The GATA-type transcription factors GNC and GNL/CGA1 repress gibberellin signaling downstream from DELLA proteins and PHYTOCHROME-INTERACTING FACTORS

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The phytohormone gibberellin (GA) regulates various developmental processes in plants such as germination, greening, elongation growth, and flowering time. DELLA proteins, which are degraded in response to GA, repress GA signaling by inhibitory interactions with PHYTOCHROME-INTERACTING FACTOR (PIF) family transcription factors. How GA signaling is controlled downstream from the DELLA and PIF regulators is, at present, unclear. Here, we characterize *GNC* (*GATA, NITRATE-INDUCIBLE, CARBON-METABOLISM INVOLVED*) and *GNL/CGA1* (*GNC-LIKE/CYTOKININ-RESPONSIVE GATA FACTOR1*), two homologous GATA-type transcription factors from *Arabidopsis thaliana* that we initially identified as GA-regulated genes. Our genetic analyses of loss-of-function mutants and overexpression lines establish that *GNC* and *GNL* are functionally redundant regulators of germination, greening, elongation growth and flowering time. We further show by chromatin immunoprecipitation that both genes are potentially direct transcription targets of PIF transcription factors, and that their expression is up-regulated in *pif* mutant backgrounds. In line with a key role of GNC or GNL downstream from DELLA and PIF signaling, we find that their overexpression leads to gene expression changes that largely resemble those observed in a *ga1* biosynthesis mutant or a *pif* quadruple mutant. These findings, together with the fact that *gnc* and *gnl* loss-of-function mutations suppress *ga1* phenotypes, support the hypothesis that GNC and GNL are important repressors of GA signaling downstream from the DELLA and PIF regulators.

[*Keywords*: *Arabidopsis thaliana*; DELLA protein; GATA factor; gibberellin; LLM domain; phytochrome-interacting factor; PIF]

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The phytohormone gibberellin (GA) controls many important aspects of plant development such as germination, greening, elongation growth, and flowering time (Ueguchi-Tanaka et al. 2007; Schwechheimer and Willige 2009). GA is bound by members of the GA-INSENSITIVE DWARF1 (GID1) receptor family that heterodimerize with DELLA proteins and thereby induce their degradation by the 26S proteasome (Gomi et al. 2004; Ueguchi-Tanaka et al. 2005; Willige et al. 2007; Murase et al. 2008; Shimada et al. 2008). In *Arabidopsis thaliana*, proteasomal DELLA protein degradation is promoted by the F-box protein SLEEPY1 (SLY1), which induces DELLA protein ubiquitylation as a subunit of the E3 ubiquitin ligase SCF^{SLY1} (Dill et al. 2004; Ariizumi et al. 2008; Ueguchi-Tanaka et al. 2008). *Arabidopsis* encodes three functionally redundant GID1 receptors (GID1A–C) and five DELLA proteins (namely, GA-INSENSITIVE [GAI], REPRESSOR OF *ga1*-3 [RGA], RGA-LIKE1 [RGL1], RGL2, and RGL3) (Peng et al. 1997; Silverstone et al. 1998; Dill and Sun 2001; King et al. 2001; Lee et al. 2002; Cheng et al. 2004; Griffiths et al. 2006; Willige et al. 2007).

The mode of action of the N-acetylglucosamine (GlcNAc) transferase SPINDLY (SPY) in GA signaling is, at present, unresolved, but several observations clearly position SPY in the GA pathway. First, *spy* mutants morphologically resemble plants that lack DELLA repressors or that have been grown in the presence of high doses of GA (GA overdose phenotype). Second, *spy* alleles suppress the phenotypes of mutants with a constitutively repressed GA pathway. Third, *spy* mutants accumulate DELLA proteins, suggesting, in view of

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their GA overdose phenotype, that the DELLA repressors or as-yet-unidentified downstream regulators are not active in *spy* (Jacobsen and Olszewski 1993; Wilson and Somerville 1995; Dill and Sun 2001; King et al. 2001; Silverstone et al. 2007). Taken together, these findings suggest that SPY activates DELLA proteins by GlcNAc modification.

DELLA proteins repress GA responses, at least in part, by interfering with the DNA-binding activity of PHYTOCHROME-INTERACTING FACTOR (PIF) basic helix–loop–helix (bHLH) transcription factors (de Lucas et al. 2008; Feng et al. 2008). In *Arabidopsis*, PIFs constitute a multiprotein family with seven members, and the developmental roles of individual PIFs in isolation and in combination with other PIF family members have been elucidated in recent years; e.g., through the analysis of a *pif1 pif3 pif4 pif5* quadruple mutant (Castillon et al. 2007; Leivar et al. 2008). These analyses revealed that the individual family members have, at least in part, redundant functions (Castillon et al. 2007; Leivar et al. 2008). PIF function is also negatively regulated by interactions with light-activated phytochrome B that induce PIF proteolysis in the light (Bauer et al. 2004; Park et al. 2004; Shen et al. 2005). PIFs thus integrate light and GA signals to control developmental responses. The inhibitory role of DELLA proteins in the regulation of PIFs is particularly prominent in the dark, when phytochromes are inactive, and during the transition from skotomorphogenic to photomorphogenic seedling growth, when DELLA protein levels increase due to decreasing GA levels and reduced DELLA protein turnover (Alabadi et al. 2008; de Lucas et al. 2008; Feng et al. 2008).

How GA responses are regulated downstream from DELLA and PIF proteins is, at present, not well understood. Here, we characterize the role of two functionally redundant *A. thaliana* GATA family transcription factors, which we initially identified as GA-regulated transcripts. We now demonstrate that the two GATA factors are critical regulators of GA signaling that control germination, greening, elongation growth, and flowering downstream from DELLA and PIF proteins.

Results

GNC *(*GATA, NITRATE-INDUCIBLE, CARBON-METABOLISM INVOLVED*) and* GNL *(*GNC-LIKE*) expression is regulated by GA*

With the goal of identifying novel GA pathway regulators, we generated and systematically screened a set of homozygous T-DNA insertion mutants of GA-regulated genes for germination and hypocotyl elongation defects (Willige et al. 2007; data not shown). During this analysis, we detected germination defects in mutants of two closely related GATA family transcription factors: GNC and GNL/CGA1 (hereafter GNL) (Fig. 1A). It had been noted previously that mutants of *GNC* and *GNL* have greening defects, and that the two genes are transcriptionally regulated by nutrient availability, cytokinin, and light (Bi et al. 2005; Naito et al. 2007; Mara and Irish 2008). Their important role as regulators of GA signaling, as elucidated by our studies, had, however, been overlooked previously.

Our initial microarray analysis had revealed that *GNC* and *GNL* transcript levels are reduced in the GA biosynthesis mutant *ga1* following a 1-h GA treatment (Willige et al. 2007). We could subsequently also confirm by quantitative real-time PCR that their transcript levels are repressed by GA in wild-type and *ga1* mutant seedlings (Fig. 1B,C). Since, at the same time, GA had no effect on *GNC* and *GNL* transcript abundance in the GA receptor mutant *gid1abc*, and since the basal transcript levels of *GNC* and *GNL* were elevated in *ga1* and *gid1abc*, we reasoned that *GNC* and *GNL* repression requires DELLA protein degradation (Fig. 1B–D). When we examined the dynamics of GA-dependent *GNC* and *GNL* repression over time, we noted that the reduction in transcript abundance of the two genes, which initially correlated with the GA-induced degradation of the DELLA proteins RGA and GAI, was attenuated after prolonged GA treatment (Fig. 1E,F). At the same time, we observed that neither RGA nor GAI were completely degraded by the end of the experiment, that GAI protein even reaccumulated over time, and that the transcript abundance of DELLA regulators increased in response to GA (Fig. 1F,G). Neither *GNC* and *GNL* nor *RGA* and *GAI* genes were regulated in the GA-insensitive *gid1abc* mutant, supporting the notion that the observed gene expression responses require DELLA protein turnover (Fig. 1E,F). We thus concluded that *GNC* and *GNL* transcript levels correlate with DELLA protein turnover, and that negative feedback mechanisms control the abundance of DELLA repressors, which may be responsible for the attenuation of *GNC* and *GNL* repression after prolonged GA treatment. However, we also cannot rule out that the attenuation of *GNC* and *GNL* repression is controlled by other regulators whose presence or activity depends on GA signaling or DELLA protein degradation.

GNC and GNL redundantly regulate germination

GA controls a number of important developmental processes, including germination, greening, elongation growth, and flowering time. Our T-DNA insertion mutant screen revealed that *gnc* and *gnl* loss-of-function mutant seeds germinate faster than wild-type seeds, suggesting that GNC and GNL repress germination. We subsequently analyzed the expression of *GNC* and *GNL* during germination in detail and characterized the germination defect of *gnc* and *gnl* mutants with regard to individual germination stages.

When we examined the accumulation of *GNC* and *GNL* transcripts during the cold imbibition of nondormant seeds—thus, in unfavorable germination conditions—we observed a strong transcriptional activation of both genes that was attenuated following the transfer of the imbibed seeds to ambient temperatures (Fig. 2A). The DELLA proteins GAI and RGL2 had been shown previously to be critical repressors of germination in the GA-deficient *ga1* mutant (Lee et al. 2002; Cao et al. 2005). We therefore examined their transcription and protein abundance during the stratification and germination process (Fig. 2B,C). Interestingly, the transcriptional activation of *GNC* and *GNL* during stratification correlated well with an increase of GAI protein abundance—and, to a minor extent, also of RGL2 protein abundance—suggesting that these DELLA proteins may

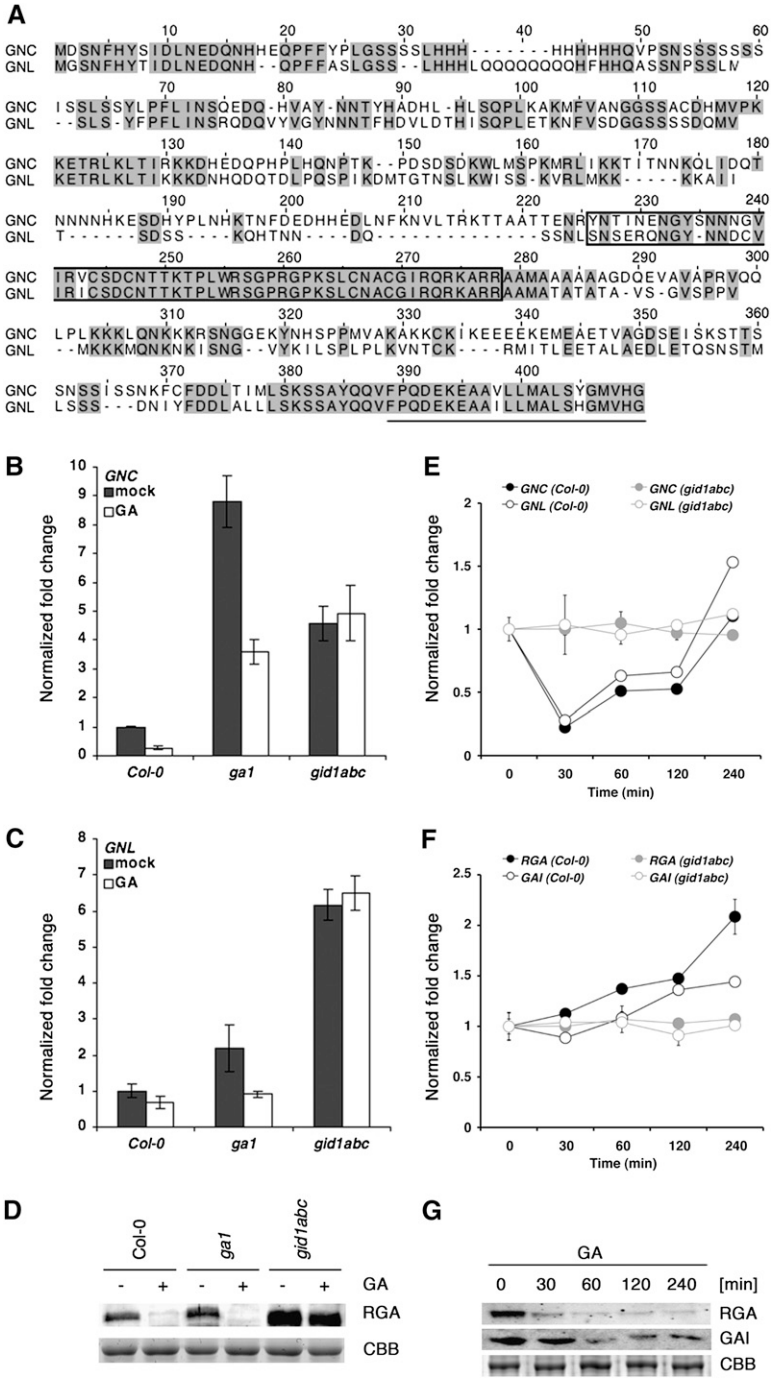


Figure 1. *GNC* and *GNL* transcription is repressed by GA and requires DELLA repressor degradation. (*A*) ClustalW alignment of the GATA transcription factors GNC and GNL (Larkin et al. 2007). Identical amino acids are shaded (BLOSUM62 score), and the GATA DNA-binding domain is framed. The conserved C-terminal LLM domain, which is also found in GATA transcription factors from other plant species (Supplemental Fig. S1), is underlined. (*B*,*C*) Expression of *GNC* and *GNL*, respectively, as determined by quantitative real-time PCR in light-grown mock-treated and GA-treated (100 μM GA3, 1 h) wild-type and mutant seedlings. (*D*) Immunoblot with an anti-RGA antibody of protein samples prepared and treated in the same way as the RNA samples used in *B* and *C* to confirm the efficiency of the GA treatment (100 μM GA3, 1 h). (CBB) Coomassie Brilliant blue-stained gel, loading control. (*E*,*F*) Time-dependent transcription of *GNC* and *GNL* (*E*) or *RGA* and *GAI* (*F*) in response to 100 μM GA3 in light-grown wild-type (Col-0) and *gid1abc* mutant seedlings, as quantified by real-time PCR. (*G*) Immunoblot with anti-RGA and anti-GAI antibodies to monitor their abundance during the GA treatment (100 μM GA3) used in *E* and *F*. (CBB) Coomassie Brilliant blue-stained gel, loading control.

regulate the expression of *GNC* and *GNL* in cold-imbibed seeds (Fig. 2C). Interestingly, the DELLA protein RGA accumulated only after transfer to ambient temperatures (Fig. 2C). This suggests that RGA is not involved in the regulation of *GNC* and *GNL* transcription in the cold. However, RGA may, together with RGL2, which was detectable at all time points of the experiment, be responsible for the continued expression of *GNC* and *GNL* during germination at ambient temperatures (Fig. 2A–C). Importantly, DELLA protein accumulation was not detectably altered in the *gnc gnl* mutants, indicating that changes in DELLA protein abundance are not the molecular cause for the germination phenotypes that will be described in due course (Fig. 2C). Furthermore, although the overall increase in DELLA protein abundance correlated well with the overall induction of DELLA protein gene expression in cold-imbibed seeds, we did not observe an obvious correlation between DELLA protein abundance and DELLA protein gene expression at later stages of the experiment (Fig. 2B,C). These observations thus support the notion that DELLA protein abundance is regulated by feedback mechanisms that balance between DELLA protein de novo synthesis and DELLA protein degradation.

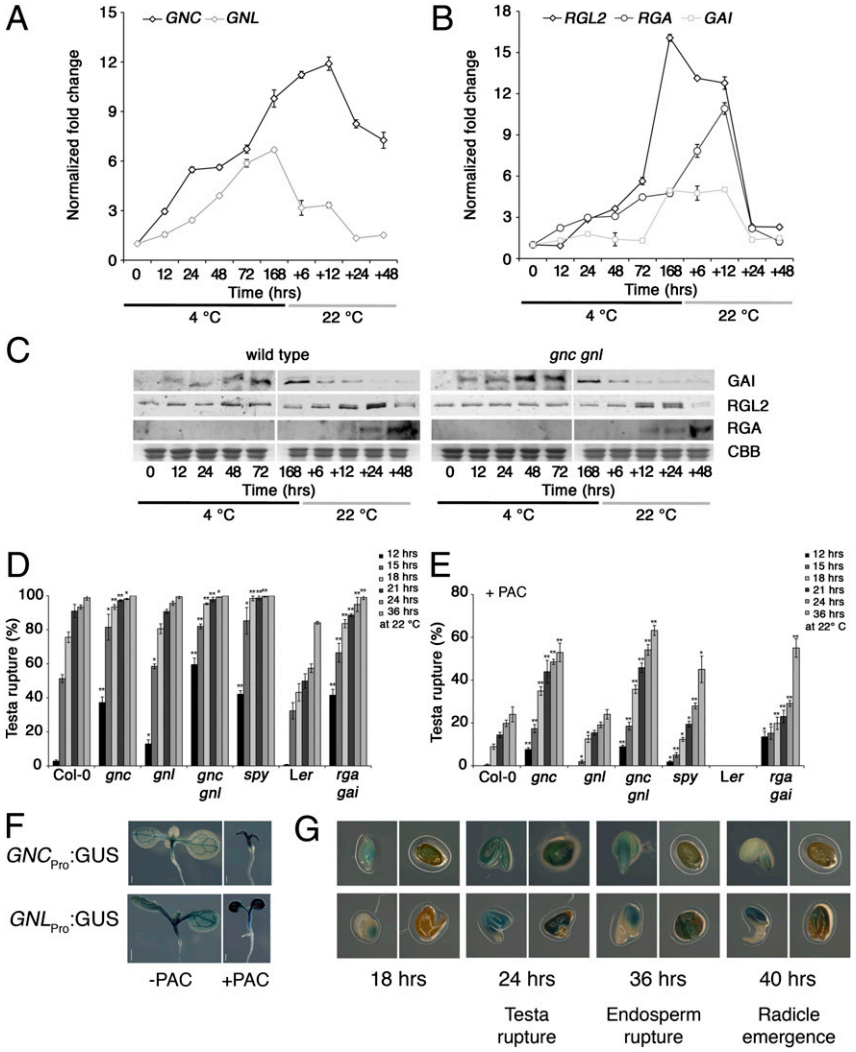


Figure 2. GNC and GNL repress germination. (*A*,*B*) Time-dependent transcription of *GNC* and *GNL* (*A*) or *GAI*, *RGL2*, and *RGA* (*B*) during cold imbibition at 4°C and subsequent germination at 22°C. (*C*) Immunoblots with anti-RGA, anti-GAI, and anti-RGL2 antibodies using protein extracts prepared from wild-type and *gnc gnl* mutant seeds to detect the abundance of the DELLA proteins under the conditions employed in *A* and *B*. (CBB) Coomassie Brilliant blue-stained gel, loading control. (*D*,*E*) Quantification of the occurrence of testa rupture in imbibed seeds grown on 0.5× MS in the absence (*D*) or presence (*E*) of 1 μM PAC. The graphs in *D* and *E* show the averaged result of three independent germination experiments. $n \geq 80$; Student's *t*-test $P \leq 0.01$ (**) and $0.01 \leq P \leq 0.05$ (*). (*F*) GUS staining of 7-d-old transgenic seedlings expressing the promoter:GUS fusions GNC_{Pro}:GUS and GNL_{Pro}:GUS grown in the absence or presence of 1-μM PAC. (*G*) Histological GUS staining of imbibed GNC_{Pro}:GUS and GNL_{Pro}:GUS seeds during germination. For each time point, a dissected embryo (*left* panel) and seed coat (*right* panel) are shown. The stage of germination is indicated *below* the relevant time points.

Our subsequent mutant analyses revealed germination defects in *gnc* and *gnl* loss-of-function mutants, which, in summary, suggest that GNC and GNL are germination repressors. In these analyses, we determined that *gnc* and *gnl* mutants reach each germination stage—thus testa rupture, endosperm rupture, and radicle emergence—earlier than the wild type (Fig. 2D; Supplemental Fig. S2). Furthermore, we found that the germination defects of the *gnc gnl* double mutant are generally stronger than those of the single mutants, suggesting that the two proteins have redundant functions. In quantitative terms, the *gnc gnl* germination defects were comparable with those observed in mutants with derepressed GA signaling. This was also true with regard of the ability of *gnc gnl* mutants to germinate efficiently on media containing concentrations of the GA biosynthesis inhibitor paclobutrazol (PAC) that strongly repress germination in the wild type (Fig. 2E). We thus concluded that GNC and GNL are functionally redundant repressors of germination that act downstream from the DELLA proteins in GA signaling.

To gain an understanding of the tissue-specific expression of *GNC* and *GNL* during germination, we generated transgenic lines expressing *GNC* and *GNL* promoter:GUS fusions. Since we found that both reporter genes responded well to PAC treatments, we reasoned that the constructs recapitulate the GA responsiveness of the respective promoters (Fig. 2F). Our subsequent analyses then revealed that both gene promoters are expressed in the endosperm, a staining pattern that was most pronounced at the time of testa rupture (Fig. 2G). At later stages, we found *GNL* expression to be largely restricted to the cotyledons, while *GNC* expression was particularly prominent in the embryonic root, a region that elongates during radicle emergence (Fig. 2G; Belin et al. 2006; Piskurewicz et al. 2008). We thus concluded that *GNC* and *GNL* are expressed in the germinating embryo as well as in the endosperm, and that the two genes may act in the same but also in distinct tissues during specific stages of germination.

gnc *and* gnl *mutants are defective in greening*

Our phenotypic analysis of *gnc* and *gnl* mutants revealed that the mutants have visibly reduced chlorophyll levels (Fig. 3A). Reduced chlorophyll accumulation is also known as a phenotype of mutants with derepressed GA signaling,

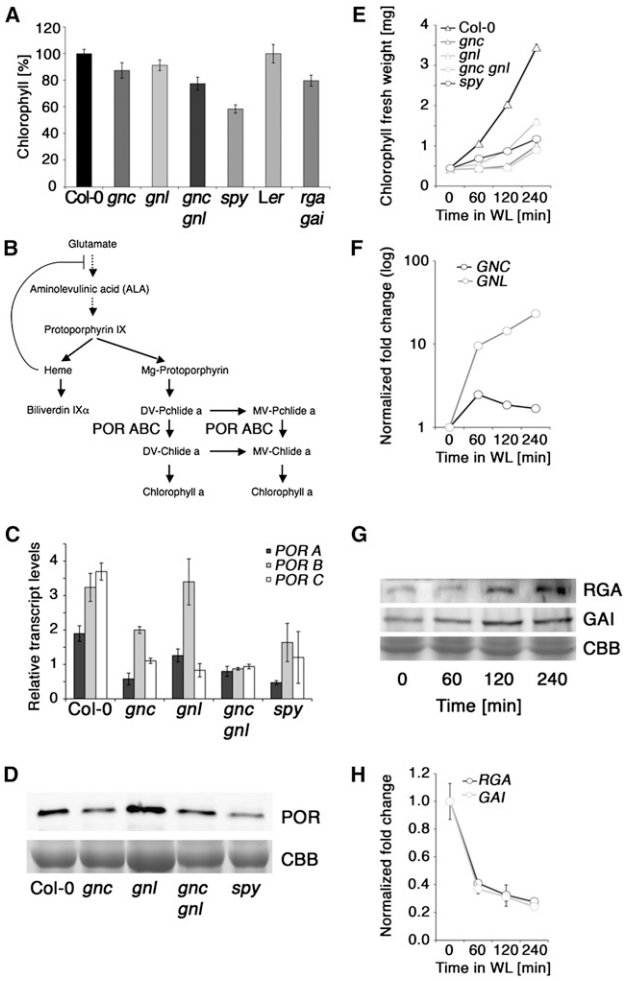


Figure 3. Loss of GNC and GNL impairs chlorophyll biosynthesis. (*A*) Quantification of chlorophyll content in *gnc* and *gnl* mutants as well as *spy* and *rga gai* mutants in comparison with the respective wild types. The reduction in chlorophyll content as shown here corresponds in all cases to a visible reduction in greening. (*B*) Overview of the chlorophyll biosynthesis pathway with modifications from Thomas (1997). The functionally redundant POR A, POR B, and POR C promote chlorophyll biosynthesis in *Arabidopsis*. (*C*,*D*) The chlorophyll biosynthesis defect of *gnc* and *gnl* mutants correlates with reduced *POR* transcription (*C*) and reduced POR protein levels (*D*). Note that the POR antibody does not distinguish between the three POR homologs. (CBB) Coomassie Brilliant blue-stained gel, loading control. (*E–H*) Chlorophyll accumulation (*E*) and *GNC* and *GNL* transcription (*F*), as well as RGA and GAI protein accumulation (*G*) and gene transcription (*H*), as detected by quantitative RT–PCR and immunoblots after the transfer of dark-grown seedlings to 100 μmol m^{-2} sec^{-1} white light (WL). Note that the reduction in *GAI* and *RGA* transcription (*H*) does not correlate with the increase in the abundance of RGA and GAI protein (*G*). (CBB) Coomassie Brilliant blue-stained gel, loading control.

such as *spy* and *rga gai* (Fig. 3A). Reduced chlorophyll accumulation already had been noted previously as a phenotype of *gnc* and *gnl* mutants, but their greening defect had not been recognized or discussed as a defect related to GA signaling (Fig. 3A; Bi et al. 2005; Mara and Irish 2008). We thus questioned whether a causal relationship exists between DELLA proteins and *GNC* or *GNL* expression in the context of chlorophyll accumulation.

Wild-type seedlings start greening during the transition from skotomorphogenic to photomorphogenic growth when protochlorophyllide oxidoreductases (PORs) are induced and promote the conversion of protochlorophyllide to chlorophyllide (Fig. 3B; Thomas 1997). We hypothesized that a reduction of POR levels may be the molecular cause for the greening defect of *gnc* and *gnl* mutants. Our subsequent analysis showed indeed that the levels of the three *Arabidopsis POR* isoforms are reduced in the *gnc* and *gnl* mutants as well as in *spy* (Fig. 3C). The reduced abundance of *POR* transcripts further correlated with reduced POR protein levels and reduced chlorophyll accumulation rates when dark-grown mutant seedlings were transferred to the light (Fig. 3A–E). Interestingly, this phenotype was accompanied by an increase in *GNC* and *GNL* transcript abundance as well as by an increase in the abundance of GAI and RGA protein (Fig. 3F,G). At the same time, we found the levels of *RGA* and *GAI* transcript to be down-regulated, supporting the existence of a negative feedback regulatory mechanism (Fig. 3H). We therefore concluded that greening responses require GNC and GNL as well as DELLA regulators, and that reduced protochlorophyllide metabolism and POR protein levels may be a common cause for the greening defects in their mutants.

GNC and GNL repress flowering time and leaf elongation growth

Next, we examined the role of *GNC* and *GNL* in flowering time control. Our quantification of several flowering time parameters showed that *gnc* and *gnl* mutants as well as their double mutants flower earlier than the wild type (Table 1). When grown in long-day conditions (16-h light/8-h dark), *gnc gnl* double mutants, similarly to *rga gai* or *spy* mutants, bolted and flowered ~3 d earlier than their wild-type counterparts. At the same time, we noticed an increase in rosette leaf size in the *gnc* and *gnl* mutants, suggesting that GNC and GNL also negatively control leaf size in the wild type (Table 1).

Since DELLA proteins, particularly GAI and RGA, are critical repressors of flowering in GA-deficient mutant backgrounds, we asked whether GNC and GNL also are critical for the repression of flowering in the absence of GA (Dill and Sun 2001; King et al. 2001). To this end, we introduced the *gnc* and *gnl* mutations into the GA biosynthesis mutant *ga1*. Interestingly, *gnc* and, even more so, *gnc gnl* mutations were able to partially suppress the flowering defect and the leaf elongation defect of *ga1* mutants (Fig. 4). We therefore concluded that GNC and GNL repress, at least in part, flowering and leaf expansion phenotypes in a GA-deficient background.

GNC or GNL overexpression impairs germination, greening, and flowering

The experiments with the *gnc* and *gnl* loss-of-function mutants had indicated that both proteins redundantly

Table 1. *Flowering time of* gnc *and* gnl *mutants in comparison with the wild-type and GA pathway mutants grown in long-day conditions*

Genotype	Bolting time (d)	Rosette leaves (number)	Flowering time (d)	Rosette leaf length 30 DAG (cm)	Rosette leaf length 35 DAG (cm)
Col-0	25.0 ± 0.8	10.7 ± 0.6	29.1 ± 0.7	3.93 ± 0.42	4.21 ± 0.21
gnc	23.4 ± 1.1[b]	10.3 ± 0.6[a]	28.3 ± 0.9[b]	4.36 ± 0.45[b]	4.63 ± 0.30[b]
gnl	24.4 ± 0.8[a]	11.1 ± 0.7[a]	29.4 ± 0.8[b]	4.33 ± 0.43[b]	4.63 ± 0.34[b]
gnc gnl	22.2 ± 1.1[a]	10.1 ± 0.7[b]	26.9 ± 1.0[b]	4.82 ± 0.46[b]	4.96 ± 0.37[b]
spy-3	21.9 ± 1.0[b]	9.2 ± 0.7[b]	25.9 ± 1.0[b]	4.15 ± 0.28[a]	4.30 ± 0.26[a]
GNC:GFP	34.6 ± 0.8[b]	18.3 ± 0.6[b]	40.3 ± 1.1[b]	1.54 ± 0.14[b]	1.88 ± 0.22[b]
YFP:GNL	33.3 ± 0.5[b]	17.5 ± 0.7[b]	37.7 ± 1.1[b]	1.58 ± 0.18[b]	1.94 ± 0.22
L*er*	23.2 ± 0.7	9.5 ± 0.9	27.5 ± 0.9	4.43 ± 0.32	4.75 ± 0.31
rga-24 *gai*-t6	21.0 ± 0.9[b]	7.0 ± 0.9[b]	25.1 ± 0.8[b]	2.60 ± 0.29[b]	3.17 ± 0.31[b]
n =	23	23	23	23	23

[a]Student's *t*-test: $P \leq 0.01$.
[b]Student's *t*-test: $0.01 \leq P \leq 0.05$.
(DAG) Days after germination.

control germination, greening, flowering, and elongation growth in the wild type. We next generated plants that overexpress the two proteins as fluorescent protein-tagged variants: GNC:GFP and YFP:GNL (Supplemental Fig. S3). Interestingly, we found that GNC as well as GNL overexpression lines are dark-green and accumulate chlorophyll also in tissues such as the lower part of the hypocotyl, where chlorophyll does not visibly accumulate in the wild type (Fig. 5A,B). Furthermore, we found adult GNC or GNL overexpressors to be clearly distinguishable from the wild type due to their reduced rosette diameter and late-flowering phenotype (Fig. 5C,D; Table 1). In fact, several first-generation GNC:GFP transgenic plants failed to flower completely, so that only GNC:GFP plants with a presumably weaker phenotype could be propagated. The flowering time delay was suggestive for reduced GA responses in the overexpression lines. In line with this hypothesis, we found that seeds descending from the GNC or GNL overexpression lines were hypersensitive to PAC treatment. Following treatment with critical PAC concentrations, GNC:GFP seedlings accumulated visibly more chlorophyll than the wild type, while YFP:GNL seeds were unable to germinate (Fig. 5A). Furthermore, we observed a significant germination delay in GNC:GFP and YFP:GNL transgenic seeds as well as a reduced responsiveness to GA (Fig. 5E,F). Furthermore, we measured reduced responses of YFP:GNL seedlings—but, interestingly, not of GNC:GFP seedlings—with regard to GA-induced cotyledon expansion (Fig. 5G). In summary, we conclude that the overexpression of GNC and GNL leads to growth defects that are opposite to those observed in their loss-of-function mutants, and that the overexpression lines are, at least in part, GA-insensitive.

With regard to their defects in germination, greening, and flowering time, GNC and GNL overexpressors resemble mutants with repressed GA signaling, such as *ga1* or *gid1abc*. Using microarray analysis, we could subsequently demonstrate that these phenotypic similarities also extend to similarities at the gene expression level. Most strikingly, we found that approximately half of the genes (3659 out of 7211) that are differentially expressed in *ga1* are also differentially expressed in GNC or GNL overexpression lines (Fig. 5H; Supplemental Table S2). Within this gene set, we identified, among a total of 1925 differentially regulated transcripts, 1348 genes that are transcriptionally induced and 465 genes that are repressed in all three genotypes when compared with the wild type. Thus, GNC and GNL overexpression lines and GA pathway mutants share not only morphological and physiological phenotypes, but also molecular phenotypes, a finding that further supports the notion that GNC and GNL are important targets downstream from GA signaling and the DELLA regulators.

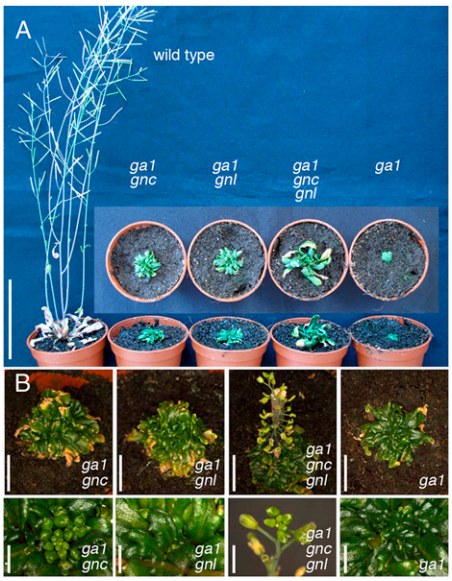
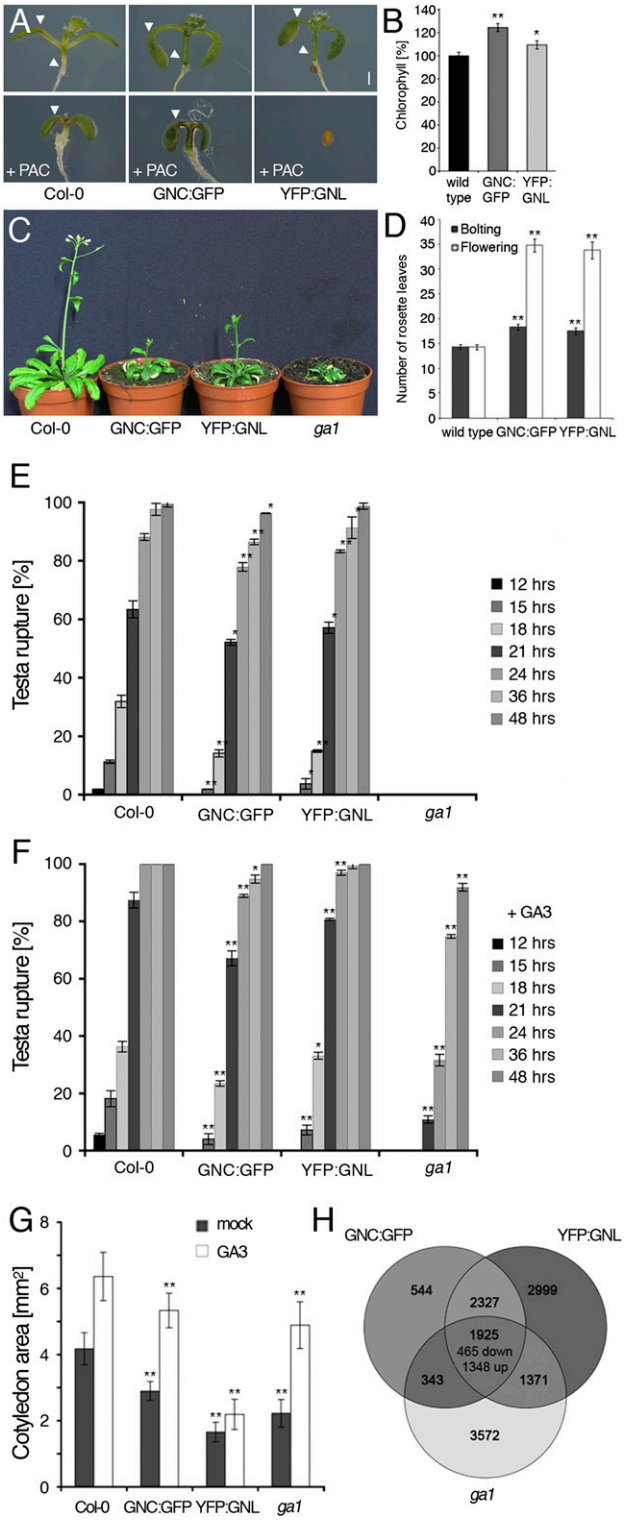


Figure 4. *gnc* and *gnl* mutations suppress the *ga1* phenotype. (*A*, *B*) Partial suppression of the rosette leaf expansion and flowering time phenotype of the GA biosynthesis mutant *ga1* by *gnc* and *gnl* loss-of-function alleles. The phenotypes of 7-wk-old (*A*) and 14-wk-old (*B*) plants are shown. The *bottom* panel in *B* shows a magnification of the meristems and inflorescence meristems of the plants shown in the *top* panel. Note the presence of inflorescences in *ga1 gnc* and *ga1 gnc gnl*. Bars: *A*, 5 cm ; *B*, *top* panel, 1 cm; *B*, *bottom* panel, 2 mm.

Feedback regulation of GA signaling components

We next examined for the existence of feedback control mechanisms in the *gnc* and *gnl* mutant backgrounds and overexpression lines. First, we could show that *GNC* and *GNL* transcripts are more abundant in *gnl* and *gnc* mutants, respectively, indicating that a homeostasis mechanism controls the transcript abundance of the two functionally redundant proteins (Fig. 6A). In turn, we found that none of the GA signaling components tested was differentially expressed in the *gnc gnl* double mutant, indicating that the respective loss-of-function mutant phenotypes are not caused by the misexpression of the respective genes (Fig. 6B). Interestingly, however, we found that the abundance of transcripts of GA3 oxidase 1 (GA3OX1), a GA-anabolizing enzyme, and GA2OX2, a GA-catabolizing enzyme, are reduced and increased, respectively, in *gnc gnl* mutants, suggesting that GA hormone levels are reduced due to increased GA turnover (Fig. 6C,D). Reduced GA levels may in fact be the cause for the increased RGA protein levels that we detected in the *gnc* and *gnc gnl* mutants (Fig. 6E). We thus concluded that the loss of GNC and GNL function triggers a negative feedback mechanism that may lead to increased GA turnover and the stabilization of RGA as well as that of other DELLA proteins not tested here. Since the phenotypes of *gnc* and *gnl* mutants are indicative of a derepression of the GA pathway, the concomitant accumulation of the DELLA repressor RGA further supports the notion that GNC and GNL function downstream from the DELLA repressors.



GNC *and* GNL *are target genes of PIF transcription factors*

The DELLA repressors of the GA signaling pathway control GA-regulated gene expression, at least in part, by

Figure 5. Transgenic GNC and GNL overexpression lines are PAC-hypersensitive and have increased chlorophyll biosynthesis and delayed flowering. (*A*) Ten-day-old seedlings overexpressing GNC (GNC:GFP) and GNL (YFP:GNL) grown on 1× MS and 1% sucrose in the absence and presence of 0.5 μM PAC. The downward-pointing arrowheads indicate the enhanced petiole angle of the overexpressor seedlings that is also observed in the wild type after PAC treatment. YFP:GNL-overexpressing seedlings fail to germinate at this PAC concentration. The other arrowheads point at the increased chlorophyll accumulation in the lower half of the hypocotyl that is observed in the overexpression lines but not in the wild type. (*B*) Quantification of the chlorophyll content of 8-d-old seedlings grown on 1× MS and 1% sucrose in the overexpression lines and the wild type. The overexpression lines are visibly darker green than the wild type. (*C*,*D*) Phenotypes of 5-wk-old GNC- and GNL-overexpressor plants of the T2 generation (*C*), and quantification of their delayed flowering time (*D*) (n = 12). (*E*,*F*) Quantification of the occurrence of testa rupture in imbibed seeds grown on 0.5× MS in the absence (*E*) or presence (*F*) of 10 μM GA3. The graphs in *E* and *F* show the averaged result of three independent germination experiments. $n \geq 50$; Student's t-test $P \leq 0.01$ (**) and $0.01 \leq P \leq 0.05$ (*). (*G*) Quantification of cotyledon expansion of 10-d-old seedlings grown in the absence (mock) and presence of 1 μM GA3. (*H*) Venn diagram comparing transcriptome changes that are detected in 5-d-old GNC:GFP, YFP:GNL, and *ga1* seedlings. Numbers indicate the number of differentially expressed genes in the respective genotypes and their intersections. Among the 1925 genes that are differentially expressed in all three genotypes, 465 are repressed and 1348 are induced in all three genotypes.

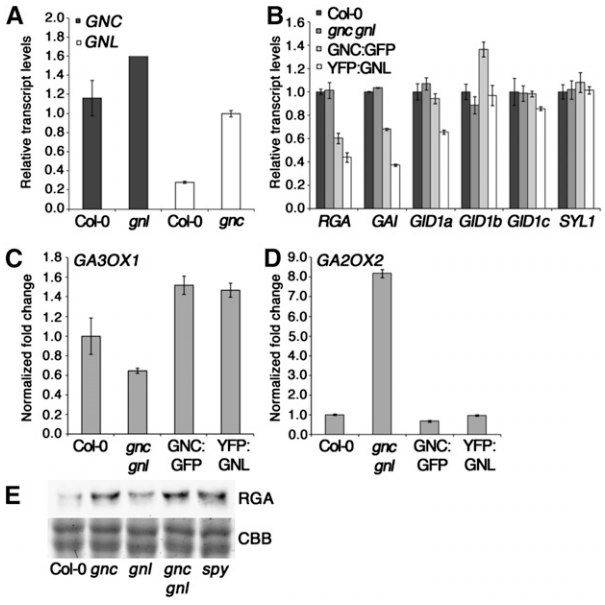


Figure 6. *GNC* and *GNL* homeostasis and feedback regulation on the transcription of GA signaling components. (*A*) Transcription of *GNC* and *GNL* in the *gnl* and *gnc* mutants, respectively, as detected by quantitative RT-PCR. (*B–D*) Expression levels of different GA signaling genes (*B*), *GA3OX1* (*C*), and *GA2OX2* (*D*) in *gnc gnl* loss-of-function mutants and *GNC* and *GNL* overexpression lines. (*E*) Immunoblot with an anti-RGA antibody from protein extracts of 5-d-old seedlings reveals elevated RGA protein levels in *gnc* and *gnc gnl* mutants. (CBB) Coomassie Brilliant blue-stained gel, loading control.

preventing PIF transcription factors from binding to their target genes (de Lucas et al. 2008; Feng et al. 2008). Since *PIF* mutants and overexpressors share phenotypes with *GNC* and *GNL* overexpressors and mutants—e.g., with regard to germination and chlorophyll accumulation—we hypothesized that *GNC* and *GNL* may be direct PIF targets (Monte et al. 2004; Stephenson et al. 2009). We therefore tested *GNC* and *GNL* expression in different *pif* loss-of-function mutant combinations and found increasing *GNC* and *GNL* expression with increasing *pif* mutant complexity (Fig. 7A). In turn, PIF overexpression led to a slight decrease in *GNC* and *GNL* expression (Fig. 7A). In summary, this suggests that PIFs are repressors of *GNC* and *GNL* expression, and that the correlation between DELLA abundance and *GNC* or *GNL* expression may be explained by the repression of PIFs by DELLA proteins (Fig. 7A). Strikingly, a direct comparison of the transcriptome of a *pif1 pif3 pif4 pif5* (*pif1345*) mutant with *GNC* and *GNL* overexpression lines revealed that >80% of all genes that are differentially expressed in *pif1345* are also differentially expressed in GNC and/or GNL overexpressors (Fig. 7B; Supplemental Table S2). Among 1624 genes that were differentially expressed in all three genotypes, we identified 1448 induced and 176 repressed transcripts. Thus, GNC or GNL overexpression phenocopy the loss of PIF function at the molecular level. Furthermore, the data show that PIFs and the GATA factors GNC and GNL regulate the transcription of their target genes in an antagonistic manner.

In further support of the proposed regulation of *GNC* and *GNL* by PIFs, we identified three G-/E-boxes—cognate binding sites of PIF transcription factors—upstream of the *GNC* and *GNL* coding regions (Fig. 7C; Frazer et al. 2004). We then immunoprecipitated PIF3:MYC(6x) to test whether *GNC* and *GNL* promoter fragments can be recognized by PIF3, at least when expressed from a high-level constitutive promoter (Clack et al. 2009). Indeed, we found that one predicted element of the *GNC* promoter as well as three predicted elements of the *GNL* promoter precipitate with PIF3:MYC(6x) (Fig. 7D,E; Supplemental Fig. 4). In summary, these data suggest that *GNC* and *GNL* are direct transcription targets of PIF3 (and possibly also of other PIFs), that their expression is repressed by PIFs, and that the two GATA transcription factors are important PIF target genes.

Discussion

In the present study, we identify the two GATA family transcription factors GNC and GNL as important downstream targets of DELLA proteins and PIF transcription factors. Our conclusions are supported by the observation that the loss of *GNC* and *GNL* and, conversely, their overexpression result in a number of phenotypic changes that identify GNC and GNL as activators of greening and repressors of germination, elongation growth, and flowering time. These processes have been shown previously to be regulated by the phytohormone GA and by PIF transcription factors. In addition to phenotypic similarities, our conclusions are further supported by the fact that *gnc* and *gnl* mutations partially suppress the GA biosynthesis defect of *ga1*, and that GNC or GNL overexpression induces gene expression changes that strongly resemble those of the GA biosynthesis mutant *ga1*. Thus, GNC and GNL repress developmental processes downstream from GA signaling and DELLA proteins.

Since the abundance of *GNC* and *GNL* transcript is reduced following GA treatment, and since we did not find any evidence for GA-mediated changes in GNC and GNL protein behavior, our data at present suggest that GNC and GNL activity is regulated at the transcriptional level. DELLA proteins regulate the activity of PIF transcription factors, and, in line with the demonstrated regulation of *GNC* and *GNL* transcript abundance by DELLA proteins, we could show that the expression of both genes is regulated by PIF transcription factors. This is manifested by the direct binding of PIF3 (at least when overexpressed) to the promoters of *GNC* and *GNL*, by the misexpression of *GNC* and *GNL* in *pif* mutants, and by the striking overlap in gene expression changes between a *pif1345* quadruple mutant and GNC or GNL overexpression lines. The fact that *GNC* and *GNL* transcript levels are increased in *pif* mutant backgrounds suggests that *GNC* and *GNL* may be direct transcriptional repression targets of PIFs. This model is also supported by a recent gene expression study in which *GNC* and *GNL* were found to be repressed following PIF1 overexpression (Oh et al. 2009), and another study that reveals that PIFs can act as transcriptional repressors as

well as transcriptional activators (Toledo-Ortiz et al. 2010). Since *gnc* and *gnl* mutants have a germination defect, and since PIF1 is the major PIF factor controlling germination, it may be that PIF1 controls germination by regulating *GNC* and *GNL* expression. We thus show that GNC and GNL act downstream from DELLA repressors and PIF transcription factors. In view of the fact that PIFs are also regulated by phytochrome B, it is also interesting to note that GNL was identified previously as a gene whose expression is strongly activated by light in a phytochrome-dependent manner (Naito et al. 2007). Thus, GNL and, most likely, also GNC integrate GA and light signals and mediate growth responses downstream from the PIFs.

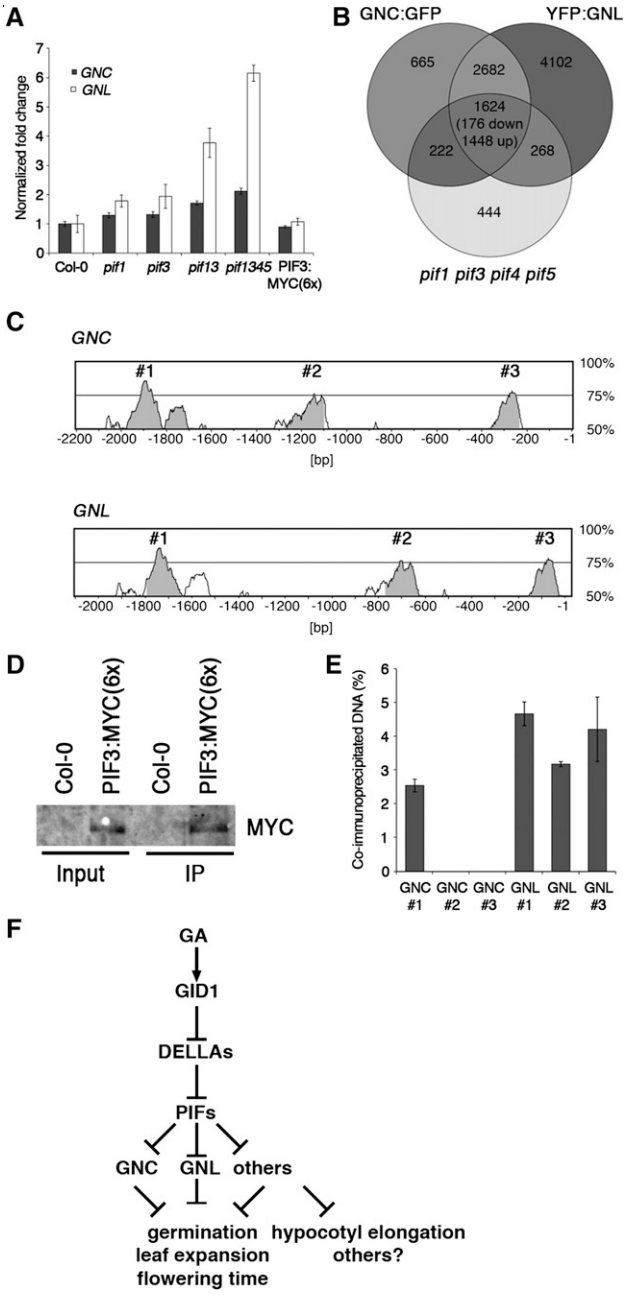


Figure 7. *GNC* and *GNL* are direct targets of PIF transcription factors. (*A*) Differential expression of *GNC* and *GNL* in 5-d-old dark-grown *pif* mutant seedlings and the PIF overexpression line PIF3:MYC(6×) as detected by quantitative RT–PCR. (*B*) Venn diagram comparing transcriptome changes that are detected in 5-d-old GNC:GFP, YFP:GNL, and *pif1 pif3 pif4 pif5* mutant seedlings. Numbers indicate the number of differentially expressed genes in the respective genotypes and their intersections. Among the 1624 genes that are differentially expressed in all three genotypes, 176 are repressed and 1448 are induced in all genotypes. (*C*) Presence of E-/G boxes in the upstream promoter regions of *GNC* and *GNL* as predicted by rVISTA (Loots et al. 2002). (*D*) Immunoblot for the detection of the MYC-tagged PIF3:MYC(6x) protein in the total extract (input) and following chromatin immunoprecipitation (IP). (*E*) Binding of the predicted E-/G boxes by PIF3:MYC(6x) as shown in *C* by quantitative RT–PCR (see also Supplemental Fig. S4). (*F*) Model of the predicted mode of action of the repressors GNC and GNL in the context of GA and PIF signaling as demonstrated based on genetic data from this study.

An important aspect of the present study resides in our finding that the two GATA transcription factors are essential for the repression of GA responses in *ga1*. To the best of our knowledge, to date, only loss-of-function mutants of DELLA proteins and SPY as well as a gain-of-function allele of the F-box protein SLY1 have been identified as *ga1* mutant suppressors (Wilson and Somerville 1995; Dill and Sun 2001; King et al. 2001; Lee et al. 2002). The fact that the effects of the loss of GNC and GNL on the *ga1* phenotype are most pronounced in the absence of both proteins may have prevented the identification of *GNC* and *GNL* mutant alleles in *ga1* suppression screens as yet. While the loss of DELLA protein genes is sufficient to largely normalize plant growth in *ga1*, the suppression of the *ga1* phenotype by *gnc* and *gnl* is comparatively less pronounced (Dill and Sun 2001; King et al. 2001; Lee et al. 2002). This may suggest that other proteins, possibly proteins related to GNC and GNL, repress GA signaling in *ga1*. In fact, our search for putative functional homologs of GNC and GNL in *Arabidopsis* and other species has led to the identification of additional candidate proteins with functional homology in *Arabidopsis* as well as in other plant species (Supplemental Fig. S1). We identified these proteins from monocots, dicots, and gymnosperms based on the presence of a highly conserved GATA DNA-binding domain as well as a highly conserved C-terminal domain (Supplemental Fig. S1B,C). We designated this domain, which is found exclusively in the proposed GNC and GNL homologs, the LLM domain, since all proteins identified share the consensus A-A-X-L-L-M-X-L-S (sequence listed using the one-letter code for amino acids, where X is any amino acid). In turn, we could not find additional proteins with similarity to the very N-terminal domain that is well conserved between GNC and GNL, suggesting that the similarity at the N terminus may be evidence for a recent gene duplication rather than evidence for functional significance (Fig. 1A). Although we cannot rule out that proteins unrelated to GNC and GNL have repressive function in the *ga1* mutant, the putative functional homologs identified here clearly represent candidate repressors.

Our mutant and overexpression analysis revealed a role for GNC and GNL in the control of germination, greening, elongation growth, and flowering time. However, we also identified at least two GA-controlled pathways that are seemingly not controlled by GNC and GNL. First, we did not find a strongly shortened hypocotyl in dark-grown seedlings of GNC and GNL overexpression lines, as could be expected based on the known hypocotyl phenotypes of GA pathway mutants and *pif* mutants such as *pif1345*. This suggests that GNC and GNL do not repress GA-controlled hypocotyl elongation. In line with this hypothesis, we found that the hypocotyls of GNC or GNL overexpression lines are still largely responsive to GA (data not shown). Second, we did not observe a derepression of the late-flowering phenotype of the *ga1* mutant in the *ga1 gnc gnl* background when the plants were grown in short-day conditions (data not shown). Thus, repressors other than GNC and GNL may repress GA responses in these tissues or growth conditions.

In summary, our identification of GNC and GNL as repressors in the GA signaling pathway introduces a new level of regulation to the pathway. Interestingly, GNC and GNL as well as all of their upstream signaling components are repressors of GA signaling. Thus, GA induces a cascade of derepression events that ultimately results in the transcriptional repression of *GNC* and *GNL* (Fig. 7F). Since *GNC* and *GNL* expression has also been shown previously to be regulated by cytokinin, nutrients, and light, GNC and GNL may mediate the cross-talk between these growth-regulating signals.

Materials and methods

Biological material

GNC and *GNL* T-DNA insertion lines were obtained from the Nottingham *Arabidopsis* Stock Centre (*gnc*, SALK_001778, AT5G56860; *gnl*, SALK_003995, AT4G26150); homozygous mutants were established by PCR-based genotyping (see Supplemental Table S1 for primer sequences) (Bi et al. 2005; Naito et al. 2007; Mara and Irish 2008). We failed repeatedly to confirm the T-DNA insertion in an additional previously reported *gnl* allele, SALK_021362 (Mara and Irish 2008). *ga1* (SALK_109115), *gid1abc*, *pif1*, *pif3*, *pif1 pif3*, *pif1 pif3 pif4 pif5* (*pif1345*), PIF3:MYC(6×), *rga*-24 *gai*-t6, and *spy*-3 mutants were described previously (King et al. 2001; Silverstone et al. 2007; Willige et al. 2007; Leivar et al. 2008; Clack et al. 2009).

Physiological experiments

For germination assays, all seeds were harvested from 8-wk-old plants and kept for 2 wk in paper bags in complete darkness at 23°C for after-ripening. Surface-sterilized seed were plated on Murashige and Skoog medium (pH 5.8), supplemented with 0.8% agar and PAC or GA3 as specified in the text. To examine germination, seeds were generally first incubated for 2 d at 4°C and then grown at 21°C in continuous white light (100 μmol m^{-2} sec^{-1}). Seed germination was quantified by scoring testa rupture, endosperm rupture, and radicle emergence at the time points indicated in the figures. Presented are the averaged results of three independent experiments ($n \geq 80$). Chlorophyll of 8-d-old seedlings was extracted and quantified as described previously; three independent replicates and measurements were performed (Inskeep and Bloom 1985). For flowering time analysis, plants were arranged randomly and grown in 150 μmol m^{-2} sec^{-1} white light in MobyLux GroBanks (CLF Plant Climatics) in long-day conditions (16 h/8 h, 21°C at 18°C). The time of bolting was scored by counting the number of rosette leaves and the number of days when the main stem had bolted 1 cm. Flowering time was scored as the number of days until visible buds had differentiated on the main stem.

Molecular techniques

To generate GNC:GFP and YFP:GNL, the ORFs of *GNC* and *GNL* were amplified by RT–PCR with GNC-GFP-attB1/attB2 and YFP-GNL-attB1/attB2 and cloned via pDONR201 (Invitrogen) into the Gateway-compatible vectors 35S-GW-GFP and pExtag-YFP, respectively. At least 10 transgenic lines were generated in the Col-0 ecotype and analyzed at the phenotypic level. Confocal miscroscopy of GNC:GFP and YFP:GNL was performed on 5-d-old seedlings using an Olympus FV1000/XI81 laser scanning confocal microscope (Olympus). To generate GNC_{Pro}:GUS and GNL_{Pro}:GUS, 2-kb promoter fragments were amplified from Col-0 genomic DNA using GNC Pro-LP/RP and GNL Pro-LP/RP, respectively, and inserted as EcoRI–BglII fragments into pCAMBIA1391Z. At least six transgenic lines were generated in the Col-0 ecotype and analyzed at the histochemical level as described previously (Dohmann et al. 2008). For GUS staining, seeds were fixed in 20% acetone for >12 h at −20°C prior to staining. All plant transformations were performed as described previously (Clough and Bent 1998). See Supplemental Table S1 for a list of primers.

Quantitative real-time PCR

Total RNA from stratified, imbibed, and germinating seeds was extracted as described previously (Vicient and Delseny 1999). Total RNA from 5-d-old etiolated and deetiolated seedlings was isolated with a NucleoSpin RNA plant kit (Machery-Nagel). DNA was removed by an on-column treatment with rDNase (Machery-Nagel), and 2 μg of total RNA was reverse-transcribed with an oligo(dT) primer and M-MuLV Reverse Transcriptase (Fermentas). The cDNA equivalent of 60–80 ng of total RNA was used in a 10-μL PCR reaction on a CFX96 Real-Time System Cycler with iQ SYBR Green Supermix (Bio-Rad). A 40-cycle two-step amplification protocol (10 sec at 95°C, 25 sec at 60°C) was used for all measurements. See Supplemental Table S1 for a list of relevant primers.

Immunoblots and chromatin immunoprecipitation

Immunoblots with anti-DELLA protein antibodies were preformed as described previously except that cycloheximide was omitted to test for DELLA protein accumulation rather than protein degradation (Willige et al. 2007; Piskurewicz et al. 2008). POR immunoblots were performed overnight at 4°C with an anti-POR antibody (Agrisera). Note that this antibody did not allow us to distinguish between the different POR isoforms. All immunoblots were incubated with SuperSignal Femto West substrate (Thermo Fisher Scientific) and detected with a LAS-4000 Mini-image analyzer (Fujifilm). For chromatin immunoprecipitation, seedlings were grown for 6 d in low light (5 μmol m^{-2} sec^{-1}) and then transferred to the dark for 2 d to allow for PIF protein accumulation. Chromatin immunoprecipitation quantitative RT–PCR was performed and analyzed as described previously (Oh et al. 2007; Fode and Gatz 2009). PIF3:MYC(6×) was precipitated with anti-c-Myc agarose and detected using a monoclonal anti-c-Myc antibody (Sigma-Aldrich).

Microarray analysis

Microarray analysis was performed using total RNA extracted from 5-d-old seedlings grown in 100 μmol m^{-2} sec^{-1} light using the NucleoSpin RNA plant kit (Machery-Nagel). Five-hundred nanograms of total RNA was prepared and labeled with Cy3 using the Quick Amp labeling protocol (Agilent Technologies). Three biological replicate samples were prepared for each genotype and *Arabidopsis* arrays (V4, design ID 21169; Agilent Technologies) were hybridized for 17 h at 65°C in rotating hybridization chambers (Agilent Technologies). Subsequently, the arrays were washed according to the manufacturer's instructions and scanned using an Agilent Microarray Scanner (Agilent Technologies). Total RNA and probe quality were controlled with a Bioanalyzer 2100 (Agilent Technologies). Raw data were extracted using the Feature Extraction software, version 10.5.1.1. (Agilent). Raw data files were imported into GeneSpring GX (version 11) and normalized choosing the scale-to-median and baseline-to-median options. Data were then filtered using the fold change algorithm (twofold change) and subsequently subjected to a one-way ANOVA analysis ($P \leq 0.05$) (Supplemental Table S2). Microarray data were deposited to GeneExpressionOmnibus and are accessible as GSE21256.

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References

Alabadi D, Gallego-Bartolome J, Orlando L, Garcia-Carcel L, Rubio V, Martinez C, Frigerio M, Iglesias-Pedraz JM, Espinosa A, Deng XW, et al. 2008. Gibberellins modulate light signaling pathways to prevent *Arabidopsis* seedling de-etiolation in darkness. *Plant J* **53:** 324–335.

Ariizumi T, Murase K, Sun T-P, Steber CM. 2008. Proteolysis-independent down-regulation of DELLA repression by the gibberellin receptor GIBBERELLIN INSENSITIVE DWARF 1. *Plant Cell*. **20:** 2447–2459

Bauer D, Viczian A, Kircher S, Nobis T, Nitschke R, Kunkel T, Panigrahi KC, Adam E, Fejes E, Schafer E, et al. 2004. Constitutive photomorphogenesis 1 and multiple photoreceptors control degradation of phytochrome interacting factor 3, a transcription factor required for light signaling in *Arabidopsis*. *Plant Cell* **16:** 1433–1445.

Belin C, de Franco PO, Bourbousse C, Chaignepain S, Schmitter JM, Vavasseur A, Giraudat J, Barbier-Brygoo H, Thomine S. 2006. Identification of features regulating OST1 kinase activity and OST1 function in guard cells. *Plant Physiol* **141:** 1316–1327.

Bi YM, Zhang Y, Signorelli T, Zhao R, Zhu T, Rothstein S. 2005. Genetic analysis of *Arabidopsis* GATA transcription factor gene family reveals a nitrate-inducible member important for chlorophyll synthesis and glucose sensitivity. *Plant J* **44:** 680–692.

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:** 105–113.

Castillon A, Shen H, Huq E. 2007. Phytochrome interacting factors: Central players in phytochrome-mediated light signaling networks. *Trends Plant Sci* **12:** 514–521.

Cheng H, Qin L, Lee S, Fu X, Richards DE, Cao D, Luo D, Harberd NP, Peng J. 2004. Gibberellin regulates *Arabidopsis* floral development via suppression of DELLA protein function. *Development* **131:** 1055–1064.

Clack T, Shokry A, Moffet M, Liu P, Faul M, Sharrock RA. 2009. Obligate heterodimerization of *Arabidopsis* phytochromes C and E and interaction with the PIF3 basic helix-loop-helix transcription factor. *Plant Cell* **21:** 786–799.

Clough SJ, Bent AF. 1998. Floral dip: A simplified method for Agrobacterium-mediated transformation of *Arabidopsis thaliana*. *Plant J* **16:** 735–743.

de Lucas M, Daviere JM, Rodriguez-Falcon M, Pontin M, Iglesias-Pedraz JM, Lorrain S, Fankhauser C, Blazquez MA, Titarenko E, Prat S. 2008. A molecular framework for light and gibberellin control of cell elongation. *Nature* **451:** 480–484.

Dill A, Sun T. 2001. Synergistic derepression of gibberellin signaling by removing RGA and GAI function in *Arabidopsis thaliana*. *Genetics* **159:** 777–785.

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:** 1392–1405.

Dohmann EM, Levesque MP, De Veylder L, Reichardt I, Jurgens G, Schmid M, Schwechheimer C. 2008. The *Arabidopsis* COP9 signalosome is essential for G2 phase progression and genomic stability. *Development* **135:** 2013–2022.

Feng S, Martinez C, Gusmaroli G, Wang Y, Zhou J, Wang F, Chen L, Yu L, Iglesias-Pedraz JM, Kircher S, et al. 2008. Coordinated regulation of *Arabidopsis thaliana* development by light and gibberellins. *Nature* **451:** 475–479.

Fode B, Gatz C. 2009. Chromatin immunoprecipitation experiments to investigate in vivo binding of *Arabidopsis* transcription factors to target sequences. *Methods Mol Biol* **479:** 261–272.

Frazer, K.A., Pachter, L., Poliakov, A, Rubin, E.M., and Dubchak, I. 2004. VISTA: Computational tools for comparative genomics. *Nucleic Acids Res* **32:** W273–W279. doi: 10.1093/nar/gkh458.

Gomi K, Sasaki A, Itoh H, Ueguchi-Tanaka M, Ashikari M, Kitano H, Matsuoka M. 2004. GID2, an F-box subunit of the SCF E3 complex, specifically interacts with phosphorylated SLR1 protein and regulates the gibberellin-dependent degradation of SLR1 in rice. *Plant J* **37:** 626–634.

Griffiths J, Murase K, Rieu I, Zentella R, Zhang ZL, Powers SJ, Gong F, Phillips AL, Hedden P, Sun TP, et al. 2006. Genetic characterization and functional analysis of the GID1 gibberellin receptors in *Arabidopsis*. *Plant Cell* **18:** 3399–3414.

Inskeep WP, Bloom PR. 1985. Extinction coefficients of chlorophyll a and b in N,N-dimethylformamide and 80% acetone. *Plant Physiol* **77:** 483–485.

Jacobsen SE, Olszewski NE. 1993. Mutations at the SPINDLY locus of *Arabidopsis* alter gibberellin signal transduction. *Plant Cell* **5:** 887–896.

King KE, Moritz T, Harberd NP. 2001. Gibberellins are not required for normal stem growth in *Arabidopsis thaliana* in the absence of GAI and RGA. *Genetics* **159:** 767–776.

Larkin MA, Blackshields G, Brown NP, Chenna R, McGettigan PA, McWilliam H, Valentin F, Wallace IM, Wilm A, Lopez R, et al. 2007. Clustal W and Clustal X version 2.0. *Bioinformatics* **23:** 2947–2948.

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 Dev* **16:** 646–658.

Leivar P, Monte E, Oka Y, Liu T, Carle C, Castillon A, Huq E, Quail PH. 2008. Multiple phytochrome-interacting bHLH transcription factors repress premature seedling photomorphogenesis in darkness. *Curr Biol* **18:** 1815–1823.

Loots GG, Ovcharenko I, Pachter L, Dubchak I, Rubin EM. 2002. rVista for comparative sequence-based discovery of functional transcription factor binding sites. *Genome Res* **12:** 832–839.

Mara CD, Irish VF. 2008. Two GATA transcription factors are downstream effectors of floral homeotic gene action in *Arabidopsis*. *Plant Physiol* **147:** 707–718.

Monte E, Tepperman JM, Al-Sady B, Kaczorowski KA, Alonso JM, Ecker JR, Li X, Zhang Y, Quail PH. 2004. The phytochrome-interacting transcription factor, PIF3, acts early, selectively, and positively in light-induced chloroplast development. *Proc Natl Acad Sci* **101:** 16091–16098.

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

Naito T, Kiba T, Koizumi N, Yamashino T, Mizuno T. 2007. Characterization of a unique GATA family gene that responds to both light and cytokinin in *Arabidopsis thaliana*. *Biosci Biotechnol Biochem* **71:** 1557–1560.

Oh E, Yamaguchi S, Hu J, Yusuke J, Jung B, Paik I, Lee HS, Sun TP, 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:** 1192–1208.

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:** 403–419.

Park E, Kim J, Lee Y, Shin J, Oh E, Chung WI, Liu JR, Choi G. 2004. Degradation of phytochrome interacting factor 3 in phytochrome-mediated light signaling. *Plant Cell Physiol* **45:** 968–975.

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 Dev* **11:** 3194–3205.

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

Schwechheimer C, Willige BC. 2009. Shedding light on gibberellic acid signalling. *Curr Opin Plant Biol* **12:** 57–62.

Shen H, Moon J, Huq E. 2005. PIF1 is regulated by light-mediated degradation through the ubiquitin-26S proteasome pathway to optimize photomorphogenesis of seedlings in *Arabidopsis*. *Plant J* **44:** 1023–1035.

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:** 520–523.

Silverstone AL, Ciampaglio CN, Sun T. 1998. The *Arabidopsis* RGA gene encodes a transcriptional regulator repressing the gibberellin signal transduction pathway. *Plant Cell* **10:** 155–169.

Silverstone AL, Tseng TS, Swain SM, Dill A, Jeong SY, Olszewski NE, Sun TP. 2007. Functional analysis of SPINDLY in gibberellin signaling in *Arabidopsis*. *Plant Physiol* **143:** 987–1000.

Stephenson PG, Fankhauser C, Terry MJ. 2009. PIF3 is a repressor of chloroplast development. *Proc Natl Acad Sci* **106:** 7654–7659.

Thomas H. 1997. Chlorophyll: A symptom and a regulator of plastid development. *New Phytol* **136:** 163–181.

Toledo-Ortiz G, Huq E, Rodriguez-Concepcion M. 2010. Direct regulation of phytoene synthase gene expression and carotenoid biosynthesis by phytochrome-interacting factors. *Proc Natl Acad Sci* **107:** 11626–11631.

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:** 693–698.

Ueguchi-Tanaka M, Nakajima M, Motoyuki A, Matsuoka M. 2007. Gibberellin receptor and its role in gibberellin signaling in plants. *Annu Rev Plant Biol* **58:** 183–198.

Ueguchi-Tanaka M, Hirano K, Hasegawa Y, Kitano H, Matsuoka M. 2008. Release of the repressive activity of rice DELLA protein SLR1 by gibberellin does not require SLR1 degradation in the gid2 mutant. *Plant Cell* **20:** 2437–2446.

Vicient CM, Delseny M. 1999. Isolation of total RNA from *Arabidopsis thaliana* seeds. *Anal Biochem* **268:** 412–413.

Willige BC, Ghosh S, Nill C, Zourelidou M, Dohmann EM, Maier A, Schwechheimer C. 2007. The DELLA domain of GA INSENSITIVE mediates the interaction with the GA INSENSITIVE DWARF1A gibberellin receptor of *Arabidopsis*. *Plant Cell* **19:** 1209–1220.

Wilson RN, Somerville CR. 1995. Phenotypic suppression of the gibberellin-insensitive mutant (gai) of *Arabidopsis*. *Plant Physiol* **108:** 495–502.