Convergence of auxin and gibberellin signaling on the regulation of the GATA transcription factors *GNC* and *GNL* in *Arabidopsis thaliana*

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Plant growth is regulated by a complex network of signaling events. Points of convergence for the signaling cross-talk between the phytohormones auxin and gibberellin (GA), which partly control overlapping processes during plant development, are largely unknown. At the cellular level, auxin responses are controlled by members of the AUXIN RESPONSE FACTOR (ARF) family of transcription factors as well as AUXIN/INDOLE-3-ACETIC ACID INDUCIBLE (AUX/IAA) proteins that repress the activity of at least a subset of ARFs. Here, we show that the two paralogous GATA transcription factors *GATA, NITRATE-INDUCIBLE, CARBON-METABOLISM INVOLVED* (*GNC*) and *GNC-LIKE* (*GNL*)/*CYTOKININ-RESPONSIVE GATA FACTOR1* (*CGA1*) are direct and critical transcription targets downstream from ARF2 in the control of greening, flowering time, and senescence. Mutants deficient in the synthesis or signaling of the phytohormone GA are also impaired in greening, flowering, and senescence, and interestingly, *GNC* and *GNL* were previously identified as important transcription targets of the GA signaling pathway. In line with a critical regulatory role for *GNC* and *GNL* downstream from both auxin and GA signaling, we show here that the constitutive activation of GA signaling is sufficient to suppress *arf2* mutant phenotypes through repression of *GNC* and *GNL*. In addition, we show that GA promotes ARF2 protein abundance through a translation-dependent mechanism that could serve to override the autoinhibitory negative feedback regulation of ARF2 on its own transcription and thereby further promote GA signaling.

The phytohormone auxin [indole-3-acetic acid (IAA)] regulates virtually all aspects of plant growth and development (1). At the cellular level, auxin responses are mediated by auxin response factors (ARFs) that are identified based on their ability to bind to promoter elements that confer auxin responsive gene expression [so-called auxin response elements (AuxREs)] (2, 3). Auxin responses also require the auxin-induced degradation of Aux/IAAs, which are repressors of a subgroup of ARF family transcription factors that are targeted for auxin-dependent degradation by the auxin receptor and E3 ubiquitin ligase SCF^{TIR1} and functionally related E3 ligase complexes (4, 5). Aux/IAAs also interact with the corepressor TOPLESS and its relatives, and ARF-targeted gene loci are transcriptionally inactive when ARFs are bound by Aux/IAAs and TOPLESS (6). Several independent studies as well as a larger genome-wide survey of pairwise ARF–Aux/IAA interactions have contributed to a picture where the ARF transcription factor family can be subdivided into ARF+ transcription activator ARFs that are negatively regulated by Aux/IAAs and ARF– repressor ARFs that interact only rarely with selected Aux/IAAs and therefore, would not be expected to be controlled by auxin and Aux/IAAs (3, 4, 7, 8).

Mutants expressing stabilized auxin-insensitive Aux/IAA variants have been described that, at least in some cases, mimic the loss-of-function phenotypes of their ARF interactors (9–13). Based on such phenotypic similarities, the Aux/IAA protein SOLITARY ROOT (SLR/IAA14) was recognized as the repressor of the functionally redundant ARF+ proteins ARF7 and ARF19 (positive regulators of lateral root formation) (7, 10, 11, 14, 15). Loss-of-function mutants of the ARF– protein ARF2 were described as mutants with defects in greening, senescence, flowering time, and floral organ abscission (16–18). Interestingly, *arf2* mutant phenotypes are enhanced in mutants of the ARF+ proteins ARF7 and ARF19, suggesting that the Aux/IAA-independent ARF– ARF2 and Aux/IAA-dependent ARF+ ARF7 and ARF19 function together to control the same growth processes, presumably by regulating the same target genes (the identity remains to be determined) (16–19).

Gibberellins (GAs) are another family of plant hormones, and they are well known for their role in the control of germination, greening, and flowering time (20). The GA-labile DELLA proteins are key regulators of GA signaling that function by repressing different types of transcription factors, including the PHYTOCHROME INTERACTING FACTORs (PIFs) (21–23). We have recently identified the two paralogous GATA family transcription factors *GATA, NITRATE-INDUCIBLE, CARBON-METABOLISM INVOLVED* (*GNC*) and *GNC-LIKE* (*GNL*)/*CYTOKININ-RESPONSIVE GATA FACTOR1* (CGA1) as critical transcription targets downstream from GA, DELLAs, and PIFs in *Arabidopsis* (24). *GNC* and *GNL* expression is repressed in response to GA, and this repression is important for proper germination, greening, flowering, and elongation growth.

Here, we show that several phenotypes of *GNC* and *GNL* overexpressors can also be observed in mutants of the auxin pathway regulators *ARF2* and *SLR*. This observation has triggered our interest in examining a possible relationship between the two GATA transcription factors and the auxin signaling components. Interestingly, we find that *GNC* and *GNL* are critical transcription targets downstream from ARF2 and the Aux/IAA repressor SLR. Importantly, we also find that constitutive activation of GA signaling allows compensation for the loss of *ARF2*, thus strongly indicating that *GNC* and *GNL* integrate auxin and GA signals for the control of plant growth.

Results

GNC and GNL Restrain Growth in the Absence of ARF2. We have previously generated and characterized loss-of-function mutants and overexpression lines of the two GATA factors *GNC* (GNC:GFP) and *GNL* (YFP:GNL) (24). During the characterization of the GNC:GFP and YFP:GNL overexpression lines, we noticed that the GATA-overexpressing plants share a number of phenotypes

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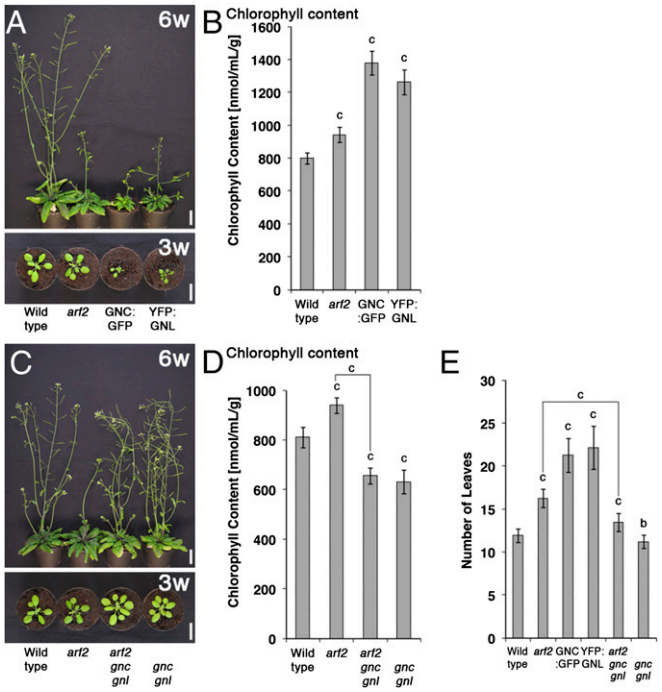

Fig. 1. Genetic interaction of *ARF2* with *GNC* and *GNL*. (*A* and *C*) Representative photographs of 6- (6w) and 3-wk-old (3w) plants. (Scale bars: 2 cm.) (*B* and *D*) Absolute chlorophyll content of 10-d-old light-grown seedlings. (*E*) Number of leaves until bolting as a measure of flowering time. The average and SE of three replicate measurements are shown in *B*, *D*, and *E*. Student *t* test: b = $P \leq 0.01$; c = $P \leq 0.001$.

with previously described *arf2* loss-of-function mutants (16, 18). Similarly to *arf2* mutants, *GNC* and *GNL* overexpressors accumulate chlorophyll and are delayed in senescence as judged by the degradation of chlorophyll after the transfer of leaves from light-grown plants to the dark (Fig. 1*A* and *B* and Fig. S1*A*) (25). Importantly, these *arf2* phenotypes are strongly suppressed when we introduce *gnc* and *gnl* loss-of-function alleles into *arf2*, indicating that *GNC* and *GNL* are important regulators downstream from *ARF2* (Fig. 1 *C* and *D* and Figs. S1*B* and S2). Additional phenotypic analyses revealed additional shared phenotypes between the *GNC* and *GNL* overexpressors on the one side and *arf2* on the other side in the control of flowering time, stamen elongation, and floral organ abscission as well as seed size; in each case, the defects of the *arf2* mutant are suppressed in *arf2 gnc gnl* triple mutants (Fig. 1*E* and Fig. S1 *C–E*). The suppression of the *arf2* phenotype in *arf2 gnc gnl* is also apparent at the gene expression level, because an expression analysis of the *PROTOCHLOROPHYLLIDE OXIDOREDUCTASE* genes (*PORA*, *PORB*, and *PORC*) that encode important enzymes in chlorophyll biosynthesis as well as a global gene expression analysis showed a partial or full suppression of the gene expression defects of *arf2* in *arf2 gnc gnl* (Fig. S1 *F* and *G*) [e.g., 45% (376 genes) of 840 genes that we found to be differentially regulated in *arf2* compared with the WT are antagonistically regulated in *arf2 gnc gnl* (Fig. S1*G* and Dataset S1)].

***GNC* and *GNL* Promoters Are Bound by ARF2 and ARF7.** The phenotypic similarities between *GNC* and *GNL* overexpressors and *arf2* mutants could potentially be explained by increased expression of the GATA genes in *arf2*, and indeed, we detected increased transcript levels of both GATAs in *arf2* mutants (Fig. 2*A*). To test whether ARF2 can bind to the *GNC* and *GNL* promoters, we performed ChIP with ARF2:GFP using a transgenic line that expresses the ARF2:GFP fusion protein under control of the *ARF2* promoter (26). In this experiment, we detected binding of ARF2:GFP to two promoter regions that span a total of four predicted AuxREs (TGTCTN) in the *GNC* promoter and one predicted AuxRE in the *GNL* promoter (Fig. 2*B* and Table S1) (2). We also performed ChIP analysis using an antibody directed against K9-acetylated HISTONE3 (H3K9Ac), which marks open chromatin, to find evidence on whether increased transcript abundance is a result of increased transcription (27). In this experiment, we detected increased H3K9Ac binding in *arf2* mutants at promoter sites (Hb) located in proximity of the *GNC* and *GNL* transcription start sites (Fig. 2*C*). At the same time, H3K9Ac binding was unaltered at distal promoter sites (Ha), and therefore, we concluded that the increased *GNC* and *GNL* abundance in *arf2* is the result of increased *GNC* and *GNL* transcription (Fig. 2*C*). In summary, these findings invite the conclusion that ARF2 binds directly to the *GNC* and *GNL* promoters and represses the expression of the two GATA genes.

To understand the regulation of *GNC* and *GNL* transcription by ARF2 and auxin, we examined the effect of auxin on the expression of *GNC* and *GNL* in the WT and *arf2* mutants. We found that *GNC* and *GNL* transcript abundance is strongly decreased after auxin treatment, suggesting that the expression of the two GATAs is under control of an auxin-regulated transcription repressor (Fig. 2 and Fig. S3). Interestingly, the effect of auxin on the repression of the GATAs was much more

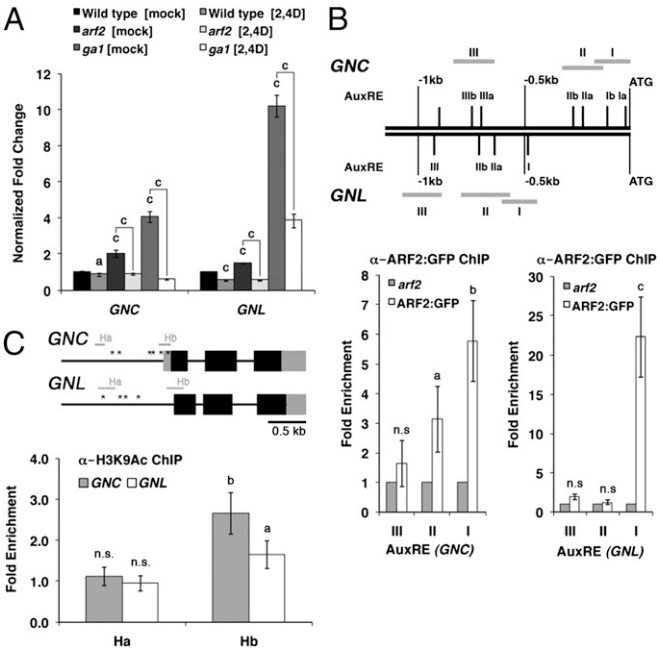

Fig. 2. Transcriptional regulation of *GNC and GNL* by ARF2. (*A*) Quantitative RT-PCR (RT-qPCR) analyses of *GNC* and *GNL* expression in WT and *arf2* as well as *ga1* mutant seedlings after a 30-min treatment with 5 μM 2,4D (2,4D) or mock (mock) solution. The fold change relative to transcript levels of mock-treated WT seedlings is shown. (*B*) Schemes of the *GNC* and *GNL* promoter regions (*Upper*). Roman numbers indicate the predicted AuxREs; gray bars indicate promoter regions selected for amplification after ChIP. The fold enrichment (ARF2:GFP/*arf2*) of AuxRE amplification after ChIP-PCR (*Lower*). (*C*) Schemes of the *GNC* and *GNL* genomic loci (*Upper*). Black boxes indicate exons; gray boxes indicate UTRs. Ha and Hb correspond to two regions amplified after H3K9Ac ChIP. Asterisks indicate the positions of the AuxRE binding sites examined in *B*. Please note that there is no 5′ UTR known for *GNL*. The fold enrichment (*arf2*/*wild type*) of Ha and Hb amplification after ChIP-qPCR (*Lower*); 10-d-old seedlings were used for all experiments, and the average and SE of three replicate measurements are shown. Student *t* test: a = $P \leq 0.1$; b = $P \leq 0.05$; c = $P \leq 0.01$; n.s., not significant.

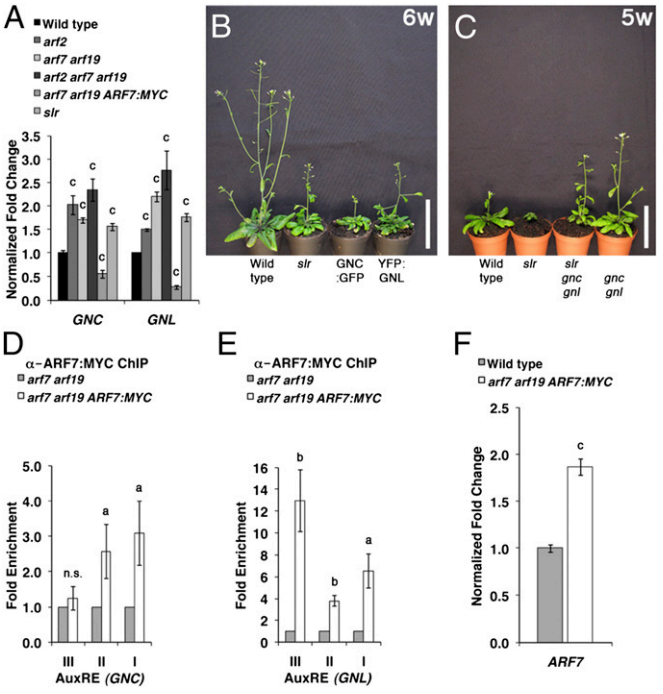

Fig. 3. Interaction between *SLR1/IAA14* and *ARF7* with *GNC* and *GNL*. (*A*) Detection of *GNC* and *GNL* by RT-qPCR from 10-d-old light-grown WT, *slr*, and *arf* mutant seedlings. Shown is the fold change relative to WT levels. (*B* and *C*) Representative photographs of 6- (6w) and 5-wk-old (5w) plants grown in long-day conditions. (Scale bars: 5 cm.) (*D* and *E*) qPCR result after ChIP with an anti-MYC antibody (ARF7:MYC). Shown is the fold enrichment (ARF7:MYC/*arf7 arf19*) by qPCR after ChIP of individual AuxRE-containing promoter regions present in the (*D*) *GNC* and (*E*) *GNL* promoters (Fig. 2*B*). (*F*) RT-qPCR analysis of *ARF7* expression in WT and *arf7 arf19 ARF7:MYC*; 10-d-old seedlings were used for all experiments, and the average and SE of three replicate measurements are shown. Student *t* test: a = $P \leq 0.1$; b = $P \leq 0.05$; c = $P \leq 0.01$; n.s., not significant.

pronounced in the *arf2* and *ga1* mutants, where *GNC* and *GNL* expression is derepressed (Fig. 2*A*). Together, these observations gave rise to the hypothesis that other auxin-dependent regulators must control *GNC* and *GNL* transcription in the absence of *ARF2*. When examining the phenotypes of other relevant mutants, we noticed that *slr* gain-of-function mutants also share phenotypes with the *GNC* and *GNL* overexpressors as well as *arf2* mutants (11, 16); *slr* mutants were similar to *GNC* and *GNL* overexpressors at least with regard to their increased chlorophyll content, delayed flowering, and increased seed size (Fig. S4) (11). In line with the idea that SLR/IAA14 as well as its established interacting ARFs ARF7 and ARF19 are involved in GATA regulation, we detected increased *GNC* and *GNL* transcript levels in the *slr* gain-of-function mutant and the *arf7 arf19* loss-of-function mutant, and compared with *arf2* or *arf7 arf19*, even greater increased transcript levels were detected in the *arf2 arf7 arf19* triple mutant (Fig. 3*A*). We then tested genetically whether the *slr* phenotypes are caused by increases in *GNC* and *GNL* abundance and found that *slr* mutant phenotypes are partially or fully suppressed in *slr gnc gnl* (Fig. 3*C* and Fig. S4). Taken together, these data suggested that *GNC* and *GNL* repress growth downstream from *SLR/IAA14*. At the same time, the transcript abundance of the GATAs was decreased to levels even below those levels detected in the WT in a transgenic line expressing ARF7:MYC in an *arf7 arf19* background (Fig. 3*A*). Because these data indicated that ARF7 and ARF19 may act as repressors of *GNC* and *GNL* expression, we examined next whether ARF7 can bind to the *GNC* and *GNL* promoters. Indeed, ChIP studies provided proof for the direct binding of ARF7:MYC to five of six AuxRE-containing sites of the *GNC* and *GNL* promoters (Fig. 3 *D* and *E*). Because we used a 35S cauliflower mosaic virus (CaMV) promoter-driven ARF7:MYC line for this experiment and because transcription factor overexpression may lead to the binding and regulation of off-target gene promoters, we also examined the degree of *ARF7* overexpression in ARF7:MYC. However, because only a twofold increase in the expression of *ARF7* was detectable in the ARF7:MYC transgenic line compared with WT, we consider it very unlikely that the observed repression of *GNC* and *GNL* is the result of an off-target binding event (Fig. 3*F*).

Suppression of Auxin Mutant Phenotypes by Promoting GA Signaling. We had previously shown that *GNC* and *GNL* are repressors of GA signaling downstream from DELLAs and PIFs (20, 24). Because our analysis of *GID1* GA receptor gene expression and GA-induced DELLA protein degradation provided no evidence for an impairment of GA signaling in *arf2* mutants (Fig. S5), we hypothesized that it may be possible to suppress *arf2* growth defects by promoting GA and PIF signaling. Indeed, when we examined the *arf2* flowering phenotype in mock and GA-treated *arf2* mutant plants, we observed a suppression of the *arf2* flowering time delay, which correlated with a decrease in *GNC* and *GNL* transcript abundance in the GA-treated *arf2* mutants (Fig. 4 *A*–*C*). To obtain additional support for a suppression of auxin pathway defects through activation of the GA signaling cascade, we introduced the constitutive GA response mutant *spindly* (*spy*) into *arf2* (28); *spy* mutants are deficient in the function of the O-linked N-acetylglucosamine transferase SPY, they resemble plants that have been grown in the presence of high doses of

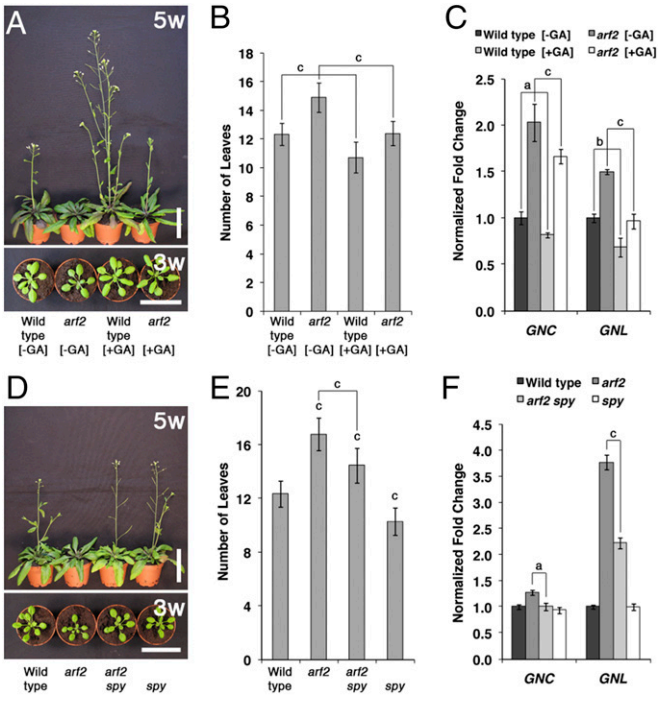

Fig. 4. Suppression of *arf2* by activation of the GA signaling pathway. (*A* and *D*) Representative photographs of 5- (5w) and 3-wk-old (3w) plants. Plants in *A* were watered every other day with a mock (–GA) or 100 μM GA3-containing solution (+GA). (*B* and *E*) Flowering time analysis. Number of leaves until bolting. (*C* and *F*) RT-qPCR analyses to examine the levels of *GNC* and *GNL* transcript after a mock (–GA) or 30-min treatment with 10 μM GA3 (+GA). Fold change relative to (mock-treated) WT levels; 10-d-old seedlings were used for all experiments, and the average and SE of three replicate measurements are shown. Student *t* test: a = $P \leq 0.05$; b = $P \leq 0.01$; c = $P \leq 0.001$.

GA, and they accumulate DELLA proteins in a seemingly inactive state (28–30). Interestingly, we found that the loss of SPY function is sufficient to at least partially suppress the flowering time phenotype of *arf2* mutants and that the suppression of the *arf2* phenotype correlates with decreases in *GNC* and *GNL* transcript abundance (Fig. 4 *D–F*). In summary, these findings suggested that the repression of *GNC* and *GNL* gene expression through constitutive activation of the GA pathway can, indeed, suppress defects of mutants with a defect in auxin signaling.

To verify that auxin and GA signaling act independently on the control of *GNC* and *GNL* expression, we additionally examined the effects of ARF2 and PIF1 (as a representative PIF protein) on *GNC* and *GNL* expression. To this end, we introduced a *PIF1* overexpression line (*PIF1-TAP*) into the *arf2* background and examined the effects of the presence and absence of the ARF2 and PIF1 repressors on the transcript abundance of the GATAs. In line with ARF2 and PIF1-TAP being independent repressors of *GNC* and *GNL*, we observed intermediate *GNC* and *GNL* expression levels in the *PIF1-TAP arf2* background compared with the *arf2* mutant and the *PIF1-TAP* line, where *GNC* and *GNL* expression is derepressed and strongly repressed, respectively (Fig. S6). Additionally, we found, as presented earlier in this study, that the auxin-induced repression of *GNC* and *GNL* does not require GA, because the transcript abundance of the *GATAs* could be efficiently downregulated by auxin in the GA-deficient *ga1* mutant (Fig. 2*A*). In summary, our findings strongly support the notion that GA and auxin signaling act independently to control *GNC* and *GNL* expression and that these two GATAs are critical for the control of GA- and auxin-controlled growth responses.

GA Promotes ARF2 Abundance. We also examined putative effects of auxin and GA on ARF protein abundance. Interestingly, although we found no evidence for a regulation of ARF2:GFP or ARF7:MYC protein by auxin (Fig. S7), we noted that the abundance of ARF2:GFP but not ARF7:MYC increases in response to GA treatment (Fig. 5*A* and Fig. S8). Importantly, ARF2:GFP abundance decreased after treatment with the protein biosynthesis inhibitor cycloheximide (CHX) (Fig. 5*A*). Because concomitant treatment with the proteasome inhibitor MG132 could prevent this degradation, we concluded that ARF2 is degraded by the 26S proteasome (Fig. 5*A*). Additionally, because combined treatments with CHX and GA were not sufficient to inhibit the ARF2:GFP turnover, we concluded that ARF2:GFP translation may be under GA control or alternatively, that a de novo synthesized and unknown GA-responsive protein may regulate ARF2:GFP abundance (Fig. 5*A*). In agreement with the role of ARF2 as a repressor of *GNC* and *GNL*, we found that the GA-promoted increase in ARF2:GFP abundance correlated with an increased binding of ARF2:GFP to AuxREs of the *GNC* and *GNL* promoters on the one side and increased *GNC* and *GNL* repression on the other side (Fig. 5 *B–D*). Importantly, this GA-induced transcriptional repression of *ARF2* could not be detected in the GA-insensitive *gid1abc* triple mutant, where ARF2 abundance cannot be promoted by GA because of the absence of all three *GID1* GA receptor genes that confer complete GA insensitivity (Fig. 5 *C* and *D*) (31). We, therefore, concluded that ARF2:GFP levels are positively regulated by the GA signaling pathway. Interestingly, we also found that the increase in ARF2:GFP abundance after GA treatment and the decrease in ARF2:GFP abundance after GA/CHX treatment correlated negatively and positively, respectively, with a decrease in *ARF2* transcript abundance (Fig. 5*E*). Because our subsequent ChIP analyses then revealed that ARF2:GFP can bind to AuxREs in the *ARF2* promoter, we concluded that ARF2 protein may repress its own transcription as part of a negative feedback regulatory loop (Fig. 5*F*). Thus, the GA-dependent increase in ARF2 abundance may serve as a translation-dependent mechanism that promotes ARF2 abundance and overrides the autoinhibitory negative feedback of ARF2 on its own transcription in the presence of GA.

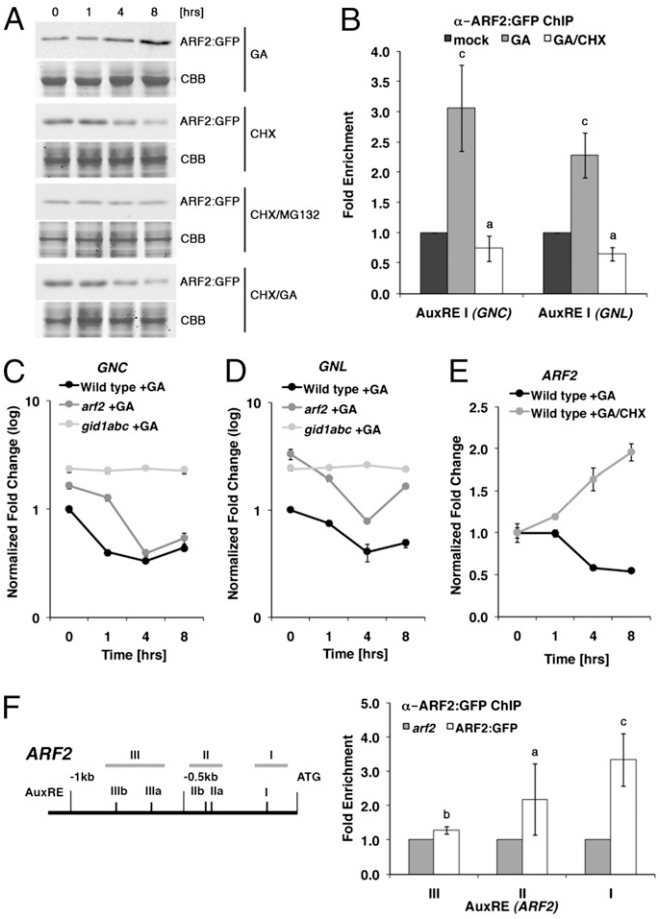

Fig. 5. GA promotes ARF2 abundance. (*A*) ARF2:GFP immunoblot analyses of 10-d-old light-grown seedlings treated with 10 μM GA3, 50 μM CHX, and 100 μM MG132 as indicated. CBB, Coomassie Brilliant Blue (loading control). (*B*) ARF2:GFP ChIP on selected AuxRE-containing promoter elements of the *GNC and GNL* promoters (Fig. 2*B*). The fold enrichment (ARF2:GFP/*arf2*) of AuxRE amplification is shown. (*C–E*) RT-qPCR analyses for *ARF2*, *GNC*, and *GNL* after 10 μM GA3 treatment; *gid1abc* is a GA receptor loss-of-function triple mutant (31). (*F*) Scheme of the *ARF2* promoter with predicted AuxREs and promoter regions used for ChIP. The fold enrichment (ARF2:GFP/*arf2*) of regions containing AuxREs after qPCR amplification is shown. Student *t* test: a = $P \leq 0.05$; b = $P \leq 0.01$; c = $P \leq 0.001$.

Discussion

In our study, we identify the *Arabidopsis* GATA transcription factors *GNC* and *GNL* as critical transcription targets downstream from auxin signaling in the control of different physiological growth processes, including greening, flowering time control, and senescence (Fig. 6). We have previously established *GNC* and *GNL* as transcription targets of GA signaling and show here that the two GATAs are also critical and direct transcription targets of the auxin pathway (24). We reveal that both GA and auxin signaling function independently in the repression of *GNC* and *GNL* and that the activation of the GA pathway allows suppressing phenotypes of auxin response mutants (Fig. 6). Based on our observations, we conclude that GNC and GNL are threshold-dependent regulators that are targeted by both pathways to control plant growth.

Most research on auxin signal transduction is concerned with the role of auxin in the control plant development, differentiation,

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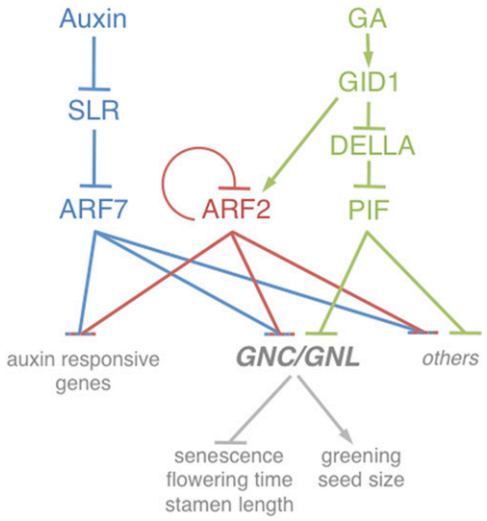

Fig. 6. Model of the control mechanisms acting on the transcriptional control of *GNC and GNL*. *GNC* and *GNL* expression is repressed by ARF2 (red) and ARF7 (blue). In addition, the abundance of their transcripts is under the direct repression control of the GA signaling cascade and the PIF repressors as well as indirect GA control by the stabilization of ARF2 (green). Other genes that are under ARF and PIF control may also be regulated in a manner similar to *GNC* and *GNL*. In addition, gene expression events that are under control of the auxin pathway may also be regulated by GA signaling because of the control of ARF2 abundance by GA. The individual pathways are shown in distinct colors.

and morphology. In the case of ARF7, ARF19, and SLR/IAA14, the control of lateral root formation by activation of the genes *LATERAL ORGAN BOUNDARIES DOMAIN16* and *LATERAL ORGAN BOUNDARIES DOMAIN18* has been given particular attention (32). Our study now emphasizes the role of auxin in the regulation of physiological growth responses, such as greening, flowering time, and senescence. Although such phenotypes were already described for *arf2* mutant combinations several years ago, the underlying molecular mechanisms remained elusive (16–18). Through the analysis of *slr* and *arf7 arf19* mutants, we emphasize here the fact that other auxin mutants have—other than their already well-understood defects in controlling lateral root development—defects in physiological growth responses (11, 16–18). At the same time, we identify *GNC* and *GNL* as critical target genes downstream from these auxin pathway regulators. Unlike the ARF+ proteins ARF7 and ARF19, several pieces of evidence support the notion that the ARF– protein ARF2 is not controlled by Aux/IAA repressors (3, 7). These findings suggest that ARF– proteins, such as ARF2, may negatively control *GNC* and *GNL* expression in an auxin-independent manner and that auxin, through derepression of ARF+-type ARFs, can only partially modulate the expression of the two GATAs (7). Obviously, it can be expected that other ARF target genes are also regulated by auxin-independent and -dependent ARFs that compete for the same AuxRE binding sites in the promoters of the respective genes (Fig. 6).

Interestingly, we show that *GNC* and *GNL* are repressed by the Aux/IAA SLR as well as ARFs. Auxin-induced transcriptional repression has been reported already in several studies (33–36) but cannot be explained by the current model of Aux/IAA repressor function, according to which the auxin-dependent degradation of the Aux/IAAs leads to the derepression of ARFs acting as transcriptional activators (6). Our data on the regulation of *GNC* and *GNL* and data in the published literature, thus, suggest that other mechanisms must exist to control the repressor activity of Aux/IAA-controlled ARFs. It can be envisioned that Aux/IAAs inhibit the DNA binding activity of repressor ARFs, Aux/IAA degradation allows for the regulation of ARFs by post-translational modifications (37), or Aux/IAA degradation allows for the formation of repressive heterodimers of ARFs with other ARFs or other transcription regulators (7, 38). Which mechanism operates in the context of the transcriptional repression of GNC and GNL is unclear at present.

Our study also suggests a molecular mechanism for the GA–auxin cross-talk, where GA promotes the abundance of ARF2 and thereby enhances the repression of *GNC* and *GNL* transcription in response to GA (Fig. 6). At the same time, we also establish that ARF2 represses its own transcription through negative feedback regulation (Fig. 6). Because the effect of GA on ARF2 protein abundance must be dominant over the repressive effects of ARF2 on its own transcription, this feedback regulatory mechanism should help to ensure the dominant role of GA pathway activation over ARF2-dependent signaling (Fig. 6). Interestingly, GA does not increase the abundance of the ARF+ protein ARF7, and therefore, the GA-dependent increase of ARF2 could represent a molecular mechanism that serves to enhance GA signaling through the recruitment of auxin and Aux/IAA-independent ARFs. This GA-dependent regulatory mechanism may, thus, allow for the uncoupling of ARF-controlled gene expression from its regulation by auxin, Aux/IAAs, and ARF+ regulators.

In summary, our analysis identifies GNC and GNL as critical repressors of plant growth downstream from auxin and GA signaling. *GNC* and *GNL* had originally been identified based on their transcriptional regulation by nitrate and by cytokinin and light, respectively (39, 40). Although the light control of *GNL* expression could be mediated by PIFs, which are degraded in response to light-dependent interactions with the phytochrome red light receptors (41), the signaling pathways that control responses to the growth-promoting nitrate and cytokinin hormone signals remain to be identified. In fact, the *spy* mutant had also been described as a cytokinin-insensitive mutant (42, 43). It may, thus, be envisioned that the regulation of *GNC* and *GNL* by cytokinin is mediated by SPY and that the role of SPY in cytokinin signaling may interfere with the interpretation of our genetic interaction analysis between *ARF2* and *SPY*. However, we found that *GNC* and *GNL* transcript levels were unaltered in *spy* compared with the WT (Fig. 4*F*), and thus, altered cytokinin signaling in *spy* should not interfere with the suppression of *arf2* in *arf2 spy*. Regardless of the underlying molecular mechanisms, the control of *GNC* and *GNL* expression by three hormone signaling pathways (auxin, GA, and cytokinin) as well as their regulation by nitrate suggest that the transcriptional regulation of these GATA factors is critical for the integration of multiple growth-controlling signals during plant growth and development. Identification of their transcription targets will further add to the understanding of this intriguing signaling network.

Materials and Methods

Biological Material. The following mutants and transgenic lines were used in this study: *arf2-5*, *arf2-8*, and *arf2 arf7 arf19* (16); ARF2:GFP (ARF2:ARF2:GFP) (26); *arf7 arf19* (10); ARF7:MYC (35S:ARF7:MYC; gift from Hidehiro Fukaki, Kobe University, Kobe, Japan); *gnc*, GNC:GFP (35S:GNC:GFP), *gnl*, YFP:GNL (35S:YFP:GNL), and *ga1* (24); *slr* (11); *gid1abc* (31); and PIF1-TAP (44). All mutants and transgenic lines are in the *A. thaliana* ecotype Columbia.

Physiological Experiments. For chlorophyll measurements, chlorophyll was extracted and quantified as previously described; three independent replicates and measurements were performed (45). Basal chlorophyll levels were quantified from 10-d-old seedlings. The senescence assay was performed using a previously established method (25). Chlorophyll was extracted from leaf numbers 3 and 5 of 21-d-old plants (set to 100%) and plants that were subsequently kept for 4 d in liquid medium in the dark. For flowering time analyses, plants were randomly arranged and grown in 150 μmol m^{-2} s^{-1} white light in MobyLux GroBanks (CLF Plant Climatics) in long-day conditions

(16/8 h at 21 °C/18 °C). The time of bolting was scored from at least 18 plants by counting the number of rosette leaves (46). Seed size was determined from at least 200 seeds per genotype. Floral organ abscission was determined as previously described by counting the last floral bud that still retained sepals and/or petals starting from the top of the inflorescence (16).

Quantitative Real-Time PCR. Total RNA 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 total RNA were reverse transcribed with an oligo(dT) primer and M-MuLV Reverse Transcriptase (Fermentas). The cDNA equivalent of 60–80 ng total RNA was used in a 10 μL PCR in a CFX96 Real-Time System Cycler with iQ SYBR Green Supermix (BioRad). A 40-cycle two-step amplification protocol (10 s at 95 °C and 25 s at 60 °C) was used for all measurements. Relevant primers are listed in Table S2.

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