Putative *Arabidopsis* Transcriptional Adaptor Protein (PROPORZ1) is required to modulate histone acetylation in response to auxin

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Plant development is highly adaptable and controlled by a combination of various regulatory circuits that integrate internal and environmental cues. The phytohormone auxin mediates such growth responses, acting as a dynamic signal in the control of morphogenesis via coordinating the interplay between cell cycle progression and cell differentiation. Mutants in the chromatin-remodeling component *PROPORZ1 (PRZ1;* also known as *AtADA2b)* are impaired in auxin effects on morphogenesis, suggestive of an involvement of PRZ1-dependent control of chromatin architecture in the determination of hormone responses. Here we demonstrate that *PRZ1* is required for accurate histone acetylation at auxin-controlled loci. Specifically, *PRZ1* is involved in the modulation of histone modifications and corresponding adjustments in gene expression of *Arabidopsis KIP RELATED PROTEIN* (*KRP*) CDK inhibitor genes in response to auxin. Deregulated *KRP* expression in *KRP* silencer lines phenocopies *prz1* hyperproliferative growth phenotypes, whereas in a *KRP* overexpression background some mutant phenotypes are suppressed. Collectively, our findings support a model in which translation of positional signals into developmental cues involves adjustments in chromatin modifications that orchestrate auxin effects on cell proliferation.

CDK inhibitor | cell proliferation | histone modification

Plant development is characterized by an astonishing plasticity, instrumental for responses to environmental changes (1). Variability in plant growth is primarily based on the regulation of postembryonic development, giving rise to the formation of organs that derive from meristem-localized stem cell niches (2, 3). This requires a tight regulation of cell proliferation and differentiation, which involves the impact of a variety of plant growth regulators (4). Sensing of and responding to such developmental signals constitutes a complex network of regulatory events, among which transcriptional and posttranslational control of regulators of cell proliferation predetermines organ initiation and successive differentiation events (2, 5, 6).

A link between control of cell proliferation and transcriptional regulation was established with the characterization of PROPORZ1, an *Arabidopsis* protein related to the yeast yADA2 transcriptional coactivator (7, 8). In *Saccharomyces cerevisiae*, yAda2 represents a subunit of Spt-Ada-Gcn5-Acetyltransferase (SAGA) and additional chromatin remodeling complexes that control histone acetylation, mediated by yGcn5 histone acetyl transferase (HAT) (9, 10). By analogy, PRZ1 was shown to interact with AtGCN5/HAG1, a plant Gcn5 ortholog, indicating related functions in yeast and *Arabidopsis* (11). Specifically, PRZ1 was suggested to modulate AtGCN5 activities, thereby affecting histone acetylation and hence expression of target loci (11).

Mutations in *PRZ1* and *AtGCN5* cause pleiotropic alterations in pattern formation and organogenesis also manifested as defects in the interpretation of signals relevant for plant morphogenesis (7, 8, 12–14). Furthermore, *PRZ1* and *AtGCN5* appear to be essential for adaptive growth, reflected in altered stress responsiveness of the corresponding mutant alleles (8, 15) Consistent with these pleiotropic phenotypes, ChIP experiments demonstrated that AtGCN5 associates with about one third of approximately 20,000 *Arabidopsis* promoter regions analyzed (16), which correlates with misexpression of a substantial fraction of the *Arabidopsis* transcriptome in *atgcn5* and *prz1* alleles (8).

Despite the large number of predicted targets for *Arabidopsis* SAGA-like activities, experimental evidence implied that misexpression of key regulators of cell proliferation and cell identity causes specific developmental defects in *Arabidopsis* SAGA complex mutants. For example, *PLT1* and *PLT2* (*PLETHORA*), two transcription factors that redundantly control stem cell niche establishment in root meristems (17), exhibit reduced expression in *atgcn5* and *prz1* (12). Overexpression of *PLT2* partially rescues *atgcn5* root meristem defects, suggesting that diminished *PLT* abundance interferes with proper meristem function in *Arabidopsis* SAGA complex mutants (12). Another report indicated that *PRZ1* determines the impact of the phytohormone auxin on cell proliferation via control of core cell cycle regulators (7). However, experimental evidence for a role of PRZ1 in integrating auxin signals into the control of chromatin architecture has not been provided so far.

Here we show that *PRZ1* is required for adjustments in hormonally controlled variations of histone acetylation and gene expression and provide mechanistic evidence for an involvement of SAGA-like activities in *Arabidopsis* morphogenesis in response to auxin.

Results and Discussion

PRZ1 Is Required for Control of Histone Acetylation. Given the role of yeast Ada2 in the control histone acetylation (9, 10), we asked whether or not a mutation in *PRZ1* causes defects in histone acetylation. Quantification of acetylated histones in chromatin preparations of wild type and *prz1-1* revealed multiple deficiencies, reflected in reduced amounts of chromatin modifications typically associated with transcribed euchromatin (18), namely histone H3-acK9, H3-acK9/14, and tetra-acetylated histone H4 (Fig. 1). Moreover, *prz1-1* exhibited diminished levels of trimethylated histone H3-K4me3 (Fig. 1), a chromatin mark that promotes transcriptional activation in conjunction with histone H4 acetylation (19). Overall, these pronounced defects in his-

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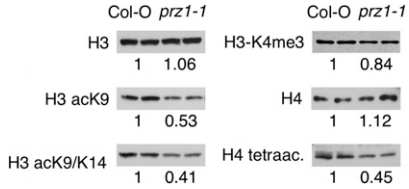

Fig. 1. PRZ1 is a positive regulator of histone acetylation. Protein extracts derived from wild type and *prz1-1* were normalized with anti-histone H3 and anti-histone H4 and probed with antibodies recognizing histone H3-acK9, histone H3-acK9/K14, and tetra-acetylated histone H4. Histone H3-K4me3 is another chromatin modification predominantly associated with transcribed loci. Signal quantification is shown below each panel (wild type = 1).

tone modifications could cause widespread alterations in gene expression, as observed in a *prz1* allele (8).

Arabidopsis PRZ1/AtADA2b might affect chromatin organization via modulation of AtGCN5 HAT activity (11). Remarkably, although in vitro analysis of AtGCN5 substrate specificities demonstrated a preference for histone H3-K14 (20), histone acetylation profiles obtained in a *gcn5* allele revealed more diverse alterations, predominantly affecting histone H3 and to a lesser extent histone H4 acetylation (13). These observations imply extended AtGCN5 specificity when acting in context of a SAGA-like complex, which resembles the situation described for yeast yGcn5 (21). Overlapping deficiencies in histone acetylation found in *atgcn5* and *prz1* alleles are thus consistent with a role of PRZ1 in recruitment or activation of AtGCN5 within a chromatin-remodeling complex. Moreover, reduced levels of histone H3-K4me3 in *prz1-1* indicate additional roles for PRZ1 in the coordination of chromatin remodeling activities.

PRZ1 Modulates Auxin Effects on Gene Expression. *Prz1-1* has been identified due to its inability to translate auxin signals into proper morphogenetic responses. For example, whereas auxin promotes formation of lateral roots in wild type, it triggers formation of tumorous callus-like tissue on *prz1-1* roots (7). These defective growth responses were suggested to arise as a consequence of altered expression of core cell cycle regulators. For example, several members of the *KIP RELATED PROTEIN* (*KRP*) family that inhibit CDK activity via reversible association with CDK-cyclin complexes (22–25) are misexpressed in *prz1-1*, which might contribute to deregulated cell proliferation upon auxin treatment (7).

To further determine how auxin and *PRZ1* might control *KRP* expression, we generated a set of *KRP* reporter constructs. Expression of translational *KRP7* fusion proteins (*KRP7p::KRP7:GUS, KRPp7::KRP7:GFP*) was found predominantly in meristems and developing organs (Fig. 2 and Fig. S1). In root meristems, KRP7:GUS/GFP exhibited strong signals in proximity of the quiescent center (QC), suggestive of a role of *KRP7* in modulating CDK activity in the stem cell niche and surrounding cells (Fig. 2*A* and Fig. S1). In addition, patchy signals enriched in adjacent pairs of cells could be observed in the cell division zone, indicating KRP7 protein accumulation in G1 phase cells (Fig. 2*C* and Fig. S1). Treatment of *KRP7p::KRP7:GUS* seedlings with the proteasome inhibitor MG132 resulted in uniform staining of the entire root meristem, suggestive of proteasome-dependent regulation of KRP7 turnover (Fig. S1). Consistent with such posttranslational control, activity of a transcriptional *KRP7* promoter-GUS reporter (*KRP7p::GUS*) was visible in the entire root meristem (Fig. 2*G*).

In agreement with diminished *KRP7* expression in *prz1-1* (7), signal intensities of translational and transcriptional KRP7-GUS reporters were reduced in the mutant (Fig. 2 *B* and *G*; and Fig. S1). Nevertheless, posttranslational control that restricts KRP7 to G1 phase cells in root meristems was not changed in

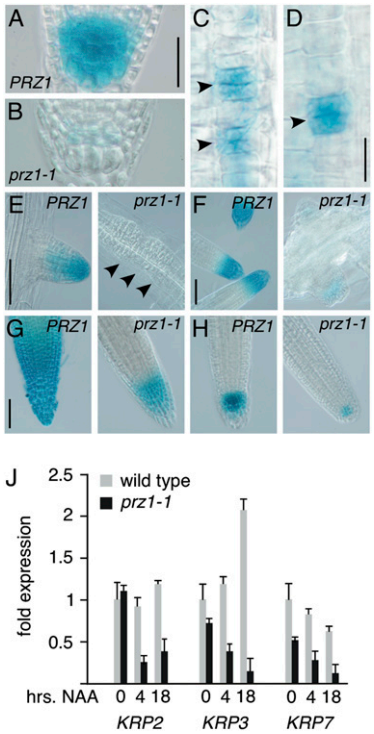

Fig. 2. Expression of *KRP* genes. (*A* and *B*) *KRP7p::KRP7:GUS* in wild type (*A*) and *prz1-1* (*B*) root meristem stem cell niches. (*C* and *D*) KRP7:GUS activity in adjacent root epidermis cells (arrowheads) of wild type (*C*) and *prz1-1* (*D*). (*E*) GUS-staining of *KRP7p::GUS* in emerging lateral roots upon incubation on 2.5 μM NAA for 60 h. Arrowheads indicate ectopic cell proliferation of *prz1-1* pericycle cells. (*F*) Same as (*E*) but after incubation on 2.5 μM NAA for 7 days. (*G*) Activity of KRP7p::GUS in wild type and in *prz1-1* at 6 days after germination (DAG). (*H*) Activity of KRP7p::GUS in 6-day-old wild type and *prz1-1* after treatment with 1 μM NAA for 18 h. (*J*) Quantitative analysis of *KRP* transcript levels in 6-day-old wild type and *prz1-1* after treatment with 1 μM NAA for 0, 4, or 18 h. Standard deviations are indicated ($n = 3$). *UBQ5* and *TUB9* were used for normalization. (Scale bars, *A*, *B*, *E*–*H* = 100 μm; *C*, *D* = 20 μm.)

prz1-1 (Fig. S1), suggesting that *PRZ1* particularly affects transcription of *KRP7*.

We then analyzed the impact of auxin on *KRP7* expression. In 4-day-old wild-type seedlings, auxin treatment resulted in the formation of lateral roots, with strong expression of the KRP7p::GUS reporter (Fig. 2 *E* and *F*). In contrast, auxin-treated *prz1-1* exhibited proliferative growth of larger sectors of the root pericycle, causing ectopic formation of dividing tissue that exhibited only limited KRP7p::GUS activity (Fig. 2 *E* and *F*). Because it cannot be excluded that *KRP7* misexpression after extended auxin treatment arises as a consequence of the severe phenotypes manifest in hormone-treated *prz1-1*, we quantified gene expression after short-term auxin treatment before marked effects on plant morphogenesis. In the wild type, treatment with 1 μM NAA for either 4 or 18 h caused a reduction in *KRP7* transcript levels that correlated with reduced activity of *KRP7p::GUS* in root meristems (Fig. 2 *G*–*J*). This inhibitory auxin effect was even stronger in *prz1-1*, causing a fourfold reduction of *KRP7* transcript levels (Fig. 2 *G*–*J*).

Analysis of other loci in *prz1-1*, demonstrated further deficiencies in the control of auxin-responsive expression. This was also the case for additional *KRP* genes. An earlier report demonstrated that high auxin concentrations cause a decrease in *KRP2* transcript levels, although those of *KRP3* were increasing (26). We did not observe a pronounced decrease in *KRP2* transcript levels in wild type, possibly due to the lower auxin concentrations used in our experiments (Fig. 2*J*). Nevertheless,

KRP3 expression in wild type exhibited a reproducible increase under our experimental conditions (Fig. 2*J*). In contrast, auxin treatment of *prz1-1* caused a decrease in transcript levels of both *KRP2* and *KRP3* demonstrating impaired auxin responses in the transcriptional control of these loci (Fig. 2*J*).

Besides *KRP* genes, we observed altered auxin effects on expression of regulators of root meristem activity. Auxin-inducible expression of *PLT2,* a potential target for *Arabidopsis* SAGA activity (12, 17), no longer exhibited a comparable response to auxin in *prz1-1* (Fig. S2). Similar defects in auxin responsiveness were found when analyzing expression of the homeobox gene *WOX5* (Fig. S2) (27, 28), although induction of the auxin-controlled Aux/IAA genes *IAA2* and *MSG2*/*IAA19* seemed less affected (Fig. S2).

Our results indicate that *PRZ1* antagonizes repressive auxin signals in the regulation of gene expression. This is exemplified by the disproportionate reduction of *KRP* transcript levels in auxin-treated *prz1-1*. On the other hand, *PRZ1* also promotes auxin-inducible gene expression as, for example, in the case of *PLT2* and *WOX5*. Given its predicted function in chromatin remodeling, PRZ1 could be involved in maintenance or establishment of a chromatin conformation that either promotes activation of gene expression or attenuates inhibitory signals in the transcriptional control of specific loci. In this context, PRZ1 would act as a positive regulator of gene expression, required for accurate interpretation of auxin signals.

PRZ1 Associates with KRP Loci in Planta. We then tested for association of PRZ1 with chromatin and generated functional, myc epitope-tagged *PRZ1* overexpression lines (*35S::myc:PRZ1*), which were used for ChIP, performed with anti-myc antibody (Fig. 3 *A*, *B*, and *D* and Fig. S3). We found that the *KRP7* locus is recognized by myc-tagged PRZ1, whereas no such interaction was apparent when using wild-type nuclear extracts for ChIP (Fig. 3*B*). In further ChIP experiments, we used affinity-purified antiserum derived against a GST:PRZ1 fusion protein, which detects PRZ1 in wild-type protein extracts (Fig. 3*C* and Fig. S3) and observed association of PRZ1 with the *KRP7* locus, preferentially in the 5′ nontranslated region and to a lesser extent in the coding region (Fig. 3 *C* and *D*). In addition, PRZ1 interacted with promoter fragments of *KRP2* and *KRP3* (Fig. S4). No such interaction was detectable in control experiments, in which we omitted to add either primary antibody or chromatin before immunoprecipitation (IP) (Fig. 3*C*). As another control, we tested for interaction between PRZ1 and a genomic fragment of the rDNA intergenic region. No enrichment of middle-repetitive rDNA in chromatin precipitated with anti-PRZ1 could be detected, which is consistent with the apparent exclusion of PRZ1 from the nucleolus (Fig. S4). Collectively, these experiments indicate that PRZ1-mediated control of gene expression involves targeting to specific loci, which could be essential for the coordination of chromatin remodeling activities.

PRZ1 Modulates Auxin Signals in Histone Acetylation. We then asked whether *prz1-1* deficiencies in auxin-responsive control of gene expression could arise as a consequence of defects in histone acetylation. To this end, we determined levels of histone H3-acK9/K14 via normalization to total amounts of histone H3.

No apparent auxin effects on histone acetylation were detectable in Western blots performed with total chromatin from seedlings (Fig. S4). We therefore analyzed specific sites by ChIP. When testing the *KRP7* locus in wild-type preparations, we found histone H3-acK9/K14 preferentially in the 5′ UTR proximal to the start codon and in the *KRP7* coding region (Fig. 3*E*). Upon treatment with 1 μM NAA for 18 h, histone H3-acK9/K14 levels decreased, demonstrating that elevated auxin levels promote a less acetylated chromatin status, which correlates with reduced *KRP7* expression under these experimental conditions (Fig. 3*F*).

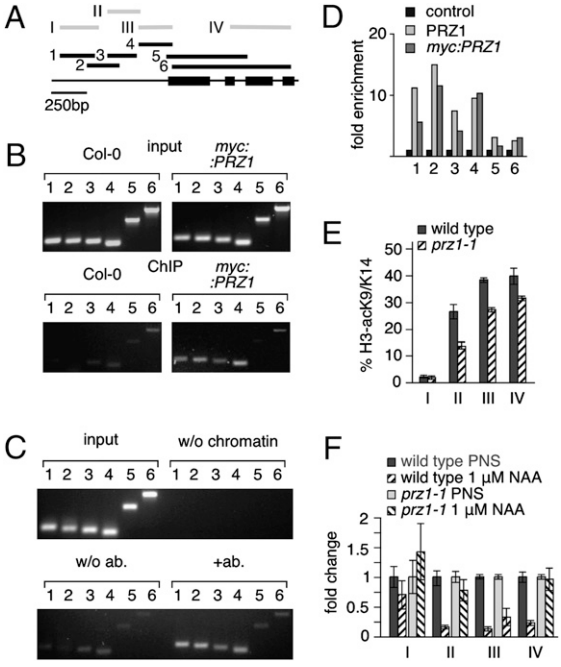

Fig. 3. PRZ1 associates with the *KRP7* locus and modulates histone acetylation. (*A*) Schematic drawing of the genomic *KRP7* locus. Dark boxes indicate exons. Dark lines labeled with Arabic numbers indicate PCR fragments tested by ChIP performed with anti-PRZ1 and anti-myc. Gray lines labeled with Roman numbers highlight PCR fragments tested in ChIP performed with histone antibodies. (*B*) anti-myc ChIP performed with chromatin from Col-0 and *35S::myc:PRZ1* seedlings was tested for *KRP7*. (*Upper*) Signals obtained in the input fractions. (*Lower*) Signals obtained in the corresponding pull-down fractions. (*C*) ChIP performed with anti-PRZ1 on chromatin preparations from 10-day-old Col-0. Before IP the input fraction was put aside. The remaining sample was divided into aliquots, which were used for IP either with anti-PRZ1 (+ab.) or by omitting the antibody (w/o ab.). w/o chromatin: control IP performed in the absence of chromatin. (*D*) Quantification of signal intensities obtained in (*B*) and (*C*) to determine the relative enrichment (arbitrary units) of DNA fragments. Enrichment was calculated as (ChIP/Input)/(ChIP control/Input control). (*E*) Histone H3-acK9/K14 levels in wild type and *prz1-1* grown on PNS. Amounts of acetylated histone H3 were determined after normalization to control ChIPs performed with non-discriminating anti-histone H3 (= 100%). Standard deviations are indicated as bars (n = 3). (*F*) Normalized amounts of histone H3 K9/K14 in wild type and *prz1-1* after treatment with 1 μM NAA for 18 h. Histone acetylation is expressed as fold change after normalization to corresponding control samples (= 1). Standard deviations are indicated as bars (n = 3).

In *prz1-1* we observed constitutively reduced histone H3-acK9/K14 levels in the 5′ UTR and in the coding region of *KRP7*, correlating with reduced *KRP7* expression in the mutant (Fig. 3*E*). Remarkably, auxin treatment of *prz1-1* caused only a limited further decrease of histone H3-acK9/14 levels, restricted to a region upstream of the *KRP7* coding region, whereas no hormone effect on histone H3-acK9/14 levels was detectable when testing additional parts of the *KRP7* locus (Fig. 3*F*). A diminished responsiveness of *prz1-1* chromatin to auxin was also observed when testing histone H3-acK9/14 levels in *WOX5* and *PLT2* promoter fragments (Fig. S2).

Similar to its yeast ortholog, PRZ1 appears to represent a subunit of an *Arabidopsis* SAGA-like complex, in which it might affect HAT activities and complex integrity (9, 29). Next to its role in histone acetylation, yeast SAGA orchestrates additional chromatin remodeling activities and recruitment of components of the core transcriptional machinery (30–34). PRZ1 could be required for coordination of such interdependent processes in *Arabidopsis*, determining crosstalk between histone acetylation and auxin-

controlled gene expression. In this scenario, the restricted auxin effect on already diminished histone acetylation at the *KRP7* locus might reflect an altered chromatin status that is poised for transcriptional repression in response to the hormone. This suggests that, apart from deficiencies in histone acetylation, a loss of *PRZ1* could also interfere with interaction or recruitment of further components of the transcriptional machinery, ultimately causing defects in dynamic adjustments in gene expression in response to auxin.

Variations in KRP Expression Determine Cell Proliferation Rates. Overexpression of *KRP* genes has been demonstrated to antagonize cell proliferation and organogenesis, which was suggested to arise as a result of perturbed cell cycle phase transition and promotion of cell cycle exit (23, 26, 35–37). Such dominant *KRP* overexpression phenotypes could be a consequence of elevated KRP activities. Accordingly, a loss of *KRP* function would be predicted to cause opposite phenotypes, namely interference with differentiation of cells and tissues. To test this hypothesis we analyzed *krp* loss of function lines.

Because no apparent phenotypes were observed in a variety of single *krp* T-DNA insertion lines, we generated *KRP* silencing lines, to affect expression of multiple *KRP* genes (Fig. S5). These lines exhibited pleiotropic phenotypes during germination and later development, suggesting that combinatorial effects of altered *KRP* expression interfere with plant morphogenesis (Fig. S5). Remarkably, after extended incubation on regular growth medium, a significant fraction of *KRP* silencer plants spontaneously started to generate callus-like tissue, although control plants never exhibited such phenotypes (Fig. 4*A* and Table S1). Thus, in agreement with their predicted function as negative regulators of cell proliferation, down-regulation of *KRP* expression seems to promote dedifferentiation and hyperplasic growth.

Our analysis of *krp* silencer lines demonstrates that control of *KRP* expression is an important determinant of cell proliferation in plant morphogenesis. This is consistent with a scenario in which deregulated *KRP* expression is involved in altered auxin responsiveness of *prz1-1*.

KRP Overexpression Partially Rescues prz1-1. To determine, how *PRZ1*-mediated regulation of *KRP* expression could affect morphogenesis, we analyzed *KRP7* overexpression lines.

In wild type, overexpression of *KRP7* under control of the 35S promoter resulted in phenotypes resembling those of published *KRP* overexpression lines, including formation of serrated true leaves, a reduced number of lateral roots and stunted growth phenotypes (Fig. S6) (23, 26, 37). Analysis of *prz1-1 KRP7ox* plants demonstrated partial rescue of *prz1-1* phenotypes: Specifically, unlike cotyledons and early true leaves of *prz1-1* that were tiny and highly epinastic, leaves of *prz1-1 KRP7ox* were larger and less epinastic, resembling the leaves of *KRP7ox* plants (Fig. 4*B*). This, together with a partial reversion of the severe dwarfism of *prz1-1* at flowering stage, indicated that some *prz1-1* defects are caused by diminished *KRP* expression (Fig. 4*C*). Nevertheless, *prz1-1 KRP7ox* still exhibited pronounced growth defects, exemplified by stunted, bushy growth and complete sterility (Fig. 4*C*), suggesting that pleiotropic *prz1* phenotypes are the combinatorial result of wide-ranging defects in transcriptional control (8).

KRP Overexpression Antagonizes prz1-1 Auxin Responses. Overexpression of *KRP2* was demonstrated to interfere with root meristem stem cell maintenance, presumably via inhibition of CDK activity in context of the Arabidopsis *RETINOBLASTOMA* pathway (38). A similar reduction in root meristem size was detectable in *KRP7ox* plants, consistent with an inhibitory role of *KRP7* in the control of cell proliferation (Fig. 4*D*).

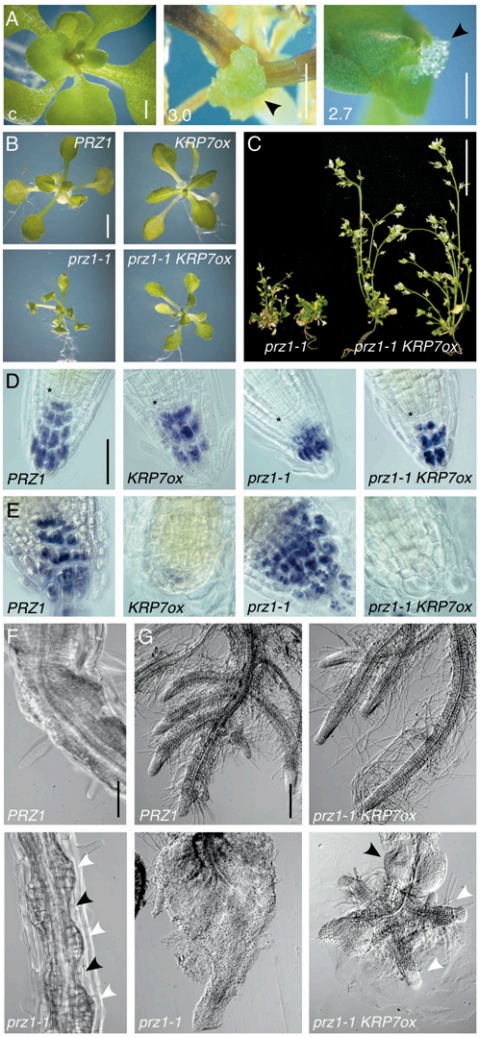

Fig. 4. Analysis of *KRP* overexpression and silencing lines. (*A Left*) Rosette leaves of a *KRP* silencer control plant (c; 22 DAG). (*Center*) Dedifferentiation of the shoot apical meristem in line 3.0 (30 DAG, black arrowhead). (*Right*) Microcallus-formation on a leaf of silencer line 2.7 (18 DAG, black arrowhead). (*B*) Comparison of Col-0, *KRP7ox*, *prz1-1*, and *prz1-1 KRP7ox* plantlets at 16 DAG. (*C*) Flowering *prz1-1* and *prz1-1 KRP7ox* at 40 DAG. (*D*) Lugol-staining of 12-day-old wild-type, *KRP7ox*, *prz1-1*, and *prz1-1 KRP7ox* primary root tips, grown on PNS. Asterisks indicate position of QC. (*E*) Lugol-staining of 12-day-old wild type, *KRP7ox*, *prz1-1*, and *prz1-1 KRP7ox* primary root tips germinated on PNS for 5 days and then transferred on medium containing 2.5 μM NAA for another 7 days. (*F*) Primary root segments of 8-day-old *prz1-1* (*Upper*) and *prz1-1 KRP7ox* (*Lower*) seedlings after incubation on 2.5 μM NAA for 4 days. *Prz1-1 KRP7ox* develops distinct lateral root primordia (white arrowheads) separated by zones with reduced pericycle proliferation (black arrowheads). (*G*) Root morphology of 12-day-old wild type, *KRP7ox*, *prz1-1*, and *prz1-1 KRP7ox* germinated on 2.5 μM NAA. Note lateral root growth in *prz1-1 KRP7ox* (white arrowheads), occasionally interspersed by callus formation (black arrowhead). (Scale bars, *A* = 2 mm; *B* = 10 mm; *C* = 20 mm; *D*, *E* = 100 μm; *F* = 50 μm; *G* = 200 μm.)

Auxin further pronounced *KRP7ox* root meristem phenotypes. In wild type and *prz1-1*, auxin caused an induction of cell proliferation resulting in enlarged root meristems, which in *prz1-1* frequently gave rise to callus formation (Fig. 4*E*). In contrast, auxin treatment of *KRP7ox* and *prz1-1 KRP7ox* triggered meristem differentiation and root meristem consumption in a dosage-dependent manner (Fig. 4*E* and Table S1).

Hormonal control of *KRP* transcription appears to act in the modulation of cell cycle progression, affecting, for example, seed

germination and lateral root formation (24, 26). The synergistic effects of auxin and *KRP* overexpression on root meristem differentiation further underline the importance of transcriptional control of *KRP* genes for interpretation of hormone signals in the regulation of cell proliferation. Moreover, the finding that overexpression of *KRP7* antagonizes auxin-induced hyperproliferation in *prz1-1* is in agreement with the suggested function of PRZ1 in the control of hormone responses.

We then tested auxin effects on lateral root formation in *prz1-1 KRP7ox*. Transfer of *prz1-1* seedlings onto auxin-containing medium resulted in the formation of a collar-like structure of proliferating cells surrounding the root vasculature (Fig. 4*F*). In *prz1-1 KRP7ox*, auxin-induced cell proliferation was less pronounced, resulting in the formation of distinct lateral root primordia, clearly distinguishable from zones that exhibited reduced proliferative growth (Fig. 4*F*). Consistent findings were made when analyzing plants germinated in the presence of auxin. Under such conditions, we observed dedifferentiation of the entire basal root pole into callus-like tissue in up to 50% of *prz1-1* seedlings (Fig. 4*G* and Table S1). In *prz1-1 KRP7ox* a smaller fraction of seedlings formed a callus-like root pole but developed differentiated lateral roots instead (Fig. 4*G* and Table S1). These results are in agreement with the reduced callus formation potential of *prz1-1 KRP7ox* when incubated on callus inducing medium (Fig. S7), indicating that elevated *KRP* expression antagonizes auxin-induced cell proliferation in *prz1-1*.

A mechanistic link between auxin signaling and variations in *KRP* expression has been suggested for the control of lateral root formation (26). Specifically, auxin was demonstrated to modulate *KRP* expression in the root pericycle, possibly predetermining lateral root primordia formation at sites with diminished *KRP* transcription. Thus, control of lateral root initiation would involve adjustments in *KRP* activity that impinges on the competence of pericycle cells to reenter the cell cycle. Results obtained with *KRP7ox* support this model, and furthermore suggest that the disproportionate reduction of *KRP* expression in *prz1-1* affects initiation and further differentiation of lateral roots.

Conclusion

In *Arabidopsis*, implementation of developmental programs involves translation of positional signals into morphogenetic switches via variations in chromatin architecture (39, 40). Auxin functions as such a signal, providing positional information via dynamic variations in hormone distribution (41). Further transmission of positional auxin signals in the control of morphogenesis has been analyzed extensively, and was suggested to involve crosstalk between auxin signal transduction components and chromatin remodeling activities (42–44).

Here we provide evidence that interpretation of developmental signals, generated by variations in auxin distribution involves active adjustments in histone acetylation that require a functional SAGA-like complex. Our analysis of *prz1-1* would suggest that such an *Arabidopsis* SAGA-like complex functions to activate transcription, thereby modulating auxin signals in the control of gene expression. In this scenario, *Arabidopsis* SAGA might antagonize TOPLESS (TPL) a transcriptional corepressor that mediates auxin-induced transcriptional repression, possibly via recruitment of histone deacetylases to chromatin (42, 44). Evidence for such crosstalk is provided by the observation that *atgnc5* suppresses severe developmental defects caused by a dominant negative allele of *TPL* (42).

Antagonistic activities of histone acetylases and deacetylases might also underlie the observed auxin-dependent transcriptional control of KRPs and additional *PRZ1* target loci. In this context, PRZ1 appears to be required for the recruitment of histone acetylating activities, thereby adjusting a homeostatic balance between activating and repressing activities in the control of gene expression.

Current models suggest that the regulatory impact of transcriptional coactivators such as PRZ1 is brought about via physical interaction with transcriptional activators (45, 46). In baker's yeast for example, interaction of yAda2 with activation domains of transcription factors appears to be essential for proper induction of gene expression (45, 46). PRZ1/AtADA2b interacts with CBF1, a transcriptional regulator of cold responses, suggestive of a similar situation in *Arabidopsis* (11). Our study now suggests a role for PRZ1 as transcriptional coactivator in hormonal control of histone acetylation and gene expression. Yet further molecular determinants acting in this hypothetical pathway remain to be identified.

Materials and Methods

Plant Growth and Material. Plants were grown on PNS medium (47), with 16 h illumination at 22 °C, and supplemented with hormones when indicated. After 2 weeks, plants were transferred to soil and grown in climate chambers under continuous illumination at 21 °C. For information on identification and analysis of mutant lines see *SI Materials and Methods*.

Constructs and Expression Analysis. Conventional techniques have been used for generation and analysis of transgenic lines (*SI Materials and Methods*).

Antisera, Protein Extraction, and Chromatin Analysis. A PRZ1-specific antibody was raised in rabbits. For histone analysis we used the following antibodies: Anti-histone H3 (Abcam; ab1791, 1:30,000) and anti-histone H4 (Upstate; 05–858, 1:400), anti-histone H3-acK9 (Upstate; 07–352, 1:3,000), anti-histone H3-acK9/K14 (Upstate; 06–599, 1:3,000), anti-histone H3-K4me3 (Abcam; ab8580, 1:40,000), and anti-tetra-acetylated histone H4 (Upstate, 06–598, 1:125). HRP-conjugated goat anti-rabbit IgG (Pierce; 1:100,000) or goat anti-mouse IgG (Dianova; 1:100,000) were used as secondary antibodies (for further details see *SI Materials and Methods* and Table S2).

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