicating that RGL2 is the main DELLA repressing seed germination. The failure to germinate in *sly1-2* can be rescued either by long after-ripening or by *GID1* gene overexpression (Ariizumi and Steber 2007, Ariizumi et al. 2013). These results have two implications; (i) GID1 can down-regulate DELLA repressors in the absence of DELLA destruction; and (ii) after-ripening may relieve seed dormancy via increased GID1 and GA levels in *sly1* mutants. This, together with the fact that *GID1a*, *GID1b* and *GID1c* are expressed in imbibing seeds, raises the question as to whether after-ripening and cold stratification lift dormancy in part through increased GID1 accumulation associated with increased GA sensitivity (Griffiths et al. 2006, Voegele et al. 2011).

This study revealed that *GID1* expression is up-regulated at the level of transcript and protein accumulation during dormancy loss. The *ga1-3* mutant was used as a tool to examine the regulation of *GID1* expression during dormancy loss in the absence of GA biosynthesis and its feedback regulation. Dormancy loss through cold stratification and after-ripening of the *ga1-3* biosynthesis mutant resulted in increased GA sensitivity. Previous studies compared mRNA levels in dormant and fully after-ripened wild-type (WT) seeds (Cadman et al. 2006, Carrera et al. 2008, Lee et al. 2010). In this study, time points early in after-ripening were used to capture changes in *GID1* mRNA and protein levels during the process of, rather than at the completion of, after-ripening. This study showed that *GID1a*, *GID1b* and *GID1c* mRNA levels all increased with cold stratification, whereas only *GID1b* mRNA levels increased with partial after-ripening. A comparison of *GID1* mRNA levels in *ga1-3* with those in the DELLA mutant *ga1-3 rgl2-1* suggested that *RGL2* switches between negative and positive regulation of *GID1* mRNA levels with dormancy-breaking treatments. Interestingly, the levels of all three GID1 proteins increased

with partial after-ripening, suggesting that increasing GA receptor protein levels may be a critical and early after-ripening event. This increase in HA:GID1 protein levels did not depend on transcript levels or proteasome activity, implying that GID1 may be regulated at the level of protein translation.

Results

After-ripening, cold stratification and *GID1* overexpression increase GA sensitivity in *ga1-3* seeds

This study examines the hypothesis that the increase in GA sensitivity with dormancy loss through cold stratification and after-ripening is due to increased expression of the GID1 GA receptors. If this is true, then *GID1* gene overexpression should mimic the effect of after-ripening by increasing GA sensitivity in imbibing *ga1-3* seeds. Seeds of the *ga1-3* deletion mutant germinate only in the presence of GA hormone in a dose-dependent manner (Koornneef et al. 1980; **Supplementary Fig. S1**). The *ga1-3* allele allowed examination of the changes in GA responsiveness in the absence of endogenous GA biosynthesis. The GA_4 dose–response during germination was compared in untransformed *ga1-3* and in *ga1-3* transformed with *HA:GID1a*, *HA:GID1b* and *HA:GID1c* overexpressed on the constitutive *Cauliflower mosaic virus* 35S promoter (*HA:GID1-OE*; Ariizumi et al. 2008). The *ga1-3 HA:GID1b-OE* line showed a highly significant increase in GA sensitivity compared with *ga1-3* both in dormant seeds imbibed without cold stratification (**Fig. 1A**; $P < 0.0001$), and in seeds after-ripened for 2 months that were cold stratified at 4°C for 3 d before imbibing in the light at 22°C for 5 d (**Fig. 1B**; $P < 0.0001$). Neither *HA:GID1a-OE* nor *HA:GID1c-OE* caused a significant increase in GA sensitivity compared with *ga1-3* in dormant seeds. Even with 2 months after-ripening and cold stratification, *HA:GID1a-OE* and *HA:GID1c-OE* only resulted in a significant increase in germination at 0.5 μM GA_4 ($P < 0.0001$). Thus, *GID1b* overexpression mimicked dormancy-breaking treatments better than either *GID1a* or *GID1c*.

If increased GA signaling triggers dormancy loss, then we would expect to see increasing GA sensitivity in *ga1-3* fairly early in cold stratification and after-ripening. The GA biosynthesis mutant *ga1-1* showed increased GA sensitivity in germination with dormancy loss resulting from either 6 months after-ripening or 7–10 d cold stratification (Karssen and Lacka 1986, Karssen et al. 1989). GA dose–response experiments were conducted to examine the effects of a shorter cold stratification period, and of partial after-ripening alone and in combination with cold stratification, on GA sensitivity in both *ga1-3* and *ga1-3 HA:GID1b-OE* seeds (**Fig. 2**). Only the *HA:GID1b-OE* line was examined because, of the three receptors, *GID1b* overexpression had the strongest effect. To examine the effects of partial after-ripening, a GA dose–response was compared in dormant (2 weeks old) and partially after-ripened (2 months old) *ga1-3* and *ga1-3 HA:GID1b-OE* seeds (**Fig. 2**). To examine the effects of cold stratification on GA dose–response, seeds were either cold stratified at 4°C in the dark for 3 d or not cold stratified before

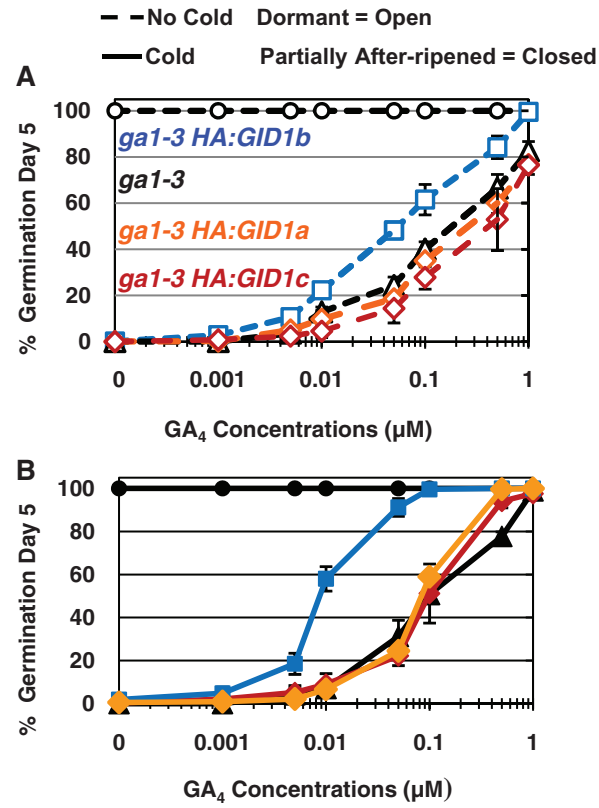


Fig. 1 The effect of 35S:*HA:GID1a* (filled diamonds, orange), 35S:*HA:GID1b* (filled circles, blue) or 35S:*HA:GID1c* (filled diamonds, red) overexpression in *ga1-3* on GA sensitivity during germination compared with untransformed *ga1-3* (filled triangles, black). Germination was scored after 5 d at 22°C in imbibing (A) dormant (open symbols) seeds without cold stratification (dashed lines) and (B) partially after-ripened (2 months after-ripened, filled symbols) seeds after cold stratification at 4°C for 3 d in the dark (solid lines). *HA:GID1a-OE* and *HA:GID1c-OE* significantly increased sensitivity to 0.5 μM GA_4 in (B) ($P < 0.0001$). *HA:GID1b-OE* significantly increased sensitivity to (A) ≥ 0.05 μM and (B) ≥ 0.005 μM GA_4 ($P < 0.0001$). *Ler* WT is shown as a control (filled circles, black). Percentage germination is the mean of three replicates of ≥ 100 seeds. Bars = SD.

incubation in the light for 5 d at 22°C. Cold stratification alone failed to result in a significant increase in GA dose–response in either dormant *ga1-3* or dormant *ga1-3 HA:GID1b-OE* seeds (**Fig. 2A**). With partial after-ripening, cold stratification resulted in a stronger increase in *ga1-3 HA:GID1b-OE* sensitivity (**Fig. 2B**; 0.01–0.1 μM GA_4, $P < 0.0001$). Without cold stratification, partial after-ripening caused a significant increase in GA sensitivity in both *ga1-3* and *ga1-3 HA:GID1b-OE* (**Fig. 2C**; to 0.1 and/or 0.5 μM GA_4, $P < 0.0001$). The effect of partial after-ripening was considerably enhanced by cold stratification in both *ga1-3* and *ga1-3 HA:GID1b-OE* (**Fig. 2D**; $P < 0.0001$). Thus, short periods of cold stratification and after-ripening have additive effects on GA sensitivity that are further enhanced by *GID1b* overexpression.

Next we compared the effects of longer after-ripening with the effects of *HA:GID1b-OE* on *ga1-3* GA sensitivity during germination. The effect of *HA:GID1b-OE* in dormant (2 week after-ripened) seeds without cold stratification was comparable with the increase in *ga1-3* sensitivity with 12 months of

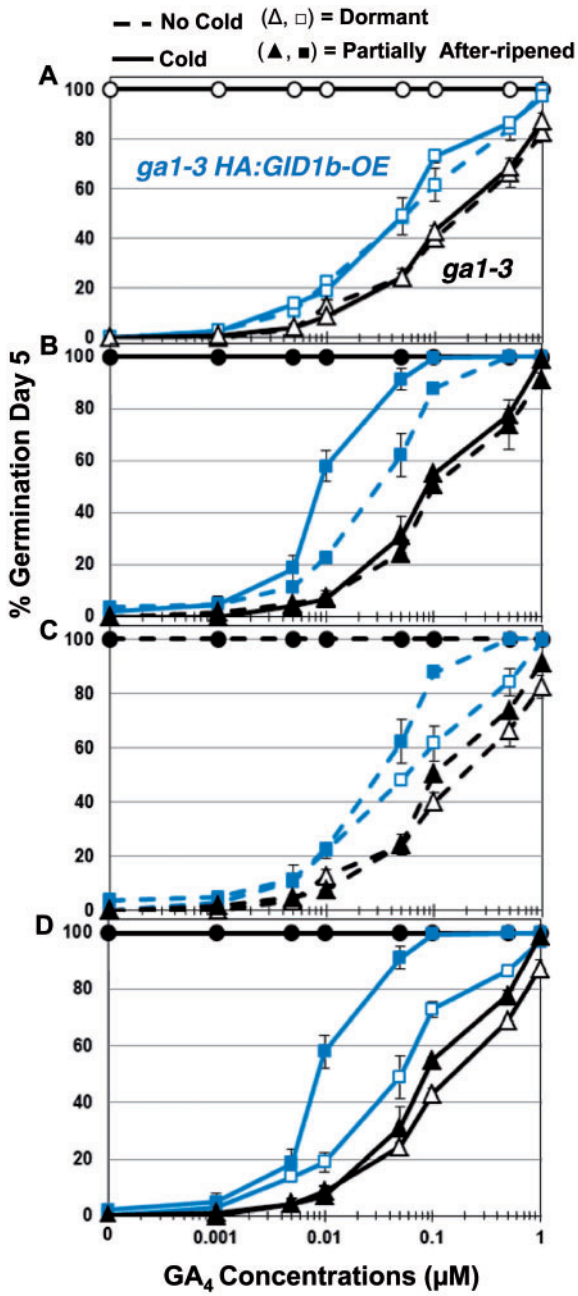


Fig. 2 Cold stratification and partial after-ripening enhance GA sensitivity in *ga1-3*. GA dose–response curves of *ga1-3* (black) and *ga1-3 HA:GID1b-OE* (blue) seeds following a 5 d incubation at 22°C under lights. (A) Dormant seeds (2 weeks after-ripened) either with (solid line) or without (dashed line) a cold stratification pre-treatment for 3 d at 4°C. (B) Partially (2 months) after-ripened seeds with and without cold stratification. (C, D) Dormant (open triangles, open squares) vs. partially after-ripened (filled triangles, filled squares) seeds incubated without (C) or with (D) cold stratification. The wild-type *Ler* control is represented with a circle (open circles, closed circles). Percentage germination is the mean of three replicates of at least 100 seeds. Bars = SD.

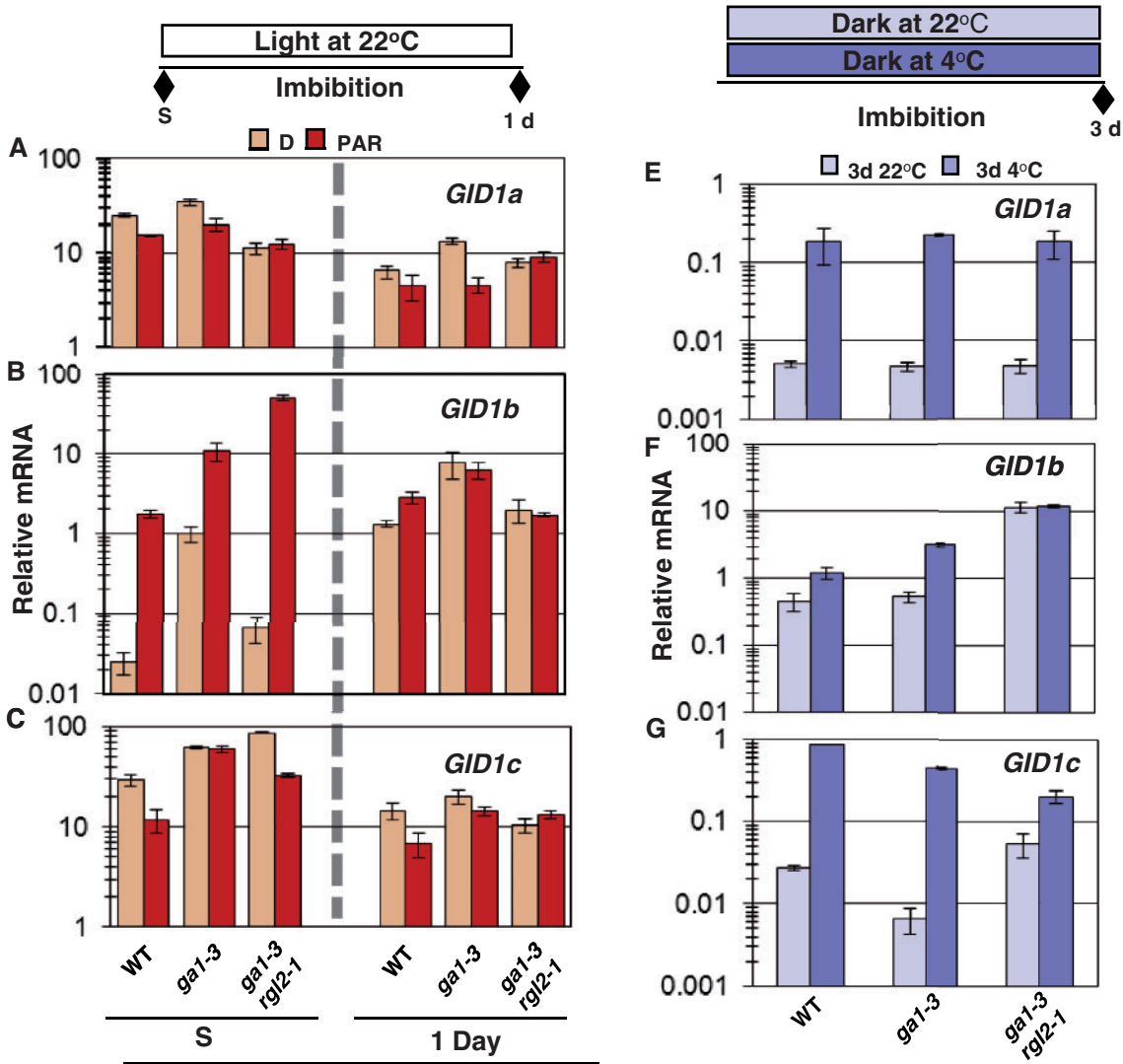
after-ripening. Thus, *GID1b* expression can phenocopy the effect of longer after-ripening. Cold stratification further enhanced the effect of after-ripening on *ga1-3* GA sensitivity (**Supplementary Fig. S2B**).

The observation that *GID1* overexpression enhanced the effects of partial after-ripening and cold stratification on *ga1-3* GA sensitivity raised the question of whether these two dormancy-breaking treatments may function in part by increasing the expression of *GID1a*, *GID1b* and *GID1c*. Thus, a series of experiments were conducted to determine whether dormancy-breaking treatments resulted in increased *GID1* mRNA and/or protein accumulation using the same *ga1-3* seeds characterized in **Fig. 2**. Previous work established that *GID1a* and *GID1b* promoters are direct DELLA REPRESSOR OF GA1-3 (RGA) targets, and that their transcripts are GA down-regulated and DELLA RGA up-regulated in seedlings (Zentella et al. 2007). In order to examine the genetic effects of endogenous GA and of the DELLA *RGL2* repressor of seed germination in each experiment, *GID1* transcript accumulation was compared in imbibing WT L*er*, *ga1-3* and *ga1-3 rgl2-1* double mutant seeds (Cao et al. 2006). If mRNA levels are higher in the WT (endogenous GA) than in *ga1-3* (no GA), then this suggests that the *GID1* transcript is GA up-regulated. If mRNA levels are higher in *ga1-3* than in *ga1-3 rgl2-1*, then this suggests that the *GID1* transcript is DELLA *RGL2* up-regulated.

After-ripening induces *GID1b* transcript accumulation in the absence of GA biosynthesis

Reverse transcription quantitative–PCR (RT–qPCR) was used to compare the accumulation of *GID1a*, *GID1b* and *GID1c* transcripts in dormant (2 week after-ripened) and partially after-ripened (2 months) seeds. Steady-state *GID1* transcript levels were measured immediately following seed sterilization (S for start at 15 min imbibition) and 1 d of imbibition at 22°C in the light in WT, *ga1-3* and *ga1-3 rgl2-1*. Partial after-ripening resulted in a significant increase in *GID1b* mRNA levels at the S time point (P-value < 0.0001; **Fig. 3B**; **Supplementary Fig. S3**); there was a 73-fold increase in the WT, a 10.9-fold increase in *ga1-3* and a 788-fold increase in *ga1-3 rgl2-1*. After-ripening was still associated with higher *GID1b* transcript levels after 1 d of imbibition in WT seeds. In contrast to *GID1b*, after-ripening was associated with a significant decrease in both *GID1a* and *GID1c* mRNA levels in imbibing WT seeds (P-value < 0.05 **Fig. 3A, C**; **Supplementary Figs S4, S5**). While *GID1a* and *GID1c* levels decreased with after-ripening, *GID1a* and *GID1c* transcript levels were initially higher than those of *GID1b* in dormant seeds. Thus, partial after-ripening stimulates *GID1b*, but not *GID1a* or *GID1c* mRNA accumulation.

GID1a, *GID1b* and *GID1c* mRNA accumulation was characterized in ungerminated *ga1-3* seeds further imbibed for up to 7 d at 22°C in the light (**Supplementary Figs. S6–S8**). In dormant seeds, *GID1b* mRNA levels continued to rise through day 3 of imbibition and then declined, whereas *GID1a* and *GID1c* decreased through day 5, and then increased. In partially after-ripened seeds, *GID1a*, *GID1b* and *GID1c* mRNA levels were highest at 15 min, then declined with further imbibition. Thus, *GID1b* mRNA levels increase with imbibition of both dormant and after-ripened *ga1-3* seeds, but partial after-ripening leads to an increase during early imbibition.



D

AR (Time)		D (S)	PAR (S)	D (1d)	PAR (1d)
GID1a	RGL2	UP	UP	UP	DN
	GA	DN	DN	DN	NE
GID1b	RGL2	UP	DN	UP	UP
	GA	DN	DN	DN	DN
GID1c	RGL2	DN	UP	UP	NE
	GA	DN	DN	NE	DN

H

Temp (°C)		22	4
GID1a	RGL2	NE	NE
	GA	NE	NE
GID1b	RGL2	DN	DN
	GA	NE	DN
GID1c	RGL2	DN	UP
	GA	UP	UP

Fig. 3 Differential regulation of the three *GID1* transcripts with after-ripening (A–D) and cold stratification (E–H). (A–C) For after-ripening, dormant (D, pink, 2 weeks after-ripened) and partially after-ripened (PAR, red 2 months) L*er* (WT), *ga1-3* and *ga1-3 rgl2-1* seeds were imbibed without cold stratification at 22°C for 15 min (S = start) and 1 d. (E–G) For cold stratification, dormant seeds were imbibed for 3 d at 22°C or at 4°C in the dark. *GID1a*, *GID1b* and *GID1c* mRNA levels are shown relative to the *IAP-Like Protein 1* constitutive control: *GID1b* in *ga1-3* S time point set to 1. Mean fold change is shown, $n = 3$, bars = SD. (D, H) Endogenous GA and DELLA *RGL2* appear to up- or down (DN)-regulate *GID1* mRNA levels differently, depending on (D) after-ripening stage of D vs. PAR and (H) cold stratification—whether or not seeds were incubated for 3 d at 22°C or at 4°C. NE indicates no effect.

GA and DELLA *RGL2* regulation of *GID1* mRNA levels was examined by comparing *ga1-3* (no GA) with L*er* WT (endogenous GA present) and with *ga1-3 rgl2-1* (germination rescued by lack of DELLA *RGL2*) at the S and 1 d time points (summarized in **Fig. 3D**). Consistent with previous research, GA generally down-regulated *GID1a*, *GID1b* and *GID1c* mRNA levels (**Fig. 3A–D**; **Supplementary Fig. S3–S5**; Zentalla et al. 2007). Interestingly, the effect of DELLA *RGL2* switched with dormancy loss. DELLA *RGL2* up-regulated *GID1b* mRNA levels in dormant seeds, but down-regulated them in after-ripened seeds (S time point; **Fig. 3B, D**; **Supplementary Fig. S3**). Similarly, DELLA *RGL2* up-regulated *GID1a* in dormant and down-regulated *GID1a* in after-ripened seeds (1 d imbibition; **Fig. 3A, D**; **Supplementary Fig. S4**). Conversely, DELLA *RGL2* down-regulated *GID1c* in dormant seeds, but up-regulated it in after-ripened seeds (S time point, **Fig. 3C, D**; **Supplementary Fig. S5**).

The effects of temperature, GA and light on *GID1* transcript accumulation

To examine the effect of cold imbibition, *GID1* mRNA levels were compared in dormant seeds imbibing: (i) in the dark for 3 d at 22°C (not cold stratified); and (ii) in the dark for 3 d at 4°C (cold stratified) (**Fig. 3E–G**; **Supplementary Fig. S9A–C**). Cold stratification resulted in significantly higher levels of all three *GID1* transcripts. *GID1a* levels were 49-fold, *GID1b* 6-fold and *GID1c* 69-fold higher with cold stratification ($P < 0.0015$).

Again, GA and DELLA *RGL2* regulation of *GID1* mRNA levels was examined by comparing *ga1-3* with the WT and with *ga1-3 rgl2-1* (**Fig. 3E–H**; **Supplementary Fig. S9A–C**). GA had no apparent effect on *GID1a* mRNA levels in the WT vs. *ga1-3* following 3 d incubation at 22 or 4°C. However, GA down-regulated *GID1b* at 4°C, and up-regulated *GID1c* at both 22 and 4°C. GA up-regulation of *GID1c* levels during imbibition in the dark contrasts strongly with GA down-regulation of *GID1a*, *GID1b* and *GID1c* during light imbibition in the after-ripening experiment (**Fig. 3A–C**). Since light stimulates GA hormone accumulation, GA down-regulation of *GID1* transcripts during light imbibition may represent a negative feedback response to consistently elevated GA hormone levels (Seo et al. 2009). To examine the effect of light vs. dark in the absence of GA biosynthesis, *GID1* transcript levels were compared in *ga1-3* seeds imbibed in the light and in the dark for 3 d at 22°C (**Fig. 4**; **Supplementary Fig. S10**). The levels of *GID1a* were 3,604-fold, *GID1b* 57-fold and *GID1c* 697-fold higher in the light than in the dark, respectively ($P < 0.0001$). This increase in *GID1* mRNA levels in the light did not require GA biosynthesis.

It appears that continuously high GA levels in the WT, as in the light, can cause feedback inhibition of *GID1* mRNA levels. Thus, the effect of 1 μM GA_4 treatment on *GID1* transcript accumulation was examined in *ga1-3* because it lacks the endogenous GA needed to trigger feedback inhibition under three conditions: (i) imbibed 3 d in the dark at 22°C; (ii) imbibed 3 d in the dark at 4°C; and (iii) imbibed 3 d in the dark at 4°C followed by 1 d in the light at 22°C (**Supplementary Figs. S11**, **S12**). GA treatment resulted in up-regulation of *GID1a*, *GID1b* and *GID1c* mRNA levels after 3 d imbibition in the dark regardless of temperature (**Supplementary Fig. S11A**, **C**). However, after 1 d in the light, GA treatment caused down-regulation of *GID1a* and *GID1b*, but up-regulation of *GID1c* (**Supplementary Fig. S11B**, **C**).

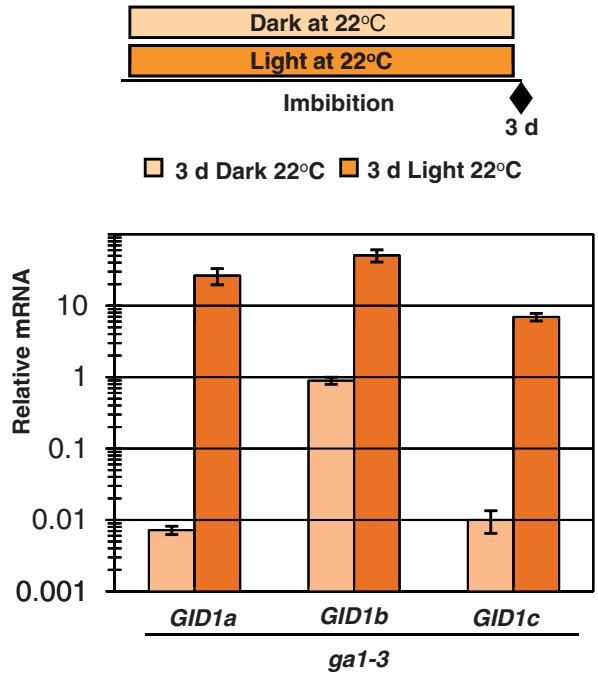


Fig. 4 The effect of dark and light imbibition on *GID1* mRNA levels in dormant (2 weeks after-ripened) *ga1-3* seeds. All seeds were imbibed 3 d at 22°C in the dark, or at 22°C in the light. RT–qPCR analysis was used to measure *GID1* mRNA levels relative to the *IAP-Like Protein 1* constitutive control ($n = 3$). The average fold change at the indicated imbibition time points is expressed relative to the level of *GID1b* in dormant, non-stratified *ga1-3* at S (set to 1). Bars = SD.

HA:GID1 protein accumulation increases with partial after-ripening

Next we examined whether partial after-ripening can regulate GID1 protein accumulation. Tagged HA:GID1a, HA:GID1b and HA:GID1c protein accumulation was examined when expressed on the constitutive 35S promoter in *ga1-3*, so that the effect of after-ripening on accumulation of each individual GID1 protein could be examined independently of the transcriptional regulation of their natural promoters. For RT–qPCR and protein blot analysis, seeds were cold stratified at 4°C for 3 d in the dark (0 d time point), and then sampled daily over 7 d imbibition under lights at 22°C.

HA:GID1 protein levels were examined by protein blot analysis with anti-HA in dormant and 2 month after-ripened *ga1-3 HA:GID1-OE* seeds. HA:GID1a, HA:GID1b and HA:GID1c protein levels were very low but detectable in imbibing dormant seeds, but increased dramatically in partially after-ripened seeds (**Fig. 5**; **Supplementary Fig. S16A**). HA:GID1a protein levels remained constant with further imbibition of partially after-ripened seeds, whereas HA:GID1b and HA:GID1c protein

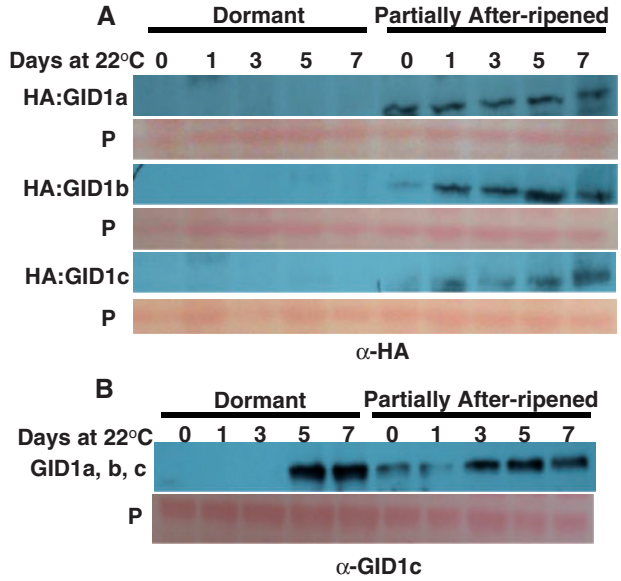


Fig. 5 Increased HA:GID1 and native GID1 protein accumulation with partial after-ripening of *ga1-3* seeds. Dormant (2 weeks after-ripened) and partially (2 months) after-ripened seeds were cold stratified in the dark at 4°C for 3 d, and then imbibed in the light at 22°C for 0–7 d. (A) HA:GID1a, HA:GID1b and HA:GID1c were detected in *ga1-3 HA:GID1-OE* seeds with anti-HA. A 60 μg aliquot of total protein was loaded per lane. (B) All three native GID1 proteins were detected as a single band in untransformed *ga1-3* seeds detected with anti-GID1c. A 20 μg aliquot of total protein was loaded per lane. The loading controls are Ponceau (P)-stained blots.

levels continued to increase with imbibition over 7 d in the light. This increase in HA:GID1 protein levels with after-ripening was not due to increasing mRNA since the steady-state *35S:HA:GID1* mRNA levels did not increase with partial after-ripening, but decreased 3- to 10 fold with 3–7 d of imbibition in the light (**Supplementary Figs. S13–S15**).

To examine whether the apparent regulation of HA:GID1 protein accumulation by seed dormancy and after-ripening might be an artifact resulting from the use of HA-tagged fusions, the same experiment was repeated using an antibody raised against Arabidopsis GID1c. Anti-GID1c detected GID1a, GID1b and GID1c as a single band that was absent in the *gid1a-1 gid1b-1 gid1c-2* triple mutant, but not in double mutants, showing specificity for GID1 protein in Arabidopsis (**Supplementary Fig. S16B**). GID1 protein was undetectable with 0, 1 and 3 d of imbibition in dormant *ga1-3* seeds, but present with 0, 1 and 3 d of imbibition of 2 month after-ripened *ga1-3* seeds (**Fig. 5B**). GID1 protein levels increased with 5 and 7 d imbibition in both dormant and after-ripened *ga1-3*. Thus, partial after-ripening stimulates the accumulation of untagged GID1 proteins during the first 3 d of imbibition.

It is possible that all seed proteins in Arabidopsis increase with partial after-ripening. To examine this possibility, protein accumulation of seed-expressed proteins CULLIN1 (CUL1) and DELLA RGL2 were measured under the same conditions in *ga1-3 HA:GID1b-OE* seeds. No apparent difference in CUL1 or DELLA RGL2 protein levels was observed in imbibing dormant and partially after-ripened seeds (**Supplementary Fig. S16C**). These experiments suggest that there is specific up-regulation of GID1 protein levels due to post-transcriptional regulation with partial after-ripening.

Low HA:GID1b protein accumulation in dormant seeds does not depend upon the 26S proteasome

If after-ripening up-regulates HA:GID1 protein accumulation through decreased degradation by the 26S proteasome, then we would expect treatment with the proteasome inhibitor MG132 to result in increased HA:GID1b protein accumulation in dormant imbibing seeds. Total protein was extracted from dormant (2 weeks) and partially after-ripened (2 months) *ga1-3 HA:GID1b* seeds imbibed for 3 d in the dark at 4°C without or with 100 μM MG132. Protein blot analysis demonstrated that MG132 treatment of dormant and after-ripened *ga1-3 HA:GID1b* seeds did not result in an apparent increase in HA:GID1b protein accumulation (**Fig. 6**). To determine whether MG132 can penetrate imbibing seeds under these conditions, we also examined a known proteasome-regulated protein, WRINKLED1 (WRI1) (Chen et al. 2013). WRI1 protein levels increased with MG132 treatment of both dormant and partially after-ripened seeds. Thus, higher HA:GID1b protein levels in partially after-ripened *ga1-3 HA:GID1b* seeds cannot be explained by proteasomal degradation of HA:GID1b protein in dormant seeds.

The effect of partial after-ripening on plant hormone accumulation

It was previously suggested that the increase in GA sensitivity with after-ripening may be due to a decrease in ABA hormone levels (Karssen and Lacka 1986). If so, then we would expect increased GA sensitivity with partial after-ripening to correlate not only with increased GID1 protein levels, but also with decreased ABA accumulation. Dormancy loss through after-ripening might also result in altered accumulation of other hormones implicated in seed germination (Jacobsen et al. 2013, Liu et al. 2013). To address this, hormone profiling measured ABA, auxin (IAA), jasmonic acid (JA) and jasmonic acid-isoleucine (JA-Ile) levels in dormant and 2 month after-ripened WT, *ga1-3* and *ga1-3 HA:GID1b-OE* seeds imbibed for 3 d at 4°C in the dark, followed by 12 h at 22°C in the light (**Fig. 7**; **Supplementary Figs. S17**, **S18**). Surprisingly, there was no significant decrease in ABA levels either with *HA:GID1b-OE* or with partial after-ripening (**Fig. 7A**). ABA levels were lower in *ga1-3* compared with WT seeds, suggesting that the reduced germination capacity of *ga1-3* was not due to increased ABA accumulation. IAA, JA and JA-Ile all accumulated at lower levels in *ga1-3* lines compared with the WT (**Fig. 7B**; **Supplementary Fig. S17**). Neither IAA nor JA levels changed significantly with after-ripening. However, JA-Ile (P-value < 0.0001) levels were higher in partially after-ripened than in dormant *ga1-3 HA:GID1b-OE*.

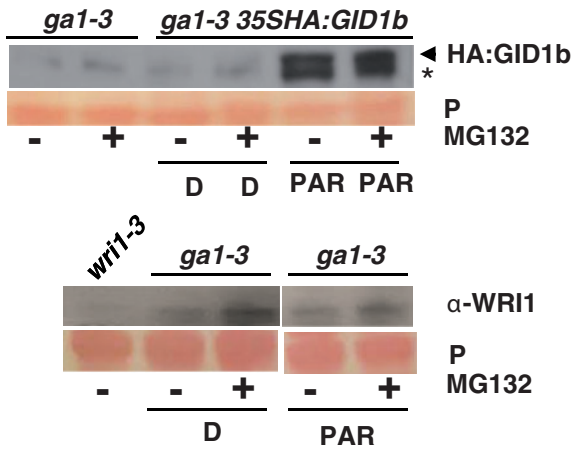


Fig. 6 Proteasome inhibitor MG132 does not increase HA:GID1b protein levels in dormant seeds. Dormant (D) and partially after-ripened (PAR) *ga1-3 HA:GID1b-OE* seeds were cold imbibed with and without MG132 in the dark at 4°C for 3 d. A 60 µg aliquot of total protein was loaded. The arrow indicates HA:GID1b protein and the asterisk indicates a non-specific band detected with α-HA. The proteasome-regulated WRI protein showed increased accumulation with MG132 treatment of D and PAR *ga1-3* seeds. The loading control is a Ponceau (P)-stained blot.

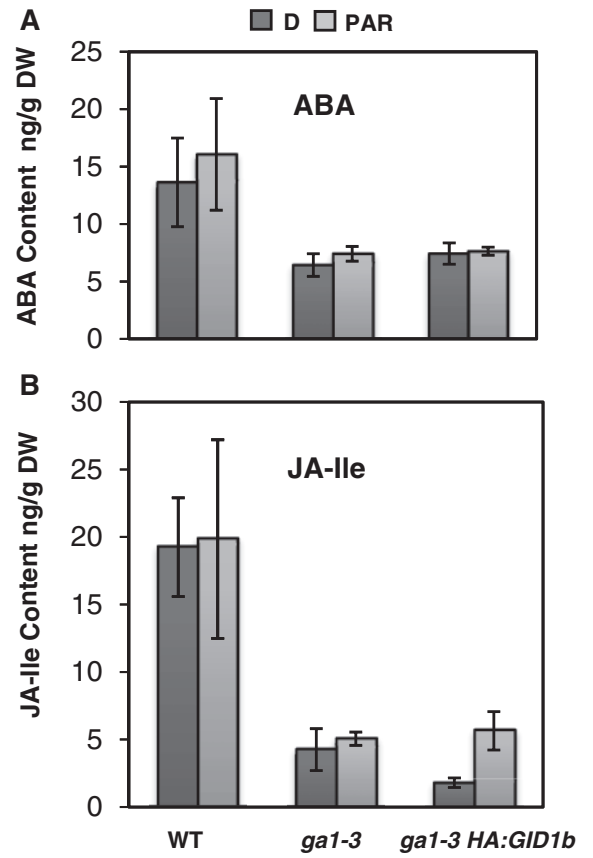


Fig. 7 The effect of partial after-ripening on endogenous ABA and JA-Ile levels in imbibed L*er* WT, *ga1-3* and *ga1-3 HA:GID1b-OE* seeds. Dormant (2 weeks after-ripened) and partially (2 months) after-ripened seeds were imbibed on MS agar in the dark at 4°C for 60 h, and then incubated at 22°C in the light for 12 h before harvesting for hormone measurement by ESI-LC-MS/MS. $n = 6$, and letters indicate statistically significant differences of < 0.07.

Discussion

After-ripening and cold stratification act additively to increase GA sensitivity in the absence of GA biosynthesis

Classic studies demonstrated that both after-ripening and cold stratification cause an increase in GA sensitivity in imbibing *ga1-3* seeds (**Fig. 2**; Karssen and Lacka 1986, Karssen et al. 1989, Derkx and Karssen 1993). Using shorter after-ripening (2 months vs. 6 months) and cold stratification (3 d vs. 7–10 d) treatments than those previously reported, we found that after-ripening and cold stratification act additively to increase GA sensitivity in *ga1-3* (**Fig. 2**). The dormancy-breaking effects of after-ripening and cold stratification were enhanced by the overexpression of the GA receptors, with *HA:GID1b-OE* showing the strongest effect on germination (**Fig. 1**). The stronger effect of *HA:GID1b-OE* on dormancy loss does not appear to be due to higher expression levels. Although 35S:*HA:GID1a*, 35S:*HA:GID1b* and 35S:*HA:GID1c* mRNA levels showed similar trends over the imbibition time course, *HA:GID1b* mRNA levels were significantly lower than both *HA:GID1a* and *HA:GID1c* at all time points ($P < 0.0001$; **Supplementary Fig. S13**). Moreover, HA:GID1b protein levels did not appear to be significantly higher than those of HA:GID1a, suggesting that the phenotypic difference is due to functional differences in the protein (**Fig. 5**). For example, GID1b has higher GA affinity and some limited capacity to bind DELLA protein in the absence of GA (Nakajima et al. 2006, Yamamoto et al. 2010).

The relative importance of the *GID1* genes in seed dormancy loss is less clear based on loss-of-function phenotypes. Because the three *GID1* genes are partly redundant in function, only the *gid1a gid1b gid1c* triple mutant shows a complete failure to germinate unless the seed coat is cut (Willige et al. 2007). Of the three *gid1* single mutants, only *gid1b* resulted in decreased GA sensitivity during seed germination (Iuchi et al. 2007). Of the *gid1* double mutants, *gid1a gid1c* had the highest seed dormancy, followed by *gid1b gid1c* and then by *gid1a gid1b*, suggesting that *GID1c* plays an important role in WT seed dormancy release (Voegele et al. 2011). The *SLY1* gene functions in seed germination based on the fact that GA-insensitive *sly1* mutants exhibit increased seed dormancy, and the *SLY1* transcript is induced during imbibition of after-ripened seeds (Ariizumi et al. 2007, Lee et al. 2010). The *sly1-2* mutant requires 2 years to after-ripen (Ariizumi et al. 2007, Ariizumi et al. 2013). The *gid1a-1* and *gid1b-1* alleles prevented *sly1-2* after-ripening, whereas the *gid1c-2* mutation only slowed after-ripening. The *sly1-2* mutant cannot destroy DELLA repressors of seed germination via the ubiquitin–proteasome pathway. Thus, it appears that *GID1c* is more important when DELLA is down-regulated via proteolysis than when DELLA cannot be destroyed. Future work should further examine if the three *GID1* genes have specialized functions.

GID1a, *GID1b* and *GID1c* transcripts are regulated by after-ripening and cold stratification

This study is the first in-depth characterization of *GID1* transcript regulation with dormancy-breaking treatments. A previous transcript profiling experiment using Arabidopsis seeds undergoing dormancy cycling under field conditions found that increasing *GID1a* transcript levels correlated with conditions leading to higher germination capacity (Footitt et al. 2011). However, no previous study has compared the effects of cold stratification and after-ripening on each of the three *GID1* transcripts. Microarray studies that examined *GID1* transcript levels with longer imbibition and after-ripening times did not detect an increase in *GID1* transcript levels (Cadman et al. 2006, Carrera et al. 2008). This study found changes early in imbibition of partially after-ripened seeds. Dormancy-breaking treatments leading to increased seed germination potential and GA sensitivity in *ga1-3* were often associated with increased *GID1* mRNA levels during early imbibition (summarized in **Fig. 8**). Both cold stratification and light stimulated the accumulation of all three *GID1* transcripts (**Figs. 3, 4**). Of the three, *GID1b* transcript levels were highest during cold imbibition. Partial after-ripening (2 months) was associated with a significant increase in *GID1b*, but not *GID1a* and *GID1c* mRNA levels in imbibing seeds (**Fig. 3**). The fact that HA GID1b more strongly and the fact that *HA:GID1b-OE* strongly stimulated GA responsiveness germination is consistent with previous work showing that GID1b has a higher affinity for GA and DELLA protein than the GID1a or GID1c receptors (**Fig. 2**; Nakajima et al. 2006, Voegele et al. 2011, Yamamoto et al. 2010). Thus, increasing GID1b accumulation with after-ripening may result in a stronger increase in GA sensitivity and germination potential, especially under conditions where GA hormone levels are low. While partial after-ripening was associated with a significant increase in relative *GID1b* mRNA levels during early imbibition, future work will need to examine whether these changes result from differential decay of mRNA during dry seed after-ripening or from rapid transcription during imbibition (Bazin et al. 2011).

Differential regulation of GA receptors by GA and DELLA *RGL2*, a regulatory switch

Interestingly, GA and DELLA *RGL2* had different effects on *GID1a*, *GID1b* and *GID1c* transcript levels, and the effects of GA and DELLA *RGL2* switched with dormancy-breaking treatments (**Fig. 3**; **Supplementary Fig. S11**). *GID1a* and *GID1b* promoters are direct DELLA RGA targets, showing GA down-regulated and DELLA up-regulated expression in seedlings (Zentella et al. 2007, Fukazawa et al. 2014, Yoshida et al. 2014). Similarly, *GID1a* and *GID1b* mRNA levels are GA down-regulated and DELLA up-regulated in seeds imbibed for 4 d in the cold (Cao et al. 2006). Consistent with this, all three *GID1* transcripts were GA down-regulated with imbibition in the light (**Fig. 3D**). However, some *GID1* transcripts switched to being GA up-regulated and/or DELLA *RGL2* down-regulated with dormancy loss (**Fig. 3D**, H; **Supplementary Fig. S11**). For example, at 15 min of imbibition, *GID1b* was DELLA *RGL2* up-regulated in both dormant and after-ripened seeds. Conversely, *GID1c* was DELLA *RGL2* down-regulated in dormant seeds but up-regulated in partially after-ripened seeds (**Figs. 3D**, 8). Therefore, the same DELLA gene may act as both a positive and a negative regulator of a *GID1* target. The effects of GA and DELLA *RGL2* also varied with cold stratification. Whereas *GID1b* is GA down-regulated with cold stratification, *GID1c* is GA up-regulated. *GID1c* also appears to switch from being DELLA *RGL2* down-regulated to up-regulated with cold stratification. The three *GID1* genes are clearly differentially regulated, suggesting that they may have specialized functions under different environmental conditions. This study focused on the genetic effects of *rgl2* because it is the only single DELLA mutation that allows *ga1-3* to germinate in the light (Tyler et al. 2004, Cao et al. 2005). However, mutations in the three DELLA genes, *RGL2*, *RGA* and *GAI*, are needed to rescue *ga1-3* germination in the dark (Cao et al. 2005). Future work should also examine if different DELLA genes have different effects on *GID1a*, *GID1b* and *GID1c* regulation during imbibition in the light or dark.

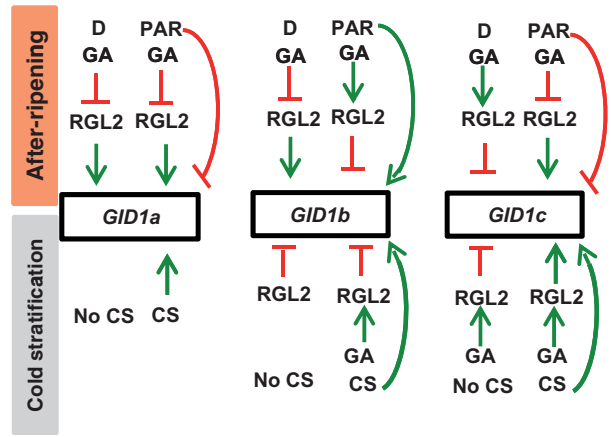


Fig. 8 The three *GID1* genes are differentially regulated by DELLA *RGL2* and dormancy loss. DELLA *RGL2* switched from a negative regulator to a positive regulator or vice versa in response to after-ripening or cold stratification. The diagram summarizes the regulatory effects at the S time point. While cold stratification induced all three *GID1* genes, dry partial after-ripening strongly induced *GID1b* but repressed *GID1a* and *GID1c* mRNA accumulation in imbibing seeds. D, dormant; PAR, partially after-ripened; CS, cold stratified; No CS, no cold stratification.

DELLA RGL2 can switch from positive to negative regulation of the same *GID1* gene in response to dormancy-breaking treatment. Changes in DELLA protein binding partners may result in altered DELLA regulation of *GID1b* transcript levels, and allow DELLA to behave as both an activator and a repressor of gene transcription depending on the dormancy-breaking conditions. There is an example where DELLA function in gene activation or repression depends upon its binding partner (reviewed by Hauvermale et al. 2012). DELLA protein binding blocks the activity of basic helix–loop–helix transcription factors PHYTOCHROME INTERACTING FACTOR1 and 4 (PIF1 and PIF4). PIF1 functions as a repressor of light-induced genes, whereas PIF4 functions as an activator of dark-induced genes

(deLucas et al. 2008, Cheminant et al. 2011). DELLA binds PIF1 to activate PIF1-regulated transcripts in the light, and binds PIF4 to repress PIF4-regulated transcripts in the dark. However, this is the first study to suggest that a DELLA may switch from functioning as an activator to a repressor (or vice versa) of transcription at the same promoter.

The effect of GA application on *GID1a*, *GID1b* and *GID1c* transcript levels also varied. Voegele et al. (2011) previously found that GA application caused *GID1b* mRNA levels to rise and *GID1a* and *GID1c* levels to decrease during WT seed imbibition. In this study, GA treatment resulted in increased accumulation of all three *GID1* transcripts in dark-imbibed *ga1-3* seeds, and this effect was enhanced at 4°C (**Supplementary Fig. S11A, C**). After *ga1-3* seeds were transferred to the light, GA treatment resulted in decreased *GID1a* and *GID1b* mRNA levels (**Supplementary Fig. S11B, C**). These results suggest that when there is no or low GA signal in the dark, GA treatment initially induces *GID1* transcript accumulation, providing positive feedback regulation. However, once GA signaling is induced by light, further GA application down-regulates *GID1* transcripts, resulting in negative feedback regulation. This is reminiscent of the feed-forward and feed-back regulation of GA biosynthesis genes encoding GA-3-oxidases and GA-20-oxidases (reviewed by Yamaguchi 2008).

Partial after-ripening enhances GID1 protein accumulation

Partial after-ripening resulted in increased GID1 protein accumulation, apparently due to post-transcriptional regulation. No GID1 protein was detected in dormant seeds at the 0 h time point immediately after 3 d of cold stratification, indicating that cold stratification alone is not sufficient to stimulate GID1 protein accumulation. A requirement for after-ripening in order to translate GID1 proteins efficiently might explain why cold stratification alone was insufficient to stimulate GA sensitivity in imbibing dormant *ga1-3* and *ga1-3 HA:GID1b-OE* seeds (**Fig. 2A**). Both untagged native GID1 protein and the HA-tagged HA:GID1a, HA:GID1b and HA:GID1c protein levels were much higher in partially after-ripened than in dormant imbibing seeds (**Fig. 5A, B**; **Supplementary Fig. S16A**). After-ripening acts post-transcriptionally to up-regulate GID1 protein accumulation because after-ripening stimulated HA:GID1 accumulation when expressed on the 35S promoter. Whereas no HA:GID1 protein was detected at 5 and 7 d of imbibition in dormant seeds, untagged GID1 protein was. This difference may be due to the HA-tag or to the fact that expression of HA:GID1 mRNAs on the 35S promoter greatly declined with 3–7 d of imbibition (**Supplementary Fig. S13A–C**). It does not appear that the proteasome down-regulates GID1 protein accumulation in dormant seeds since proteasome inhibitor did not lead to increased HA:GID1b protein levels (**Fig. 6**). Future work will need to examine if after-ripening stimulates GID1 protein accumulation via increased protein translation or increased protein stability. The notion that GID1 may be regulated at the level of protein translation is supported by a recent study showing increased ribosomal loading of *GID1* transcript with after-ripening of sunflower seeds (Layat et al. 2014). It is also interesting to consider whether GID1 may function in the control of GA signaling outside of the nucleus since GID1 fused to a nuclear exclusion signal could partially complement a *gid1a gid1c* double mutant (Livne and Weiss 2014).

Partial after-ripening is associated with increased GID1b accumulation but not decreased ABA accumulation

The increase in GID1 protein levels with after-ripening was expected to correlate with decreased endogenous ABA levels because: (i) after-ripening triggers decreased ABA hormone accumulation at 24 h of imbibition (Ala-Rachedi et al. 2004); and (ii) the GID1 down-regulated DELLA *RGL2* appears to inhibit seed germination by stimulating ABA biosynthesis (Piskurewicz et al. 2008, Lee et al. 2010). Surprisingly, no decrease in ABA content was detected in 12 h imbibed seeds with 2 months of after-ripening (**Fig. 7A**). The decreasing ABA levels detected in previous studies occurred with longer imbibition and after-ripening (3–12 months) times in WT seeds (Ali-Rachedi et al. 2004, Millar et al. 2006, Piskurewicz et al. 2008, Lee et al. 2010). However, the decrease in ABA levels with rescue of GA-insensitive *sly1-2* seed germination by 2 years of after-ripening and *GID1b* overexpression was observed under the same imbibition conditions used in **Fig. 7** (Ariizumi et al. 2013). Thus, it seems that GID1 levels may increase before ABA levels decline with after-ripening and imbibition. Future work will need to examine if increasing GID1 levels with after-ripening and imbibition are needed to trigger a decrease in ABA levels through decreased expression of *XERICO*, a DELLA-up-regulated positive regulator of ABA biosynthesis (Piskurewicz et al. 2008, Ariizumi et al. 2013).

Hormone profiling in dormant and partially after-ripened seeds resulted in several interesting observations. Seed dormancy in imbibing *sly1-2* seeds is associated with greatly elevated ABA levels, whereas ABA levels are lower in *ga1-3* than in the WT. Thus, the failure of *ga1-3* germination is not a direct result of ABA overaccumulation, suggesting that the requirement for GA in germination is not entirely due to increased ABA signaling as previously believed (Karssen and Lacka 1986). JA, JA-Ile and IAA levels were all lower in *ga1-3* and *ga1-3 HA:GID1b-OE* than in the WT, suggesting that GA biosynthesis is needed during either seed development or imbibition for normal accumulation of all of these hormones. JA-Ile has been implicated in stimulating seed germination in cereals (Barrero et al. 2009, Jacobsen et al. 2013). Interestingly, JA-Ile levels increased with after-ripening of *ga1-3 HA:GID1b-OE*. Future research will need to examine if GA signaling may act prior to and function in the increase in JA-Ile and the decrease in ABA accumulation with dormancy loss.

If GA signaling is responsible for reduced ABA accumulation in imbibing after-ripened seeds, then we would expect after-ripening to result in increased capacity to synthesize bioactive GA upon imbibition. This is difficult to test given that GA levels tend to be at the detection limit until imbibing seeds are close to germination (Jacobsen et al. 2002, Yamauchi et al. 2004). However, the GID1b receptor has high GA affinity and some ability to bind DELLA in the absence of GA (Yamamoto et al.

2010). Why some plants evolved a GA receptor capable of low level GA-independent GA signaling is a mystery. This study suggests that the GID1b receptor may induce GA signaling and dormancy loss when GA levels are very low, or in other situations where it is important to respond to a small rise in bioactive GA. It is possible that GID1b-type receptors evolved because there was a need to initiate GA signaling before GA biosynthesis during early seed imbibition to initiate the germination process. Hence, we propose that GID1b-type receptors provide a mechanism to 'prime the pump' of GA signaling under low GA hormone conditions.

This study provides several unique insights into the mechanisms of GA signaling in the context of dormancy loss (**Fig. 8**). The *GID1* transcripts can be GA up-regulated when GA hormone levels are low, but then become GA down-regulated once GA signaling is induced. The DELLA repressors can switch from being positive to negative regulators of *GID1* transcript accumulation depending upon dormancy-breaking conditions. Finally, the three *GID1* receptors do not show identical regulation by after-ripening, GA and DELLA, suggesting that there may be some functional specialization resulting from differences in expression.

Materials and Methods

Plant material and growth conditions

Genotypes used in the Landsberg *erecta* (L*er*) ecotype included the WT, *ga1-3*, *ga1-3 HA:GID1*(*a–c*) and *ga1-3 rgl2-1*, as previously described (Koornneef et al. 1980, Cao et al. 2005, Ariizumi et al. 2008). Controls for the anti-GID1c antibody in the Columbia (Col-0) background include the WT, *gid1a-1 gid1c-2*, *gid1b-1* and *gid1a-1 gid1b-1 gid1c-2* (Griffiths et al. 2006, Willige et al. 2007). The single-copy homozygous *35S:3* × *HA:GID1 ga1-3* transgenic lines were recovered by identifying T_2 lines segregating 3 : 1 for hygromycin resistance, and then identifying T_3 progeny that did not segregate for hygromycin resistance. Seeds were surface sterilized with 10% bleach/0.01% SDS for 10 min, washed six times with sterile water, plated on Murashige and Skoog (MS) agar (0.5 × MS/0.8%/5 mM MES, pH 5.5), and then imbibed for 3 d at 4°C in the dark, followed by 22°C under constant light (48–50 μmol m^{-2} s^{-1}). The germination of *ga1-3* seeds was rescued with 10 μM GA_3. Seedlings were transferred to soil at the four-leaf stage, and incubated in a Conviron™ growth chamber as in McGinnis et al. (2003). To rescue fertility, *ga1-3* and *ga1-3 HA:GID1-OE* lines were sprayed weekly with 10 μM GA_3 until the appearance of green siliques. All comparisons of dormant and after-ripened seeds were performed using single seed lots from plants grown side by side. Freshly harvested seeds were dry after-ripened for 2 weeks, and then either placed at −20°C to preserve dormancy or stored dry at room temperature under non-humid conditions for further after-ripening. Statistical significance was assessed by analysis of variance (ANOVA) with a Tukey's all pairwise comparison. No significant difference in the seed moisture content of dormant and after-ripened seeds was observed (**Supplementary Table S1**; Pandord et al. 1988).

For GA dose–response experiments (**Figs. 1**, **2**; **Supplementary Figs. S1**, **S2**), seeds were incubated as indicated on MS agar plates without GA_4 or with 0.001, 0.005, 0.01, 0.05, 0.1, 0.5 and 1 μM GA_4. Seed germination was scored daily over 9 d, and germination rates were calculated using the average of three replicates of at least 100 seeds each. Statistical significance was assessed by ANOVA with a Tukey's all pairwise comparison.

RNA extraction, cDNA synthesis and RT–qPCR analysis of mRNA expression:

Quantitative two-step RT–qPCR analysis was used to analyze *GID1* and *HA:GID1* mRNA expression in imbibing seeds harvested prior to seed germination as previously described (Ariizumi et al. 2013). A poly(T) primer was used for cDNA synthesis, and quantitative PCR was performed using gene-specific primers for *GID1a*, *GID1b*, *GID1c* (**Figs. 3**, **4**, **Supplementary Figs. S6**, **S11**) and *HA* (**Supplementary Fig. S13**), and the constitutive *IAP-Like Protein 1* (At1g17210) mRNA control (**Supplementary Fig. S20**; Griffiths et al. 2006, Ariizumi et al. 2008, Graeber et al. 2011). qPCR analysis was performed on cDNA using a Roche LightCycler (LightCycler FastStart DNA Master SYBR Green I kit) as follows: 10 min of denaturation at 95°C, followed by 45 cycles of 10 s at 95°C, 5 s at 60°C and 10 s at 72°C. For each experiment, mean mRNA levels were determined based on three biological replicates with three technical replicates each. Transcript levels were normalized against the *IAP-Like Protein 1* control that showed no statistically significant variation in seed mRNA levels in all conditions/genotypes examined (**Supplementary Fig. S19**; Graeber et al. 2011). The relative expression of *GID1* and *HA:GID1* transcripts was calculated using the Delta-Delta Ct method (Livak and Schmittgen 2001) vs. the start imbibition time point of *GID1b* in dormant *ga1-3* seeds (set equal to 1 in **Figs. 3** and **4**, and **Supplementary Fig. S6**). In **Supplementary Fig. S13**, the level of each *HA:GID1* mRNA was shown relative to the dormant seed start time point of *HA:GID1a* (set to 1). To assess statistical significance, the data for all RT–qPCR experiments were normalized using a square root transformation and analyzed with an ANOVA using a Tukey's all pairwise comparison. *P*-values of ≤ 0.07 were considered to be significant (SAS v 9.3; **Supplementary Fig. S3–S5**, **S7–S10**, and **S14** and **S15**).

Protein blot analysis of *ga1-3 HA:GID1* overexpression lines

Dormant and partially after-ripened untransformed *ga1-3* and *ga1-3 HA:GID1-OE* seeds were cold stratified for 3 d and then imbibed at 22°C. Seeds harvested at the indicated time points were ground in liquid nitrogen with extraction buffer [50 mM potassium phosphate buffer pH 7.0 with 1 × protease inhibitor cocktail (Sigma-Aldrich)]. Protein blot analysis was performed using 60 μg of total protein from *ga1-3 HA:GID1-OE* seeds, and 20 μg of total protein from untransformed *ga1-3* seeds. Proteins were separated by 10% SDS–PAGE, then transferred onto a polyvinylidene difluoride (PVDF) membrane using a semi-dry blotter (Bio-Rad Trans-Turbo Blotter) (**Fig. 5**; **Supplementary Fig. S16**). Proteins were detected using α-HA (1 : 5,000, Immuno Consultants Laboratory), α-CUL1 (1 : 10,000; Wang et al. 2002), α-RGL2 (1 : 10,000; Hussain et al. 2005) and α-WRI (1 : 1.000; Chen et al. 2013) primary antibodies. GID1a, GID1b and GID1c were detected using a polyclonal antibody to GID1c, raised using GID1c cleaved from glutathione *S*-transferase (GST)–GID1c that was purified from *Escherichia coli* using glutathione–Sepharose 4B (Agrisera, 1 : 1,000; GST–GID1c construct from Griffiths et al. 2006). Chemiluminescent detection was performed using ECL Advance (GE Healthcare) using the secondary antibody donkey anti-rabbit IgG–horseradish peroxidase (GE Healthcare, 1 : 200,000) for HA-GID1, CUL1 and RGL2 detection, and goat anti-rabbit IgG–horseradish peroxidase (Agrisera, 1 : 75,000) for GID1a, b and c detection. For proteasome inhibition studies, sterilized seeds were plated on filter paper saturated with 100 μM MG132 in MS (stock solution 2 mM in 95% ethanol; TOCRIS), and cold stratified at 4°C for 3 d. Proteins for day 0 were harvested immediately after cold stratification. Western blot analysis with anti-HA was performed as described above.

Hormone measurements

Electrospray ionization liquid chromatography tandem mass spectrometry (ESI-LC-MS/MS) was used to measure ABA, IAA, JA and JA-Ile plant hormone levels as previously described (Yoshimoto et al. 2009). Seeds (200 mg) plated on MS agar were incubated at 4°C for 60 h followed by 12 h at 22°C. Each biological replicate ($n = 6$) consisted of 60 mg of ground, lyophilized seeds. Statistical significance was assessed by ANOVA of $\log_{10}$-transformed data using Tukey's comparison (SAS v 9.3; **Supplementary Fig. S18**). In order to facilitate comparisons of hormone data across studies, the values shown in **Fig. 7** and **Supplementary Fig. S17** are the least square means from the model converted back to ng g^{-1} DW using the inverse $\log_{10}$.

Accession numbers

Arabidopsis Genome Initiative locus identifiers for the genes in this study are: *GID1a* (At3g05120), *GID1b* (At3g63010), *GID1c* (At5g27320), *RGL2* (At3g03450) and *IAP-like Protein 1* (At1g17210).

Supplementary data

Supplementary data are available at PCP online.

Funding

This research was supported by an National Science Foundation (NSF) Award [0850981 to C.M.S.]; National Science Foundation-Eastern Asian Pacific Summer Internship Program (NSF-EAPSI) in collaboration with the Japanese Society for the Promotion of Science [1209878 to K.M.T.].

Acknowledgments

The authors would like to thank members of the Steber lab, Drs. T. Ariizumi, M. Matsuoka and M. Ueguchi-Tanaka for helpful discussions; Drs. P. Okubura, A. Thompson and Y. Jikumaru for assistance with RT–qPCR, statistical analysis and hormone measurements, respectively; Drs. X.W. Deng, H. Hellmann and J. Peng for their gifts of the CUL1, WRI and RGL2 antibodies; Drs. K. Murase and T. Sun for the GST–GID1c construct; and J. Cardenas and Dr. S. Bednarek for purification of GST–GID1c protein.

Disclosures

The authors have no conflicts of interest to declare.

References

Ali-Rachedi, S., Bouinot, D., Wagner, M.H., Bonnet, M., Sotta, B., Grappin, P., et al. (2004) Changes in endogenous abscisic acid levels during dormancy release and maintenance of mature seeds: studies with the Cape Verde Islands ecotype, the dormant model of *Arabidopsis thaliana*. *Planta* 219: 479–488.

Ariizumi, T., Hauvermale, A.L., Nelson, S.K., Hanada, A., Yamaguchi, S. and Steber, C.M. (2013) Lifting DELLA repression of Arabidopsis seed germination by nonproteolytic gibberellin signaling. *Plant Physiol.* 162: 2125–2139.

Ariizumi, T., Murase, K., Sun, T.-P. and Steber, C.M. (2008) Proteolysis-independent downregulation of DELLA repression in Arabidopsis by the gibberellin receptor GIBBERELLIN INSENSITIVE DWARF1. *Plant Cell* 20: 2447–2459.

Ariizumi, T. and Steber, C.M. (2007) Seed germination of GA-insensitive *sleepy1* mutants does not require RGL2 protein disappearance in Arabidopsis. *Plant Cell* 19: 791–804.

Barrero, J.M., Talbot, M.J., White, R.G., Jacobsen, J.V. and Gubler, F. (2009) Anatomical and transcriptomic studies of the coleorhiza reveal the importance of this tissue in regulating dormancy in barley. *Plant Physiol.* 150: 1006–1021.

Bazin, J., Langlade, N., Vincourt, P., Arribat, S., Balzergue, S., El-Maarouf-Bouteau, H., et al. (2011) Targeted mRNA oxidation regulates sunflower seed dormancy alleviation during dry after-ripening. *Plant Cell* 23: 2196–2208.

Bewley, D.J. and Black, M. (2013) Seeds: Physiology of Development and Germination. Springer, New York.

Cadman, C.S.C., Toorop, P.E., Hilhorst, H.W.M. and Finch-Savage, W.E. (2006) Gene expression profiles of Arabidopsis Cvi seeds during dormancy cycling indicate a common underlying dormancy control mechanism. *Plant J.* 46: 805–822.

Cao, D., Cheng, H., Wu, W., Soo, H.M. and Peng, J. (2006) Gibberellin mobilizes distinct DELLA-dependent transcriptomes to regulate seed germination and floral development in Arabidopsis. *Plant Physiol.* 142: 509–525.

Cao, D., Hussain, A., Cheng, H. and Peng, J. (2005) Loss of function of four DELLA genes leads to light- and gibberellin-independent seed germination in Arabidopsis. *Planta* 223: 105–113.

Carrera, E., Holman, T., Medhurst, A., Dietrich, D., Footitt, S., Theodoulou, FL., et al. (2008) Seed after-ripening is a discrete developmental pathway associated with specific gene networks in Arabidopsis. *Plant J.* 53: 214–224.

Cheminant, S., Wild, M., Bouvier, F., Pelletier, S., Renou, J.-P., Erhardt, M., et al. (2011) DELLAs regulate chlorophyll and carotenoid biosynthesis to prevent photooxidative damage during seedling deetiolation in Arabidopsis. *Plant Cell* 23: 1849–1860.

Chen, L., Lee, J.H., Weber, H., Tohge, T., Witt, S., Roje, S., et al. (2013) Arabidopsis BPM proteins function as substrate adaptors to a CULLIN3-based E3 ligase to affect fatty acid metabolism in plants. *Plant Cell* 25: 2253–2264.

de Lucas, M., Daviere, J.M., Rodriguez-Falcon, M., Pontin, M., Iglesias-Pedraz, J.M., Lorrain, S., et al. (2008) A molecular framework for light and gibberellin control of cell elongation. *Nature* 451: 480–484.

Derkx, M.P.M. and Karssen, C.M. (1993) Effects of light and temperature on seed dormancy and gibberellin-stimulated germination in *Arabidopsis thaliana*: studies with gibberellin-deficient and -insensitive mutants. *Physiol Plant.* 89: 360–368.

Derkx, M.P.M., Vermeer, E. and Karssen, C.M. (1994) Gibberellins in seeds of Arabidopsis thaliana: biological activities, identification and effects of light and chilling on endogenous levels. *Plant Growth Regul.* 15: 223–234.

Finkelstein, R.R., Reeves, W., Ariizumi, T. and Steber, C.M. (2008) Molecular aspects of seed dormancy. *Annu. Rev. Plant Biol.* 59: 387–415.

Footitt, S., Douterelo-Soler, I., Clay, H. and Finch-Savage, W.E. (2011) Dormancy cycling in Arabidopsis seeds is controlled by seasonally distinct hormone-signaling pathways. *Proc. Natl Acad. Sci. USA* 108: 20236–20241.

Fukazawa, J., Teramura, H., Murakoshi, S., Nasuno, K., Nishida, N., Ito, T., et al. (2014) DELLAs function as coactivators of GAI-ASSOCIATED FACTOR1 in regulation of gibberellin homeostasis and signaling in Arabidopsis. *Plant Cell* 26: 2920–2938.

Graeber, K., Linkies, A., Wood, A.T.A. and Leubner-Metzger, G. (2011) A guideline to family-wide comparative state-of-the-art quantitative RT–PCR analysis exemplified with a Brassicaceae cross-species seed germination case study. *Plant Cell* 23: 2045–2063.

Griffiths, J., Murase, K., Rieu, I., Zentella, R., Zhang, Z.-L., Powers, S.J., et al. (2006) Genetic characterization and functional analysis of the *gid1* gibberellin receptors in Arabidopsis. *Plant Cell* 18: 3399–3414.

Groot, S.P.C. and Karssen, C.M. (1987) Gibberellins regulate seed germination in tomato by endosperm weakening: a study with gibberellin-deficient mutants. *Planta* 171: 525–531.

Hauvermale, A.L., Ariizumi, T. and Steber, C.M. (2012) Gibberellin signaling: a theme and variations on DELLA repression. *Plant Physiol.* 160: 83–92.

Hussain, A., Cao, D., Cheng, H., Wen, Z. and Peng, J. (2005) Identification of the conserved serine/threonine residues important for gibberellin-sensitivity of Arabidopsis RGL2 protein. *Plant J.* 44: 88–99.

Iuchi, S., Suzuki, H., Kim, Y.-C., Iuchi, A., Kuromori, T., Ueguchi-Tanaka, M., et al. (2007) Multiple loss-of-function of Arabidopsis gibberellin receptor AtGID1s completely shuts down a gibberellin signal. *Plant J.* 50: 958–966.

Jacobsen, J.V., Pearce, D.W., Poole, A.T., Pharis, R.P. and Mander, L.N. (2002) Abscisic acid, phaseic acid and gibberellin contents associated with dormancy and germination in barley. *Physiol. Plant.* 115: 429–441.

Jacobsen, J.V., Barrero, J.M., Hughes, T., Julkowska, M., Taylor, J.M., Xu, Q., et al. (2013) Roles for blue light, jasmonate and nitric oxide in the

regulation of dormancy and germination in wheat grain (*Triticum aestivum L.*). *Planta* 238: 121–138.

Karssen, C.M., der Brinkhorst-van Swan, D.L.C., Breekland, A.E. and Koornneef, M. (1983) Induction of dormancy during seed development by endogenous abscisic acid: studies in abscisic acid deficient genotypes of *Arabidopsis thaliana* (L.) Heynh. *Planta* 157: 158–165.

Karssen, C.M. and Laçka, E. (1986) A revision of the hormone balance theory of seed dormancy: studies on gibberellin and/or abscisic acid-deficient mutants of *Arabidopsis thaliana*. *In* Plant Growth Substances 1985: Proceedings of the 12th International Conference on Plant Growth Substances. Edited by Bopp, M. pp. 315–323. Springer-Verlag, New York.

Karssen, C.M., Zagorski, S., Kepczynski, J. and Groot, S.P.C. (1989) Key role for endogenous gibberellins in the control of seed germination. *Ann. Bot.* 63: 71–80.

Koornneef, M., Jorna, M.L., der Brinkhorst-van Swan, D.L.C. and Karssen, C.M. (1982) The isolation of abscisic acid (ABA) deficient mutants by selection of induced revertants in non-germinating gibberellin sensitive lines of *Arabidopsis thaliana* (L.) Heynh. *Theor. Appl. Genet.* 61: 385–393.

Koornneef, M. and van der Veen, J.H. (1980) Induction and analysis of gibberellin sensitive mutants in *Arabidopsis thaliana* (L.) Heynh. *Theor. Appl. Genet.* 58: 257–263.

Kushiro, T., Okamoto, M., Nakabayashi, K., Yamagishi, K., Kitamura, S., Asami, T., et al. (2004) The Arabidopsis cytochrome P450 CYP707A encodes ABA 8′-hydroxylases: key enzymes in ABA catabolism. *EMBO J.* 23: 1647–1656.

Layat, E., Leymarie, J., El-Maarouf-Bouteau, H., Caius, J., Langlade, N. and Bailly, C. (2014) Translatome profiling in dormant and nondormant sunflower (*Helianthus annuus*) seeds highlights post-transcriptional regulation of germination. *New Phytol.* 204: 864–872.

Lee, K.P., Piskurewicz, U., Tureckova, V., Strnad, M. and Lopez-Molina, L. (2010) A seed coat bedding assay shows that RGL2-dependent release of abscisic acid by the endosperm controls embryo growth in Arabidopsis dormant seeds. *Proc. Natl Acad. Sci. USA* 107: 19108–19113.

Liu, X., Zhang, H., Zhao, Y., Feng, Z., Li, Q., Yang, H.Q., et al. (2013) Auxin controls seed dormancy through stimulation of abscisic acid signaling by inducing ARF-mediated *ABI3* activation in Arabidopsis. *Proc. Natl Acad. Sci. USA* 110: 15485–15490.

Livak, K.J. and Schmittgen, T.D. (2001) Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. *Methods* 25: 402–408.

Livne, S. and Weiss, D. (2014) Cytosolic activity of the gibberellin receptor GIBBERELLIN INSENSITIVE DWARF1A. *Plant Cell Physiol.* 55: 1727–1733.

McGinnis, K.M., Thomas, S.G., Soule, J.D., Strader, L.C., Zale, J.M., Sun, T.-P., et al. (2003) The Arabidopsis *SLEEPY1* gene encodes a putative F-box subunit of an SCF E3 ubiquitin ligase. *Plant Cell* 15: 1120–1130.

Millar, A.A., Jacobsen, J.V., Ross, J.J., Helliwell, C.A., Poole, A.T., Scofield, G., et al. (2006) Seed dormancy and ABA metabolism in Arabidopsis and barley: the role of ABA 8′-hydroxylase. *Plant J.* 45: 942–954.

Murase, K., Hirano, Y., Sun, T.-P. and Hakoshima, T. (2008) Gibberellin-induced DELLA recognition by the gibberellin receptor GID1. *Nature* 456: 459–463.

Nakajima, M., Shimada, A., Takashi, Y., Kim, Y.-C., Park, S.-H., Ueguchi-Tanaka, M., et al. (2006) Identification and characterization of Arabidopsis gibberellin receptors. *Plant J.* 46: 880–889.

Ogawa, M., Hanada, A., Yamauchi, Y., Kuwahara, A., Kamiya, Y. and Yamaguchi, S. (2003) Gibberellin biosynthesis and response during Arabidopsis seed germination. *Plant Cell* 15: 1591–1604.

Oh, E., Yamaguchi, S., Hu, J., Yusuke, J., Jung, B., Paik, I., et al. (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: 1192–1208.

Okamoto, M., Kuwahara, A., Seo, M., Kushiro, T., Asami, T., Hirai, N., et al. (2006) *CYP707A1* and *CYP707A2*, which encode abscisic acid 8′-hydroxylases, are indispensable for proper control of seed dormancy and germination in Arabidopsis. *Plant Physiol.* 141: 97–107.

Pandord, J.A., Williams, P.C. and deMan, J.M. (1988) Analysis of oilseeds for protein, oil, fiber, and moisture by near-infrared reflectance spectroscopy. *J. Amer. Oil Chem. Soc.* 65: 1627–1634.

Piskurewicz, U., Jikumaru, Y., Kinoshita, N., Nambara, E., Kamiya, Y. and 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: 2729–2745.

Seo, M., Nambara, E., Choi, G. and Yamaguchi, S. (2009) Interaction of light and hormone signals in germinating seeds. *Plant Mol. Biol.* 69: 463–472.

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

Steber, C.M., Cooney, S.E. and McCourt, P. (1998) Isolation of the GA-response mutant *sly1* as a suppressor of *ABI1-1* in *Arabidopsis thaliana*. *Genetics* 149: 509–521.

Sun, T.P. and Kamiya, Y. (1994) The Arabidopsis *GA1* locus encodes the cyclase ent-kaurene synthetase A of gibberellin biosynthesis. *Plant Cell* 6: 1509–1518.

Tyler, L., Thomas, S.G., Hu, J., Dill, A., Alonso, J.M., Ecker, J.R., et al. (2004) DELLA proteins and gibberellin-regulated seed germination and floral development in Arabidopsis. *Plant Physiol.* 135: 1008–1019.

Ueguchi-Tanaka, M., Ashikari, M., Nakajima, M., Itoh, H., Katoh, E., Kobayashi, M., et al. (2005) *GIBBERELLIN INSENSITIVE DWARF1* encodes a soluble receptor for gibberellin. *Nature* 437: 693–698.

Ueguchi-Tanaka, M. and Matsuoka, M. (2010) The perception of gibberellins: clues from receptor structure. *Curr. Opin. Plant Biol.* 13: 503–508.

Wang, X., Kang, D., Feng, S., Serino, G., Schwechheimer, C. and Wei, N. (2002) CSN1 N-terminal-dependent activity is required for Arabidopsis development but not for Rub1/Nedd8 deconjugation of cullins: a structure–function study of CSN1 subunit of COP9 signalosome. *Mol. Biol. Cell* 13: 646–655.

Willige, B.C., Ghosh, S., Nill, C., Zourelidou, M., Dohmann, E.M.N., Maier, A., et al. (2007) The DELLA domain of *GA INSENSITIVE* mediates the interaction with the *GA INSENSITIVE DWARF1A* gibberellin receptor of Arabidopsis. *Plant Cell* 19: 1209–1220.

Voegele, A., Linkies, A., Müller, K. and Leubner-Metzger, G. (2011) Members of the gibberellin receptor gene family GID1 (GIBBERELLIN INSENSITIVE DWARF1) play distinct roles during *Lepidium sativum* and *Arabidopsis thaliana* seed germination. *J. Exp. Bot.* 62: 5131–5147.

Yamaguchi, S. (2008) Gibberellin metabolism and its regulation. *Annu. Rev. Plant Biol.* 59: 225–251.

Yamamoto, Y., Hirai, T., Yamamoto, E., Kawamura, M., Sato, T., Kitano, H., et al. (2010) A rice *gid1* suppressor mutant reveals that gibberellin is not always required for interaction between its receptor, GID1, and DELLA proteins. *Plant Cell* 22: 3589–3602.

Yamauchi, Y., Ogawa, M., Kuwahara, A., Hanada, A., Kamiya, Y. and Yamaguchi, S. (2004) Activation of gibberellin biosynthesis and response pathways by low temperature during imbibition of *Arabidopsis thaliana* seeds. *Plant Cell* 16: 367–378.

Yoshida, H., Hirano, K., Sato, T., Mitsuda, N., Nomoto, M., Maeo, K., et al. (2014) DELLA protein functions as a transcriptional activator through the DNA binding of the indeterminate domain family proteins. *Proc. Natl Acad. Sci. USA* 111: 7861–7866.

Zentella, R., Zhang, Z.-L., Park, M., Thomas, S.G., Endo, A., Murase, K., et al. (2007) Global analysis of DELLA direct targets in early gibberellin signaling in Arabidopsis. *Plant Cell* 19: 3037–3057.