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PROPORZ1, a Putative *Arabidopsis* Transcriptional Adaptor Protein, Mediates Auxin and Cytokinin Signals in the Control of Cell Proliferation

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Summary

Plants generate cells and organs throughout their life cycle. Plant cell proliferation relates to the activity of dividing meristematic cells, which subsequently differentiate in a position- and lineage-dependent manner [1]. The events underlying the regulation of cell division and further differentiation processes are under tight control of both intrinsic and environmental parameters [1–3]. Among the intrinsic factors, two groups of phytohormones, auxins and cytokinins, exhibit a combined regulatory impact on cell proliferation, as an important determinant for the totipotency of plant cells [4]. Classical experiments demonstrated that application of both growth regulators in appropriate concentrations promotes callus formation [4]. When the ratio between the phytohormones changes, callus cells acquire the competence to regenerate organs. Typically, a high auxin-to-cytokinin ratio promotes the formation of roots, whereas a low auxin-to-cytokinin ratio results in the regeneration of shoots. Conclusively, the concerted, proportional impact of both phytohormones functions as a determinant of plant cell proliferation; they act as cell cycle-promoting mitogens as well as morphogens that control plant organogenesis [4, 5]. Here, we describe *PROPORZ1* (*PRZ1*), an *Arabidopsis* gene, essential for the developmental switch from cell proliferation to differentiation in response to variations in auxin and cytokinin concentrations. *PRZ1* probably acts as a transcriptional adaptor protein that affects the expression of cell cycle regulators and might, thereby, mediate its effect on the control of cell proliferation.

Results and Discussion

In order to isolate effectors of auxin and cytokinin signaling, we screened T-DNA-mutagenized *Arabidopsis* seedlings for defects in cell proliferation in the presence of the hormones. The screen led to the isolation of *prz1-1*, a recessive mutation that segregates as a single nuclear locus and leads to alterations in plant responses upon hormone treatment. In the wild-type, ectopic cell proliferation is manifested as callus formation and is only observed upon incubation on callus-inducing medium (CIM; see the Supplemental Data available with this article online), containing high concentrations of both auxin and cytokinin. Wild-type hypocotyls treated with auxin alone typically respond with the formation of adventitious roots and show no apparent effect when treated with cytokinin alone (Figure 1A). Unlike the wild-type, *prz1-1* plants have a pronounced tendency to form callus-like tissue in the presence of either 5 μM 1-napthaleneacetic acid (NAA, a potent auxin analog) or 5 μM kinetin (N^6-furfuryladenine, a cytokinin). For example, hypocotyls of phytohormone-treated *prz1-1* plantlets are dedifferentiated and produce pale tissue in the presence of auxin or dark-green callus-like tissue in response to cytokinin, whereas no callus is formed without phytohormone treatment (Figure 1A). The apparent increased tendency of *prz1-1* to reenter the cell cycle upon hormone treatment is also manifested in the expression pattern of the mitotic reporter construct CYCB1;1-GUS. Expression of the reporter is induced in *prz1-1* hypocotyls by auxin or cytokinin concentrations that are insufficient to induce mitosis in wild-type hypocotyls (Figure 1B). By contrast, CYCB1;1-GUS expression is not induced in *prz1-1* or wild-type hypocotyls when they are grown without hormones, whereas both lines exhibit a similar CYCB1;1-GUS expression pattern upon incubation on CIM (Figure 1B).

The resemblance of *prz1-1* incubated on either auxin or cytokinin to wild-type grown in the presence of both hormones suggests that responses to both growth regulators are altered in the mutant. Such a scenario is also supported by the fact that both hormones exhibit an altered additive effect on *prz1-1*. Coapplication of both hormones at concentrations that are insufficient to promote callus formation in the wild-type results in pronounced callus formation in *prz1-1* (Supplemental Data, Table S1). Moreover, *de-novo* organogenesis, initiating from callus tissue when we shifted auxin and cytokinin concentrations, is severely impaired in the mutant (Supplemental Data, Table S2). Altered responses to phytohormones also became apparent when we introduced *prz1-1* into mutants defective in either *ABERRANT LATERAL ROOT FORMATION1* (*ALF1*, [6]) or *ALTERED MERISTEM PROGRAM1* (*AMP1*, [7]). Unlike the auxin overproducer *alf1-1* that generates excess lateral and adventitious roots, *alf1-1 prz1-1* double mutant seedlings fail to generate excessive numbers of secondary roots. Instead, initiating from the root-hypocotyl junction, the entire seedling starts to dedifferentiate and grows callus-like tissue (Figure 1C). Similarly, the *amp1-1 prz1-1* double mutant has an increased tendency to develop callus, a response potentially related to increased cytokinin levels associated with mutations in *AMP1* (Figure 1C). Thus, an increase (mediated by *alf1-1* or *amp 1-1*) in endogenous auxin and cytokinin concentrations gives rise to phenotypes similar to those observed upon external hormone application.

To position *PRZ1* in already characterized auxin and cytokinin signaling pathways, we generated *prz1-1*

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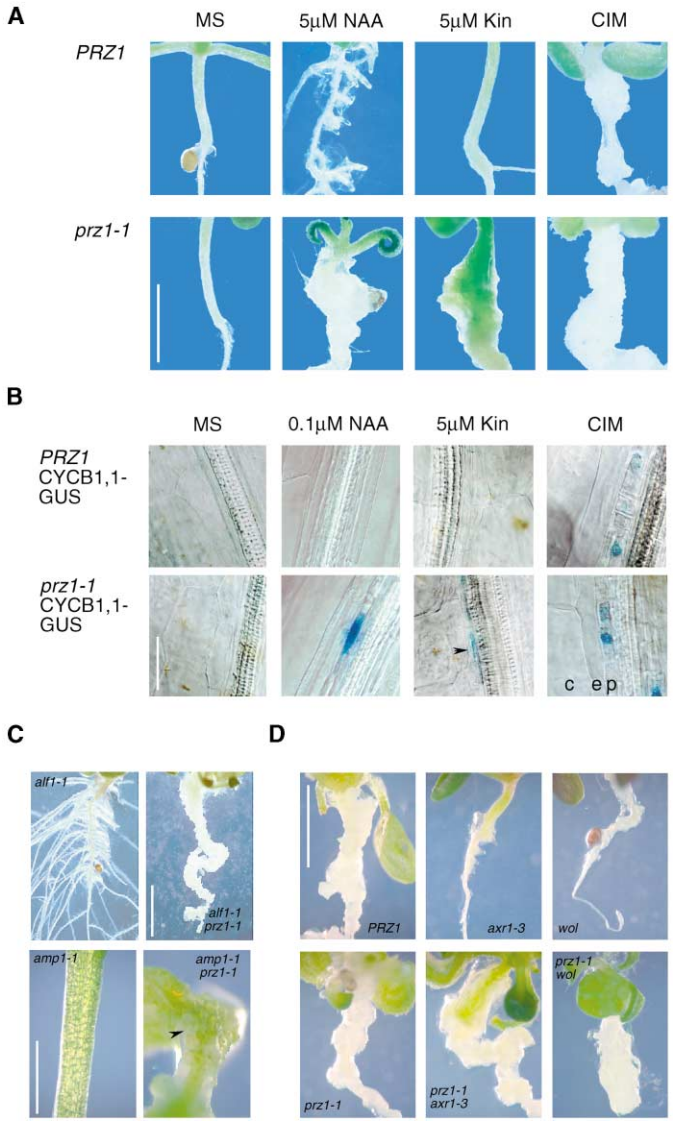

Figure 1. A Mutation in *PRZ1* Causes Defects in the Control of Cell Proliferation

(A) Hypocotyls of *PRZ1* and *prz1-1*, germinated on indicated growth media, after 20 days. From left to right: MS-medium without hormones; MS-medium supplemented with 5 μM NAA; MS-medium, supplemented with 5 μM kinetin; and CIM, MS-medium supplemented auxin and cytokinin. The scale bar represents 2.5 mm.

(B) Expression of CYCB1;1-GUS in *prz1-1* and wild-type hypocotyls (c = cortex, e = endodermis, p = pericycle; Bar = 50 μm). No GUS staining is detectable in untreated hypocotyls, whereas both lines exhibit GUS activity when incubated on CIM for 36 hr. After 36 hr on 100 nM NAA, *prz1-1* pericycle cells have started to reenter the cell cycle, whereas the wild-type has not. After 72 hr on 5 μM kinetin, *prz1-1* pericycle cells exhibit GUS activity (arrow), whereas wild-type pericycle cells remain quiescent.

(C) An increase in endogenous auxin or cytokinin concentrations leads to constitutive callus formation of *prz1-1*. (Top) *alf1-1* and *prz1-1 alf1-1*: unlike *alf1-1*, which develops excessive lateral and adventitious roots, the double mutant forms callus-like tissue instead (scale bar = 2 mm). (Bottom) Petioles of *amp1-1* and *prz1-1 amp1-1* seedlings at 20 DAG. The double mutant exhibits hyperproliferative growth and develops callus-like tissue (arrow; scale bar = 0.5 mm).

(D) Callus formation of *prz1-1 axr1-3* and *prz1-1 wol* after 16 days on CIM. Both the wild-type and *prz1-1* exhibit pronounced dedifferentiation, whereas *axr1-3* and *wol* do not. By contrast, *prz1-1 axr1-3* and *prz1-1 wol* develop callus.

axr1-3 and *prz1-1 wol* double mutants. *AUXIN RESISTANT1* (*AXR1*) is involved in the control of auxin responses via the regulation of the in vivo half-life of proteins thought to be required for auxin signaling and transport [8, 9]. As a result, a mutant defective in *AXR1* exhibits altered responses to auxin. For example, *axr1-3* seedlings do not efficiently form callus tissue when grown on CIM (Figure 1D). *WOODEN LEG* (*WOL/CRE1*) probably acts as a receptor for cytokinin and affects the differentiation of the primary root vasculature [10, 11]. In agreement with the proposed function of *WOL/CRE1*, the primary root of *wol* seedlings has a reduced tendency to dedifferentiate on CIM (Figure 1D). The situation is completely different when *prz1-1 axr1-3* and *prz1-1 wol* are grown on CIM. Unlike *axr1-3* and *wol*, both double mutants exhibit a pronounced tendency to form callus on CIM (Figure 1D). This raises the question about mechanisms that allow for perception and transduction of hormone signals even upon deficiencies in *AXR1* or *WOL/CRE1* function. One possible model would postulate that *axr1-3* and *wol* are rather weak alleles with residual activity. Such remaining functionality might be sufficient to induce cell proliferation in the hyperresponsive *prz1-1* allele. In this model, *PRZ1* could act downstream of *AXR1* and *WOL/CRE1*. Alternatively, *PRZ1* could affect mechanisms of hormone perception and transduction that are not related to the function of *AXR1* and *WOL/CRE1*.

Besides altered hormone responsiveness, *prz1-1* exhibits defects in cell and organ differentiation. These defects range from rather subtle changes in cell shape and size to severe defects in the differentiation of entire organs such as flowers (Supplemental Data). However, despite the strong effect of the phytohormones on the control of cell proliferation in *prz1-1*, no additional defects, typically related to changes in auxin or cytokinin responses, became apparent in a number of growth assays (Supplemental Data). The function of *PRZ1* thus could be restricted to a specific subset of hormone responses that are essential for the control of cell identity upon variations of auxin and cytokinin concentrations.

We cloned *PRZ1* via plasmid rescue [12]. *Prz1-1* phe-

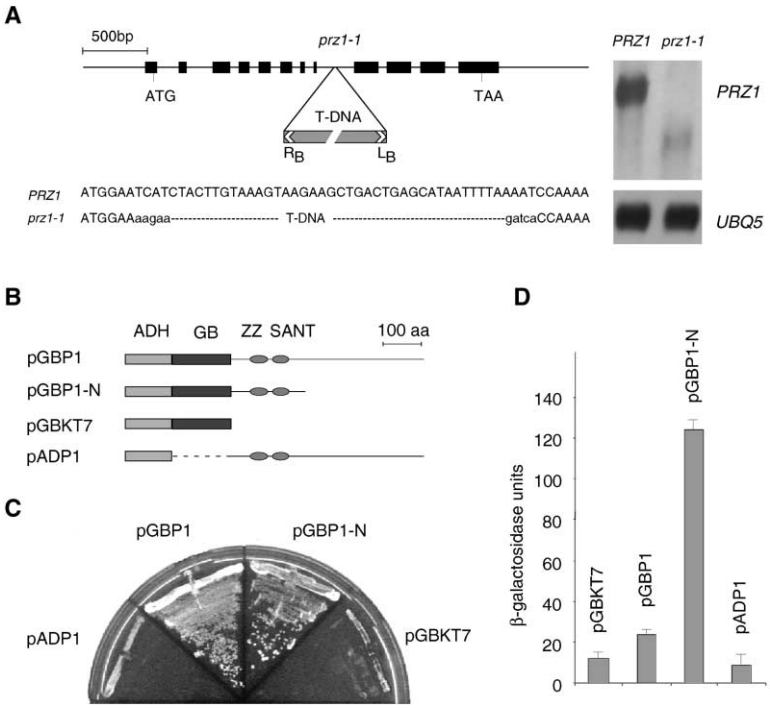

Figure 2. Organization of the *PRZ1* Locus

(A) *PRZ1* exon-intron structure as derived from the sequence of a cDNA clone. Exons are indicated as dark boxes. Positions of start and stop codons of the longest presumptive ORF are indicated. The insertion site and the orientation of the T-DNA (gray bar) in *prz1-1* are indicated below (RB: right border; LB: left border). The T-DNA insertion, associated with *prz1-1*, gives rise to a deletion of 46 bp of genomic DNA (bottom) and interferes with transcription of *PRZ1* (right; *UBQ5* was used as a control).

(B–D) Expression of *PRZ1* in *S. cerevisiae*. (B) Constructs, generated for the PRZ1 transactivation assay: Expression of all constructs is under control of the ADH promoter (ADH). pGBKT7, expressing the Gal4 DNA binding domain (GB), and pADP1 expressing the PRZ1 ORF were used as controls. pGBP1-N expresses an N-terminal portion of PRZ1, whereas pGBP1 expresses nearly full-length PRZ1 fused to the GB. The positions of the ZZ domain and the SANT domain are indicated. (C) Growth of the indicated strains on SC-HIS medium. Both GB-PRZ1 fusion proteins rescue the histidine auxotrophic strain, whereas the controls do not. (D) LacZ assay: pGBP1-N is highly active in the trans-activation assay, whereas pGBP1 expression results in lacZ activities slightly higher than in the controls (standard deviations are indicated as bars).

notypes cosegregate with a single T-DNA insertion that localizes to intron 8 of locus At4g16420 on chromosome 4 and interferes with the transcription of the corresponding gene (Figure 2A). A genomic fragment comprising the *PRZ1* genomic region fully complements the growth defects associated with *prz1-1*, suggesting that the mutant's phenotypes are caused by the T-DNA insertion. *PRZ1* is identical to *AtADA2b* (GenBank accessionnumber: AF338770), an *Arabidopsis* gene related to the transcriptional adaptor protein yAda2 of *Saccharomyces cerevisiae* [13, 14]. In baker's yeast, yAda2 represents a subunit of protein complexes that exhibit histone acetyltransferase (HAT) activity [15, 16]. The N terminus of yADA2, which contains a putative Zinc binding ZZ-domain and a Myb-related SANT-domain, was shown to be required for the interaction with the HAT yGcn5 and to be critical for yGcn5 activity in vivo [17, 18]. Moreover, yAda2 was demonstrated to interact with acidic activation domains of VP16 and Gcn4 [18, 19]. Based on these findings, it was suggested that yAda2 might function as a transcriptional coactivator that bridges the interaction between HAT activity, activation domains, and the basal transcriptional machinery [18]. The *Arabidopsis* orthologs of these yeast proteins appear to behave similarly; AtADA2b (PRZ1 throughout this report) and the *Arabidopsis* HAT AtGCN5 were shown to interact in vitro [14].

Analysis of *PRZ1* in yeast gives some indication of its function. The full-length PRZ1 does not rescue the slow-growth phenotypes of Δ*ada2* yeast cells (our unpublished data). However, chimeric constructs in which either the N-terminal portion of PRZ1, comprising the conserved N-terminal ZZ and SANT domains, or the nearly full-length PRZ1 ORF (lacking amino acids 1–4) were fused to the GAL4 DNA binding domain (Figure 2B) induce the expression of reporter constructs under the control of GAL4-dependent promoters (Figures 2C and 2D). These results parallel those made for a lexA-yAda2 fusion protein, which also activates yeast reporter gene expression in vivo [20]. Thus, PRZ1, like yeast yAda2, contains a strong, cryptic transcriptional activation domain.

To characterize the role of *PRZ1* in planta, we investigated expression and subcellular localization of PRZ1. A *PRZ1*-specific transcript is detectable in all organs tested (Figure 3A). In agreement with these findings, a functional PRZ1-GUS reporter construct, comprising the PRZ1 genomic clone fused to the β-glucuronidase gene from *Escherichia coli*, is expressed throughout the entire plant (Figure 3). PRZ1-GUS activity is strongest in meristematic zones of the shoot and root apex (Figures 3B and 3C), and predominant activity of PRZ1-GUS in meristems is also manifested by its induction during lateral-root primordia formation (Figure 3D). In whole-mount immunolocalization experiments, PRZ1-GUS colocalizes with 4',6-diamidino-2-phenylindole (DAPI), indicative for a nuclear localization of PRZ1 (Figures 3G and 3H). Expression and localization of the reporter would be in agreement with a role in transcriptional control of cell proliferation because PRZ1-GUS is detectable throughout the entire plant but is most abundant in cells such as meristematic and differentiating cells that have a high capacity to undergo cell division and subsequent differentiation.

An important determinant for the control of plant cell proliferation is phytohormone-mediated regulation of

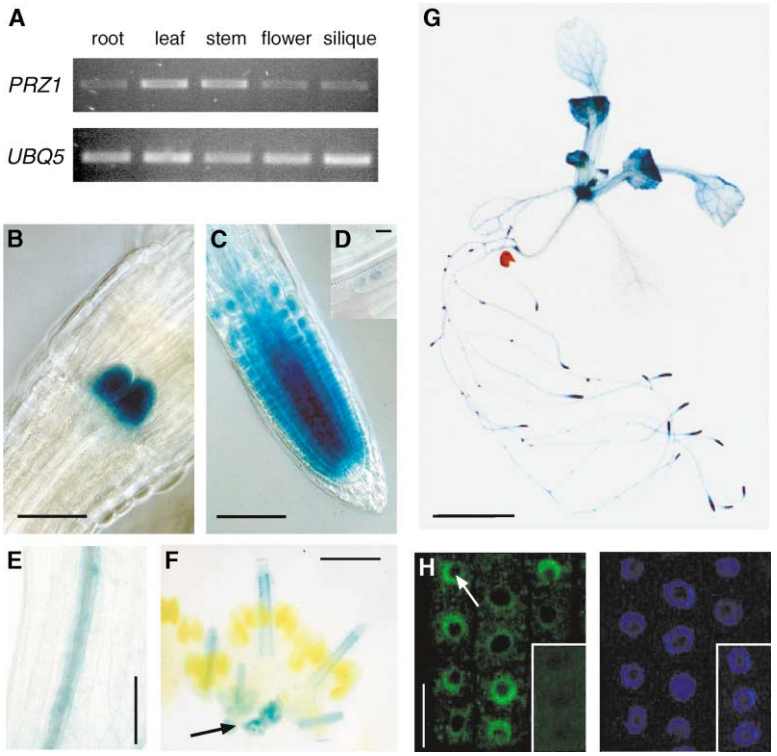

Figure 3. Expression and Subcellular Localization of PRZ1-GUS

(A) RT-PCR performed on cDNA, derived from the indicated tissues. A *PRZ1*-specific fragment is detectable in all tissues tested. *UBQ5* was used as an internal standard.

(B–D) PRZ1-GUS activity in meristematic zones at 4DAG. (B) Shoot apical meristem (scale bar = 150 μm). (C) Primary root meristem (scale bar = 100 μm). (D) Lateral root primordium (scale bar = 20 μm).

(E) PRZ1-GUS activity in the stele of the hypocotyl (scale bar = 100 μm).

(F) Inflorescences exhibit strong GUS activity in early stages of floral development (arrow; scale bar = 2 mm).

(G) PRZ1-GUS activity at 20 DAG (scale bar = 10 mm).

(H) Left: immunolocalization of PRZ1-GUS in epidermis cells of a primary root meristem (4DAG). Most of the protein (green) localizes to the nucleus, but not to the nucleolus (arrow). No signal is detectable in Col-0 controls (inlay). Right: DAPI-staining (scale bar = 30 μm).

cell cycle progression [2, 3]. Both auxin and cytokinin have been shown to control the activity of cell cycle regulators at the transcriptional and posttranscriptional level [21, 22, 23]. Given that *PRZ1* controls cell proliferation in response to phytohormones, we reasoned that genes involved in the control of cell cycle progression could represent potential targets for *PRZ1*-mediated control of gene expression. Therefore, we compared steady-state transcript levels of 34 annotated cell cycle regulators in *prz1-1* and wild-type plants [24]. For this experiment, we grew wild-type and *prz1-1* plantlets on medium containing auxin and cytokinin (CIM) as well as on medium lacking hormones. Media containing auxin or cytokinin only were not found suitable for this experiment because of the hyper-proliferative growth characteristics of *prz1-1* in response to either hormone (Figure 1 and Table S1). By contrast, upon extended incubation on CIM, wild-type and *prz1-1* plants exhibit comparable proliferation rates, also reflected in similar transcript levels of histone H4 (Figure 4). By using this experimental setup, we made certain that alterations in gene expression would not arise as a consequence of hormone-induced differences in growth characteristics between *prz1-1* and wild-type but would result from defects in the transcriptional control caused by *prz1-1*.

Depending on growth conditions, different but overlapping subsets of cell cycle regulators appear to be misexpressed in *prz1-1*, indicating that variations in hormone concentrations correlate with specific deficiencies in the transcriptional regulation of cell cycle progression. On hormone-free medium, transcript levels of a number of B- and D-type cyclins are reduced in *prz1-1* (Figure 4). In addition to altered expression of some cyclins, transcription of further cell cycle regulators is affected in *prz1-1* tissue incubated on CIM. Remarkably, four out of seven known *Arabidopsis* KIP-related proteins (KRP) appear to be misexpressed in the mutant (Figure 4). *Arabidopsis* KRP genes have been characterized as functional equivalents of members of the eukaryotic KIP/CIP family of CDK inhibitors, which affect CDK activity via reversible association with cyclin/CDK complexes and are required for both checkpoint control and the regulation of cell cycle exit [25, 26]. Expression of *AtKRP2*, *AtKRP3* as well as *AtKRP7* is no longer induced in hormone-treated *prz1-1* seedlings, suggesting that *PRZ1* affects the impact of the phytohormones on the expression of these genes. Regulation of *AtKRP1* transcription contradicts that of other KRP genes upon hormone treatment. The wild-type responds with a marked decrease of *AtKRP1* transcript levels, whereas the gene remains constantly expressed in *prz1-1*. The contrasting responses of KRP gene expression to hormone treatment indicate distinct functions for individual members of this gene family, a scenario that would parallel the findings made for mammalian $p21^{CIP1}$ and $p27^{Kip1}$ [27]. We also observed significantly increased expression of *AtCDKB1;1,* a plant-specific PPTALRE-type CDK suggested to act during G2/M transition [2], and of *AtE2Fc*, an *Arabidopsis* homolog of mammalian E2F transcription factors that is presumed to act on a wide variety of genes required for cell cycle control, DNA replication, and chromatin constitution [28].

Taken together, the observed changes in the expression of several cell cycle regulators in *prz1-1* suggest that *PRZ1* could act as a regulator of plant growth via transcriptional control of cell proliferation. However, it is currently not possible to link the variety of alterations in gene expression levels to the plethora of morphologi-

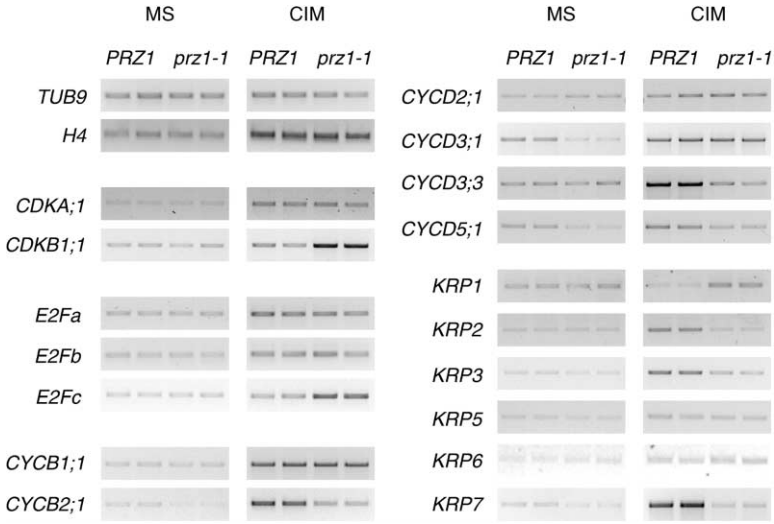

Figure 4. Expression of Cell Cycle Regulators in *prz1-1* and Wild-Type

MS, no hormones; CIM, auxin and cytokinin present in the growth medium. All experiments have been performed with parallel samples. Normalization of cDNA was performed with *TUB9*-specific primers. Equal histone H4 transcript levels indicate comparable proliferation rates of mutant and wild-type. Among the tested genes, transcript levels of *AtCYCB2;1*, *AtCYCD3;1*, and *AtCYCD5;1* are reduced in *prz1-1* on hormone-free medium. On CIM, transcript levels of *AtCYCD3;1* are induced to levels comparable to those found in the wild-type, whereas *AtCYCB2;1* and *AtCYCD5;1* remain weakly expressed in the mutant. Upon hormone treatment, transcript levels of *AtCYCD3;3* are induced in the wild-type but are unaffected in the mutant. No differences in the expression of other tested cyclins, such as *AtCYCB1;1* and *AtCYCD2;1*, are apparent. Expression of *AtKRP2, AtKRP3*, and *AtKRP7* is induced in hormone-treated wild-type, but fails to respond to the hormones in *prz1-1*. By contrast, expression of *AtKRP1* appears to be repressed in the wild-type on CIM, whereas it is not in the mutant. *AtKRP5* and *AtKRP6* are not affected in *prz1-1*. Among *Arabidopsis* CDK and E2F genes, transcript levels of *AtCDKB1;1* and *AtE2Fc* are more abundant in hormone-treated *prz1-1* than in the wild-type.

cal defects exhibited by *prz1-1*. On the one hand, our RT-PCR approach is limited by the inability to assess gene expression patterns within defined tissues or even single cells. On the other hand, the situation is complicated not only by the large variety of cell cycle regulators affected in *prz1-1* but also by the finding that *prz1-1* interferes with both activation and repression of gene expression, suggesting multifaceted transcriptional control mechanisms.

An example of flexible transcriptional control mediated by a chromatin-remodeling complex is provided by the functional analysis of the SAGA complex from *S. cerevisiae*. This protein complex is composed of several subunits, including a core element consisting of yGcn5, yAda3, and the PRZ1 ortholog yAda2 [15, 16]. In initial reports, SAGA was described as an activator of gene expression [18]. Subsequent analysis, however, indicated that the SAGA complex also mediates repression of gene expression [29]. Analysis of the protein complex revealed that the protein composition of SAGA is highly dynamic, indicating that distinct forms of the protein complex allow for flexible control of gene expression under variable growth conditions [30]. A hypothetical *Arabidopsis* chromatin-remodeling complex containing PRZ1 might behave similarly. According to this model, a mutation in *PRZ1* would result in a defective complex and give rise to deficiencies in the transcriptional regulation of genes, essential for the interplay of cell cycling and cell cycle exit in response to positional and environmental cues. Specifically, because the apparent defects in *prz1-1* hormone responses are paralleled by alterations in the transcription of cell cycle regulators, the corresponding gene product could be involved in the adjustment of gene expression levels in response to variations of auxin and cytokinin concentrations. In this model, the regulatory impact of *PRZ1* on cell cycle progression would represent an important determinant for phytohormone-mediated control of morphogenesis in *Arabidopsis*.

Supplemental Data

http://images.cellpress.com/supmat/supmatin.htm.

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Note Added in Proof

During revision of this manuscript, Vlachonasios et al., Plant Cell, *15*, 626–638 reported the characterization of an *Arabidopsis* insertion mutant that is defective in *AtADA2b* and allelic to *PRZ1*.