PICKLE Acts throughout the Plant to Repress Expression of Embryonic Traits and May Play a Role in Gibberellin-Dependent Responses[1]

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A seed marks the transition between two developmental states; a plant is an embryo during seed formation, whereas it is a seedling after emergence from the seed. Two factors have been identified in Arabidopsis that play a role in establishment of repression of the embryonic state: *PKL* (*PICKLE*), which codes for a putative CHD3 chromatin remodeling factor, and gibberellin (GA), a plant growth regulator. Previous observations have also suggested that *PKL* mediates some aspects of GA responsiveness in the adult plant. To investigate possible mechanisms by which *PKL* and GA might act to repress the embryonic state, we further characterized the ability of *PKL* and GA to repress embryonic traits and reexamined the role of *PKL* in mediating GA-dependent responses. We found that *PKL* acts throughout the seedling to repress expression of embryonic traits. Although the ability of *pkl* seedlings to express embryonic traits is strongly induced by inhibiting GA biosynthesis, it is only marginally responsive to abscisic acid and *SPY* (*SPINDLY*), factors that have previously been demonstrated to inhibit GA-dependent responses during germination. We also observed that *pkl* plants exhibit the phenotypic hallmarks of a mutation in a positive regulator of a GA response pathway including reduced GA responsiveness and increased synthesis of bioactive GAs. These observations indicate that *PKL* may mediate a subset of GA-dependent responses during shoot development.

Plants exhibit distinct differentiation characteristics during seed maturation and during subsequent seedling development. During seed maturation, the plant embryo stops growing, accumulates storage reserves, and acquires desiccation tolerance (Bewley and Black, 1994; Goldberg et al., 1994; Holdsworth et al., 1999; Baud et al., 2002). At the onset of seedling development, the plant undergoes cell expansion and division, mobilizes storage reserves, and begins photosynthesis. With the advent of genomic-based approaches, we are just beginning to appreciate the large number of genes that are differentially expressed at each of these developmental stages (Girke et al., 2000; Gallardo et al., 2001, 2002; Ruuska et al., 2002).

Characterization of *LEC1* (*LEAFY COTYLEDON1*), a gene that promotes embryonic identity in Arabidopsis, illustrates the importance of proper transcriptional regulation of stage-specific genes in specification of developmental identity. *lec1* plants exhibit defective embryo development and prematurely initiate the postembryonic differentiation program (Meinke, 1992; Meinke et al., 1994; West et al., 1994). *LEC1* codes for a putative transcriptional regulator, and the transcription of *LEC1* is seed specific (Lotan et al., 1998). Ectopic expression of *LEC1* during seedling development results in generation of supernumerary embryos on the seedling (Lotan et al., 1998). Thus, repression of *LEC1* and analogous genes is necessary to successfully make the transition from embryonic to postembryonic development.

PKL is necessary for repression of embryonic traits in Arabidopsis seedlings. The primary roots of *pkl* seedlings are capable of expressing embryonic traits after germination (Ogas et al., 1997). *pkl* primary roots that express embryonic traits are referred to as "pickle roots" based on the appearance of the distal tip of the root, which is swollen and greenish. Cloning of *PKL* revealed that it encoded a CHD3 chromatin remodeling factor (Eshed et al., 1999; Ogas et al., 1999). In animal systems, CHD3 proteins have been shown to associate in multisubunit complexes (Mi-2

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or NURD) that also contain a histone deacetylase, implying that CHD3 proteins function as negative regulators of transcription (Tong et al., 1998; Wade et al., 1998; Xue et al., 1998; Zhang et al., 1998). Consistent with such a role for CHD3 proteins in plants, *pkl* plants are defective in repression of *LEC1*; the *LEC1* transcript is expressed in germinating *pkl* seedlings (Ogas et al., 1999; Rider et al., 2003).

Expression of embryonic identity in *pkl* seedlings is dependent on the plant growth regulator GA, which plays many roles in plant growth and development (Davies, 1995; Kende and Zeevaart, 1997). In particular, the ability of GA to promote germination, shoot elongation, and flowering has been characterized in many species. In *pkl* seedlings, GA acts to repress embryonic identity; decreasing the level of GA in germinating seeds increases the penetrance of the pickle root phenotype (Ogas et al., 1997). Such a role had not been observed previously for GA, suggesting that a new GA response pathway had been identified. Furthermore, the adult shoot phenotype of *pkl* plants is reminiscent of a plant defective in GA response: *pkl* plants are dark green, time to flowering is increased, and *pkl* plants are dwarfed (Ogas et al., 1997). These phenotypes suggest that PKL itself may play a role in mediating GA-dependent responses.

We sought to further investigate the relationships between *PKL*, GA, and repression of embryonic identity. We have found that *PKL* is expressed throughout the germinating seedling and that *PKL* plays a role in repression of embryonic traits in the cotyledons, hypocotyl, and shoot apical meristem (SAM) in addition to the primary root. Although abscisic acid (ABA) and GA act during germination to repress and promote germination, respectively, we show that expression of embryonic identity in germinating *pkl* seeds is much less responsive to application of ABA. In addition, we find that although a mutation of *SPY* completely suppresses the germination defect of a plant defective in GA biosynthesis, it only slightly suppresses the derepression of embryonic traits that occurs when GA biosynthesis is perturbed in *pkl* seedlings. These observations indicate that the GA response pathway that mediates repression of embryonic traits in *pkl* seedlings appears distinct from previously characterized GA response pathways. Furthermore, we show that *pkl* plants exhibit the phenotypic hallmarks of a plant that is defective in the ability to respond to GA, including reduced responsivity to GA and elevated levels of bioactive GAs.

RESULTS

PKL Expression in Germinating Seeds

Previous work had indicated that *PKL* acted before the completion of germination (which is marked by the emergence of the radicle from the seed coat) to establish repression of embryonic identity (Ogas et al., 1997, 1999). This conjecture was based in part on the observation that the *LEC1* transcript was elevated in imbibed *pkl* seeds before germination. We analyzed *PKL* transcript levels in germinating seeds to determine if the *PKL* transcript was present before germination. Quantitative reverse transcriptase (RT)-PCR analysis was performed on total RNA isolated from desiccated seeds and from seeds that had been imbibed for up to 96 h (Fig. 1A). Germination of the seed was complete by 56 h. *PKL* transcript was detected in all of the samples, clearly indicating that the *PKL* transcript is present during imbibition before completion of germination. Furthermore, there was a significant elevation of the amount of the *PKL* transcript at the 36-h time point. Such a pattern of expression is consistent with the hypothesis that PKL acts during imbibition to repress transcription of *LEC1*.

Previous phenotypic characterization of *pkl* seedlings had revealed that *PKL* was necessary to repress embryonic identity in the primary root (Ogas et al., 1997). To examine the pattern of *PKL* expression in germinating seeds, we made use of a GUS reporter construct fused to the *PKL* upstream region, *GYM::GUS*. The expression pattern of this construct correlates with *PKL* transcript accumulation as determined by in situ hybridization (Eshed et al., 1999). GUS was detected throughout the entirety of the germinating seedling at 12, 24, and 36 h after imbibition (Fig. 1B; data not shown).

PKL Is Necessary for Repression of Embryonic Identity throughout the Seedling

Because the pattern of GUS expression suggested that *PKL* is expressed throughout the seedling, we examined whether *PKL* might be necessary for repression of embryonic identity in other parts of the seedling in addition to the primary root. Portions of the hypocotyl and/or cotyledon of 2-week-old *pkl*

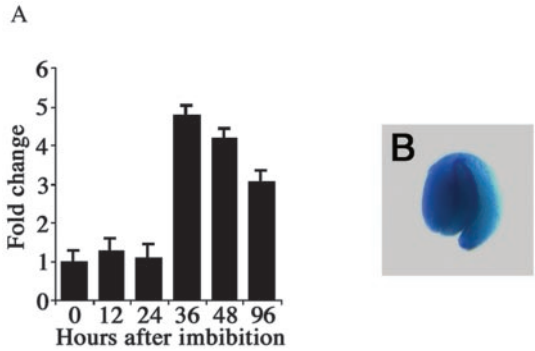
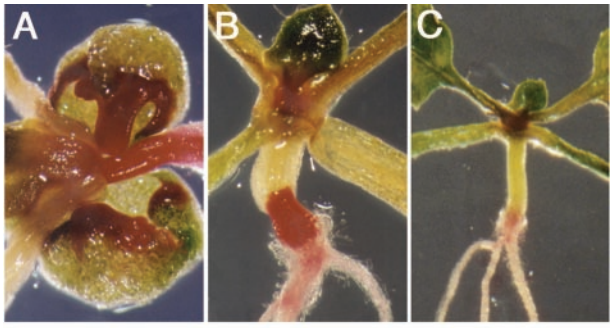


Figure 1. *PKL* is expressed throughout a germinating seed. A, Quantitative RT-PCR analysis was used to examine the *PKL* transcript level in desiccated seed and in seed that had been imbibed for up to 96 h. 18s rRNA was used as a standardization control, and expression levels are normalized to desiccated seed. Error bars represent the SD of the mean. B, GUS expression is detected throughout a transgenic seedling carrying the *GYM::GUS* reporter construct at 36 h after imbibition.

seedlings are sometimes similar in appearance to pickle root tissue. Like pickle roots, these "pickle-like" portions of the hypocotyl and cotyledons are intensely stained by Fat Red 7B, a dye that specifically interacts with neutral lipids (Brundrett et al., 1991; Fig. 2, A and B). This result suggests that these organs, like pickle roots, are capable of accumulating large amounts of triacylglycerol and, thus, are capable of expressing embryonic differentiation traits in *pkl* seedlings after germination.

To further examine the ability of various portions of the seedling to express embryogenic potential, we assayed for the ability of isolated organs to generate embryogenic callus in the absence of exogenous hormones. Pickle roots will produce embryogenic callus if excised from the plant and placed on hormone-free synthetic media (Ogas et al., 1997). We found that in addition to pickle roots, excised cotyledons and hypocotyls from *pkl* plants were also capable of forming embryogenic callus (Fig. 2D). In contrast, secondary roots and portions of primary root that did not visibly express embryonic traits did not form embryogenic callus. Excised portions of wild-type plants never gave rise to embryogenic callus.



D

Embryogenic callus produced in hormone-free synthetic medium

Genotype	Explant source	#embryogenic calli/#explants
pkl	cotyledon	15/15
	hypocotyl	5/15
	primary root (non-pickle portion)	0/15
	primary root (pickle portion)	10/15
	lateral root	0/25
WT	cotyledon	0/15
	hypocotyl	0/15
	primary root	0/25
	lateral root	0/25

E

Embryogenic cell lines derived from SAM

Genotype	# of cells in L1	%plants with embryogenic clusters
WT (Ler)	7.8 + 0.7	3.6*
pt	16.4 + 1.8	20.0
WT (Col)	9.3 + 0.9	6.9*
pkl	9.9 + 0.9	21.6
pt/pkl	18.0 + 3.3	45.0

Figure 2. *PKL* is necessary for repression of embryonic identity throughout the seedling. Ten-day-old *pkl* seedlings were stained with Fat Red 7B to visualize accumulation of triacylglycerols in the cotyledons (A) and hypocotyl (B) of 14-d-old *pkl* seedlings. C, Fourteen-day-old wild-type seedling stained with Fat Red 7B. D, Tissue explants generated from the indicated portions of *pkl*, and wild-type seedlings were transferred to hormone-free synthetic media and scored for the ability to generate embryogenic callus. E, Number of cells in the L1 layer of wild-type, *pkl*, *pt*, and *pkl pt* SAMs was determined as was the ability of the respective SAMs to give rise to embryogenic cell lines. An asterisk denotes clusters of cells that morphologically resemble embryogenic clusters of cells but that are not capable of generating embryogenic cell lines.

We also explored the potential of the SAM of *pkl* seedlings to express embryonic traits by examining the ability of the *pkl* SAM to give rise to embryogenic cell lines. We used a protocol that was used previously to characterize the embryogenic potential of the enlarged SAMs found in the *pt* (*primordia-timing*) and in two *clavata* mutants (Mordhorst et al., 1998, 2002). When seeds from these mutants are germinated in liquid media containing the synthetic auxin 2,4-dichlorophenoxyacetic acid, it is possible to identify embryogenic cell clusters on the mutant SAMs. We carried out our analysis using seeds from wild-type Columbia (Col), wild-type Landsberg *erecta* (L*er*), *pt*, *pkl*, and *pt pkl* plants. *pt* is a mutant allele of *AMP1* (*ALTERED MERISTEM PROGRAM1*), which codes for a protein with sequence similarity to a Glu carboxypeptidase that is thought to be involved in processing a signaling molecule in plants (Helliwell et al., 2001), and served as a positive control that the culture conditions were satisfactory for observing generation of embryogenic cell lines.

Under these culture conditions, neither the Col nor the L*er* SAMs gave rise to embryogenic clusters (Fig. 2E). In contrast, the *pt* and *pkl* SAMs were both capable of giving rise to such clusters. Although the *pt* seedlings had an enlarged SAM, as observed previously (Mordhorst et al., 1998), the *pkl* seedlings did not. Thus, an enlarged SAM is not a prerequisite for maintenance of embryonic identity in the *pkl* SAM. Double mutants between *pkl* and *pt* showed 2-fold more plants with embryogenic clusters, suggesting that *pkl* and *pt* act in two separate pathways to repress expression of embryonic potential in the SAM. All seeds were germinated in the absence of uniconazole-P. Thus, the *pkl* SAM is capable of expressing embryonic identity and does so in the absence of exogenous perturbation of GA biosynthesis.

In summary, all major organs that are produced during embryogenesis—the SAM, cotyledons, hypocotyl, and primary root—are impaired in repression of embryonic identity in *pkl* seedlings.

ABA Has a Modest Effect on the Penetrance of the Pickle Root Phenotype

ABA and GA act antagonistically with respect to germination (Hilhorst and Karssen, 1992; Holdsworth et al., 1999; Koornneef et al., 2002). GA pro-

motes germination, whereas ABA promotes seed dormancy and inhibits germination. Thus, treatment of a germinating seed with uniconazole-P (an inhibitor of GA biosynthesis) or with ABA has a similar phenotypic outcome: Germination is inhibited. Because ABA- and uniconazole-P-treated wild-type seedlings exhibit similar phenotypes, we examined whether ABA, like uniconazole-P, could increase penetrance of the pickle root phenotype in germinating *pkl* seedlings.

We found that ABA was much less effective than uniconazole-P in increasing the penetrance of the pickle root phenotype. *pkl* seedlings were grown in the presence of various concentrations of uniconazole-P and ABA, and penetrance of the pickle root phenotype was determined (Fig. 3A). Although 10^{-7} M uniconazole-P was able to increase pickle root penetrance to greater than 80%, 10^{-7} M ABA only increased penetrance of the pickle root phenotype to 11%. At concentrations above these, it is not possible to determine expression of the pickle root phenotype because of the inhibitory effect of uniconazole-P or of ABA on germinating seedlings. Uniconazole-P dramatically decreases germination of both wild-type and *pkl* seedlings (see below), whereas ABA-treated wild-type or *pkl* seedlings still germinate, but subsequent development is greatly impaired (data not shown). It has been demonstrated previously that GA can suppress the ability of uniconazole-P to increase the penetrance of the pickle root phenotype and that *ga1-3 pkl-1* plants exhibit higher penetrance of the pickle root phenotype in the presence of small amounts of GA (Ogas et al., 1997). Thus, these findings indicate that a reduced level of GA has a significantly greater effect on penetrance of the pickle root phenotype than the addition of ABA.

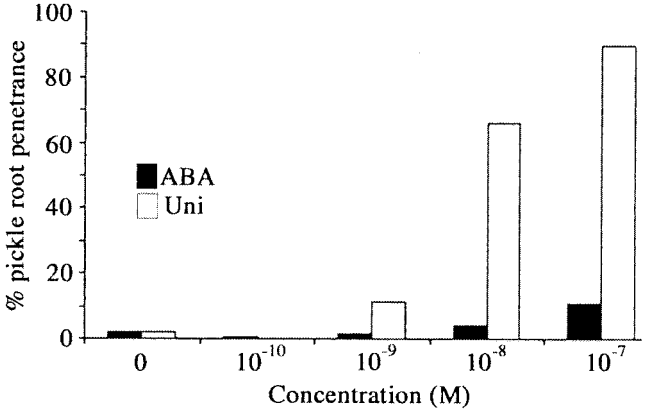


Figure 3. ABA is not as effective as uniconazole-P at increasing penetrance of the pickle root phenotype. A, Plants were grown continuously in the presence of ABA or of uniconazole-P (uni-P) and scored for penetrance of the pickle root phenotype. For each treatment, 144 seeds were incubated on synthetic media containing uniconazole-P or ABA at the indicated concentrations. The pickle root phenotype was scored at 10 d.

A Mutation in *SPY* Results in a Slight Decrease in Pickle Root Penetrance

The observation that the penetrance of the pickle root phenotype is strongly responsive to GA and relatively insensitive to ABA suggested that the GA response pathway that mediates this trait might be distinct from previously characterized GA response pathways. *SPY* is a well-characterized negative regulator of GA-dependent responses in Arabidopsis, including germination, and codes for an *O*-GlcNAc transferase (Jacobsen et al., 1996). To investigate the potential role of *SPY* in the GA response pathway that mediates penetrance of the pickle root phenotype, we examined pickle root penetrance in *pkl-1 spy-3* plants.

A mutation at the *SPY* locus suppresses the need for GA for germination (Jacobsen and Olszewski, 1993). One of the phenotypes of *pkl-1* seedlings is that they exhibit an increase in sensitivity to inhibitors of GA biosynthesis with regards to inhibition of germination. The *spy-3* mutation partially suppresses this phenotype (Fig. 4A). Although *pkl-1 SPY* seedlings do not germinate in the presence of 10^{-5} M uniconazole-P, 17% of *pkl-1 spy-3* seedlings germinate. Furthermore, *pkl-1 spy-3* seedlings are consistently less responsive to uniconazole-P than *pkl-1 SPY* seedlings with respect to the ability of uniconazole-P to promote penetrance of the pickle root phenotype (Fig. 4B).

It is worth noting, however, that these effects are not nearly as dramatic as the ability of *spy-3* to suppress the germination defect of a plant defective in GA biosynthesis. Both wild-type and *pkl-1* seeds do not germinate in the presence of 10^{-5} M uniconazole-P (Fig. 4A). Although 71% of *PKL-1 spy* seedlings germinate under these conditions, we observed that only 17% of the *pkl-1 spy-3* seedlings germinated. Similarly, *pkl-1 SPY* seedlings grown on 10^{-8} M uniconazole-P exhibit an increase in percent penetrance of the pickle root phenotype of 74%, whereas *pkl-1 spy-3* seedlings grown on 10^{-8} M uniconazole-P still exhibit an increase in penetrance of 56% (Fig. 4B). These observations indicate that a mutant allele of *SPY* has a modest effect on the GA response pathways that govern germination and repression of embryonic identity in *pkl-1* seedlings.

Characterization of GA-Dependent Responses in the *pkl* Shoot

Previous analysis of the adult phenotype of *pkl* plants revealed that they exhibit many phenotypes that are exhibited by plants that are defective in some aspect of GA biosynthesis or response. These observations suggest that PKL itself may plan a role in GA-dependent responses. An alternative hypothesis is that the shoot phenotypes exhibited by *pkl* plants are nonspecific consequences of a general alteration in transcriptional regulation. In an attempt to address

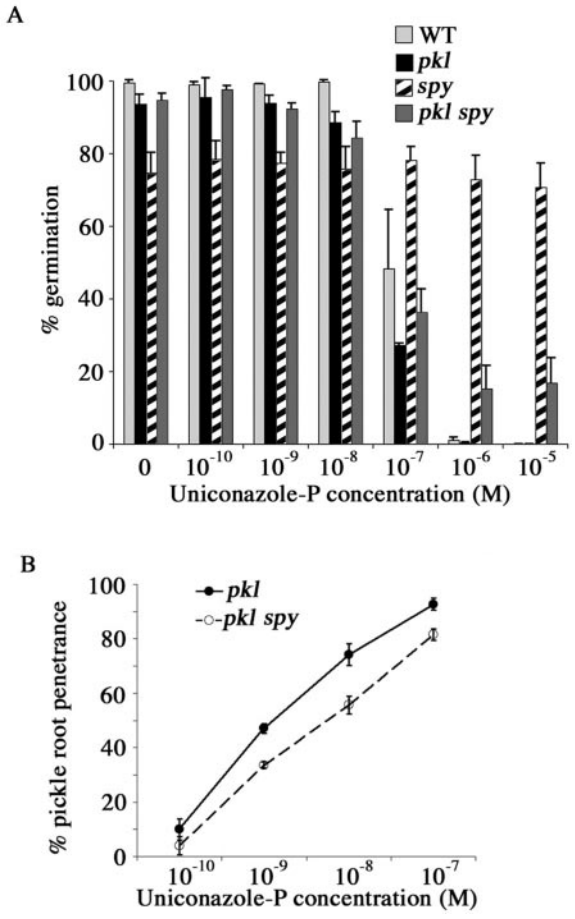


Figure 4. *spy* partially suppresses the effect of uniconazole-P on the phenotype of *pkl* seedlings. A, *spy* partially suppresses the germination defect of *pkl* in the presence of uniconazole-P. Seeds (144) were plated with at least two replicates on synthetic media containing the indicated concentration of uniconazole-P and scored for germination at 12 d after imbibition. B, *spy* partially suppresses the ability of uniconazole-P to increase penetrance of the pickle root phenotype. Plants were grown continuously on synthetic media containing the indicated concentration of uniconazole-P or in the absence of uniconazole-P and scored for penetrance of the pickle root phenotype. The *y* axis indicates the increase in penetrance of the pickle root phenotype observed at the indicated concentration of uniconazole-P as compared with penetrance of the pickle root phenotype when seedlings were grown in the absence of uniconazole-P [*y* = (percentage penetrance observed when grown at x molar uniconazole-P) − (percentage penetrance observed when grown in the absence of uniconazole-P)]. For each treatment, at least 288 seedlings were examined, and the pickle root phenotype was scored at 12 d.

whether the adult phenotypes exhibited by *pkl* plants are specifically because of a defect in some aspect of GA physiology, we examined the possibility that exogenous application of large amounts of GA might rescue some of the mutant phenotypes exhibited by adult *pkl* plants.

pkl plants are late-flowering dwarfs (Ogas et al., 1997). GA promotes the transition from vegetative to floral development in Arabidopsis, in part through promoting transcription of the floral regulator *LEAFY* (Blazquez et al., 1998; Blazquez and Weigel, 2000; Mouradov et al., 2002). To determine if exogenous application of GA might rescue the shoot phenotype of *pkl* plants, *pkl* and wild-type plants were grown in 18-h days and sprayed with GA or a mock solution (Fig. 5A). In the absence of GA, *pkl* plants flowered at 38.6 d, and wild-type plants flowered at 28.3 d. Application of GA_3 reduced the flowering time of *pkl* plants to 30.3 d and of wild-type plants to 24.3 d. This effect on flowering time can also be followed by looking at the number of rosette leaves that are produced by the plants (Fig. 5B). In the absence of exogenous GA, *pkl* plants possessed 22.6 rosette leaves upon flowering, and wild-type plants possessed 13.3 leaves. Application of GA_3 reduced the number of rosette leaves to 11.8 leaves for *pkl* plants and to 8.0 leaves for wild-type plants.

As noted above, GA promotes flowering; thus, it is not surprising that *pkl* plants flower in less time when sprayed with GA. The wild-type plants also flower in less time when sprayed with GA. The response of *pkl* plants, however, is significantly greater than that of wild-type plants with respect to flowering time and the number of rosette leaves ($P < 0.0001$). Application of GA_3 reduced flowering time by 22% for *pkl* plants and by only 14% for wild-type plants. Similarly, application of GA_3 reduced the number of rosette leaves by 48% for *pkl* plants and by 40% for wild-type plants.

Exogenous application of GA also partially rescued the reduced height of *pkl* plants, another phenotypic trait that is GA dependent. *pkl* and wild-type plants were grown as described above, and the height of the plants was measured (Fig. 5D). In the absence of GA, *pkl* plants attained a height of 29.9 cM, and wild-type plants attained a height of 55.7 cM. Application of GA_3 increased the height of *pkl* plants to 45.4 cM and that of wild-type plants to 62.1 cM. As before, GA has a significantly greater effect on *pkl* plants than on wild-type plants ($P < 0.0001$). Application of GA_3 increases the height of *pkl* plants by 52%, whereas the height of wild-type plants is only increased by 11%.

The ability of exogenous application of GA to partially rescue the shoot phenotype of *pkl* plants is consistent with a positive role for *PKL* in some aspect of GA biosynthesis or response. We specifically examined the effect of *PKL* on GA responsivity by examining the effect of the *pkl* mutation on hypocotyl elongation, a classic GA-dependent trait. *ga1-3 PKL* and *ga1-3 pkl-1* seedlings were grown in the presence of various concentrations of GA_3, and the length of the hypocotyl was determined. Although both lines exhibited a linear response to GA_3 between 5×10^{-8} and 10^{-5} M, the slope of the lines were significantly different ($P < 0.0007$; Fig. 5A). The *ga1-3 pkl-1* hypocotyls were only about one-half as responsive as the *ga1-3 PKL* hypocotyls to GA_3. Saturation of the response occurred between 5×10^{-5} and 1×10^{-4} M

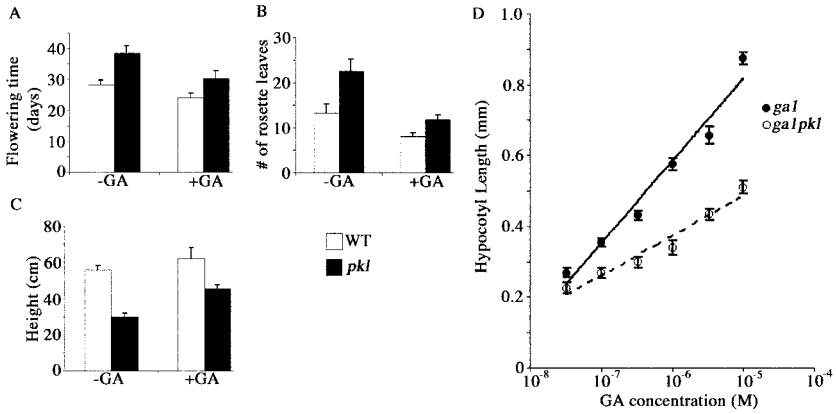


Figure 5. GA-dependent responses are altered in *pkl* plants. A, Hypocotyl length was determined at the indicated GA concentrations in *ga1* and *ga1 pkl* seedlings. The equations for the linear regressions are as follows: for *ga1*, $y = 2.0 + 0.10\ \mathrm{Ln}(x)$, $R^2 = 0.97$, indicated by a solid line; and for *ga1 pkl*, $y = 1.0 + 0.04\ \mathrm{Ln}(x)$, $R^2 = 0.95$, indicated by a dotted line. The values plotted are the mean ± SE of ≥18 seedlings for which the hypocotyl length was determined. Flowering time is defined as the number of days from imbibition to the opening of the first flower. Flowering time (B), number of rosette leaves (C), and height (D) were determined for *pkl* and wild-type plants grown in the presence or absence of GA. The values plotted are the mean ± SD of 20 plants measured.

(data not shown). At saturating concentrations of GA_3, *ga1-3 PKL* hypocotyls were 2.0 mm in length, whereas the *ga1-3 pkl-1*hypocotyls were only 1.1 mm in length. The observed decrease in responsivity of hypocotyl elongation to GA exhibited by *ga1-3 pkl-1* plants is unlikely to be a nonspecific effect of being derived from a mixed Col/L*er* background. A *ga1-3 PKL* L*er* line and a *ga1-3 PKL* L*er*/Col line do not respond differently to the GA_3 treatment gradient ($P = 0.68$; data not shown).

In light of the hypocotyl elongation results, it is intriguing to note that *pkl* seeds exhibit an increased sensitivity to uniconazole-P relative to wild-type seeds with respect to inhibition of germination (Fig. 4A). Thus *pkl* seeds also exhibit a phenotype consistent with a defect in GA response during germination.

Characterization of GA Biosynthesis in the *pkl* Shoot

Although elongation of *pkl* hypocotyls exhibits reduced responsivity to GA, this observation does not rule out the hypothesis that PKL also plays a role in biosynthesis of GA. The ability of GA to partially rescue some of the mutant shoot phenotypes of *pkl* plants is consistent with the possibility that *PKL*, a putative transcriptional regulator, promotes transcription of one or more GA biosynthetic genes and, therefore, is necessary for biosynthesis of wild-type levels of GA. We assayed endogenous GAs in *pkl* and wild-type plants to test this hypothesis. We found no evidence that *pkl* plants were defective in any step of the GA biosynthetic pathway. Instead, *pkl* plants possessed increased levels of bioactive GAs, indicating that the phenotypes exhibited by *pkl* plants are a result of a defect in the ability to respond to GA.

Table I summarizes the results of our analysis. Levels of GAs were determined by gas chromatography-mass spectrometry. The GAs in the top portion of the table are in the early 13-hydroxylation pathway, whereas the GAs in the bottom portion are in the non-13-hydroxylation pathway. GA_4 is the primary bioactive GA in Arabidopsis (Talon et al., 1990). The results revealed that *pkl* plants are not defective in GA biosynthesis. We instead found that the amounts of C_{20}-GAs were decreased in *pkl*, whereas the amounts of C_{19}-GAs—including GA_4—were increased. This experiment was repeated with similar results (data not

Table I. *GA content of wild-type Columbia and two* pkl *lines*

Nos. indicate nanograms of different GAs per gram dry wt. C_{20}-GAs are listed above the dotted line, whereas C_{19}-GAs are listed below the dotted line. GAs from the 13-hydroxlation pathway $GA_{53} \rightarrow GA_{44} \rightarrow GA_{19} \rightarrow GA_{20} \rightarrow GA_1 \rightarrow GA_8$ and from the non-13-hydroxylation pathway $GA_{12} \rightarrow GA_{15} \rightarrow GA_{24} \rightarrow GA_9 \rightarrow GA_4 \rightarrow GA_{34}$ were analyzed. GA_1 and GA_4 are bioactive GAs, whereas the other GAs are precursors or deactivated GAs.

GAs	WT	*pkl-1*	*pkl-3*
13-OH-GAs			
GA_{53}	9.7	2.8	2.4
GA_{44}	1.3	0.3	0.3
GA_{19}	11.6	7.1	6.1
GA_{20}	0.1	0.4	0.2
GA_1	0.2	0.5	0.4
GA_8	14.2	21.1	18.6
Non-13-OH-GAs			
GA_9	0.9	2.2	1.6
GA_4	1.1	1.8	2.2

shown). This pattern of accumulation of GAs is similar to that observed in *gai* plants, which are defective in perception of GA (Koorneef et al., 1985; Peng et al., 1997). Although the magnitude of the perturbation is more severe in *gai* plants (GA_4 has been reported to be elevated more than 20-fold, Talon et al., 1990; or 5-fold, Peng et al., 1999; in *gai* but is elevated only 60% in *pkl-1*), the overall trend of accumulation of GAs in *pkl* plants suggests that *PKL* is somehow involved in perception of GA.

Transcript levels of *AtGA3ox1* (*GA4*) and *AtGA20ox1* (*GA5*), which code for enzymes involved in GA biosynthesis, are subject to negative feedback regulation by GA (Chiang et al., 1995; Phillips et al., 1995; Xu et al., 1995,1999; Cowling et al., 1998). Although *gai* plants accumulate increased amounts of bioactive GAs, the level of the *AtGA20ox1* transcript is increased in *gai* plants (Xu et al., 1995; Peng et al., 1997). This observation supports the hypothesis that *GAI* is involved in feedback regulation of GA biosynthesis and that a defect in feedback regulation in *gai* plants may contribute to the elevated levels of bioactive GAs observed in *gai* plants. We examined the transcript levels of *AtGA3ox1* and *AtGA20ox1* in *pkl* plants to determine if they were similarly elevated.

We compared the relative transcript levels of *AtGA3ox1* and *AtGA20ox1* by quantitative RT-PCR in leaves from wild-type and *pkl* plants that had just began bolting (Fig. 6). We observed that the level of the *AtGA3ox1* transcript was reduced 2-fold in *pkl* plants relative to wild-type plants, whereas the *AtGA20ox1* transcript was reduced 3-fold. Thus, although both *pkl* and *gai* accumulate increased levels of C_{19}-GAs, these data suggest that *PKL* is not involved in the feedback regulation of the transcript levels of GA biosynthetic enzymes.

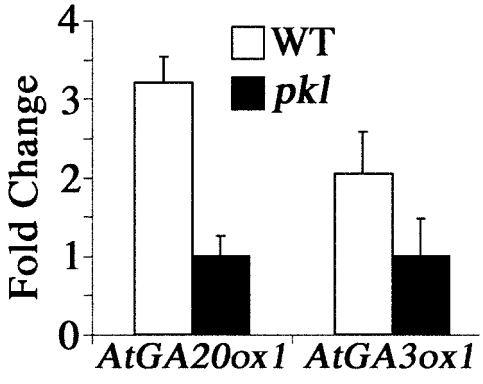


Figure 6. The *AtGA20ox1* and *AtGA3ox1* transcript levels are reduced in pkl leaves. Quantitative RT-PCR was used to determine the relative transcript level of *AtGA20ox1* and *AtGA3ox1* in wild-type leaves and *pkl* leaves. 18s rRNA was used as a standardization control, and expression levels are normalized to *pkl* leaves. Error bars = SD of the mean.

DISCUSSION

PKL Plays a Positive Role in GA-Dependent Responses

Our observations reveal that *pkl* plants exhibit two phenotypic hallmarks that are strongly associated with a defect in the ability of the plant to respond to GA. Hypocotyl elongation in *pkl* seedlings exhibits reduced responsivity to GA (Fig. 5D). *pkl* shoots accumulate C_{19}-GAs, including the bioactive GA_4 (Table I), in a manner similar to *gai* plants, which are defective in perception of GA. In addition, we find that shoot phenotypes of *pkl* plants are partially rescued by exogenous application of GA (Fig. 5, A–C). Rescue of a mutant phenotype by exogenous application of GA in a plant that is deficient in a factor that promotes a GA-dependent response has precedent. GA promotes flowering, in part by promoting transcription of *LFY*, a floral meristem identity gene that is necessary for the transition to flowering (Blazquez et al., 1998; Blazquez and Weigel, 2000; Mouradov et al., 2002). Exogenous application of GA can partially rescue the floral reversion phenotype exhibited by an *lfy-6/+* plant grown under short-day conditions (Okamuro et al., 1996).

Surprisingly, we also observed that transcript levels of *AtGA3ox1* and *AtGA20ox1* were decreased in *pkl* plants. A similar decrease in *AtGA20ox1* transcript level in *pkl* leaves has been reported previously (Hay et al., 2002). Transcript levels of both *AtGA3ox1* and *AtGA20ox1* is subject to feedback regulation; the mRNA level of both genes is reduced with the addition of GA (Chiang et al., 1995; Phillips et al., 1995; Xu et al., 1995, 1999; Cowling et al., 1998). Our interpretation of these observations (increased bioactive GAs and decreased transcript levels of *AtGA3ox1* and *AtGA20ox1*) is that *PKL* plays a role in GA homeostasis but is not necessary for feedback regulation of transcript levels. Examination of the effect of exogenous GA application on *AtGA3ox1* and *AtGA20ox1* transcript levels in *pkl* plants would help to further address this possibility.

The combination of all these observations strongly suggests that *pkl* plants are defective in some aspect of GA response, presumably as a result of a defect in transcriptional regulation.

The GA Response Pathway That Mediates Repression of Embryonic Identity in *pkl* Seedlings May Be Distinct from Previously Characterized GA Response Pathways

Penetrance of the pickle root phenotype has been demonstrated previously to be dependent on GA (Ogas et al., 1997). If GA levels are not perturbed in *pkl* seedlings, penetrance of the pickle root phenotype typically ranges from 1% to 5%. Reducing GA levels before germination results in increasing the penetrance of the pickle root phenotype to greater than 80%. We have now tested the ability of ABA,

another hormone that regulates germination, to affect pickle root penetrance. We observed that application of ABA had only a modest effect on penetrance of the pickle root phenotype (Fig. 3A). The same held true even if ABA was specifically applied during that time at which penetrance of the pickle root phenotype is most responsive to uniconazole-P (data not shown). These observations indicate that penetrance of the pickle root phenotype is substantially less responsive to application of ABA than to inhibition of GA biosynthesis. Furthermore, given that treatment with either uniconazole-P or ABA inhibits germination, the observation that treatment with uniconazole-P has a much greater effect on pickle root penetrance suggests that the ability of uniconazole-P to affect pickle root penetrance is not simply a consequence of decreasing the rate of germination.

The ability of GA to promote germination is antagonized by ABA (Hilhorst and Karssen, 1992; Holdsworth et al., 1999; Koornneef et al., 2002). The observation that penetrance of the pickle root phenotype is much less responsive to application of ABA than to inhibition of GA biosynthesis suggests that the GA response pathway that mediates repression of embryonic identity in *pkl* seedlings is distinct from previously characterized GA response pathways that function during germination. Our analysis of pickle root penetrance in *pkl spy* plants is consistent with this hypothesis. The *spy* mutation suppresses the need for GA for germination; *spy ga1* seed can germinate in the absence of GA (Jacobsen and Olszewski, 1993). We observed that mutating *SPY* only weakly suppresses the ability of uniconazole-P to increase pickle root penetrance in *pkl* seedlings (Fig. 4B). This result thus also suggests that the GA response pathway that mediates repression of embryonic identity in *pkl* seedlings is distinct from the GA response pathway that mediates germination.

It is not yet known how this *PKL*-independent pathway mediates repression of embryonic identity. One possibility is that this alternative pathway utilizes one of the other three CHD genes present in Arabidopsis. Analysis of transcript levels of the three CHD genes, however, reveals that the transcript level of all three genes is neither *PKL* dependent nor effected by the presence of 10^{-8} M uniconazole-P during germination (D. Rider, unpublished data).

PKL Is Necessary for Repression of Embryonic Identity throughout the Germinating Seedling

Previous characterization of the differentiation state of the *pkl* seedling focused on the pickle root phenotype, which was shown to result from derepression of embryonic identity in the primary root. We have now shown that all major organs derived during embryogenesis are capable of expressing embryonic traits in *pkl* seedlings (Fig. 2). Thus, *PKL* apparently functions throughout the organism to repress the potential to express the embryonic state. Consistent with this hypothesis, we observe that a *PKL* transcriptional reporter is expressed throughout a germinating seedling (Fig. 1B).

An intriguing observation regarding the embryogenic potential of the *pkl* SAM is that it is possible to generate embryogenic cell lines in the absence of uniconazole-P. These data are consistent with observations that the *LEC* class of embryonic regulators are derepressed in germinating *pkl* seedlings in the absence of uniconazole-P (Rider et al., 2003). Our interpretation of these results is that derepression of *LEC* and related genes in *pkl* seeds during germination creates the potential for generation of embryonic traits but does not ensure the subsequent expression of this potential. In the presence of other factors, however, it is possible to greatly increase the number of seedlings in which embryonic traits continue to be derepressed after germination. Thus addition of 1×10^{-8} M uniconazole-P during germination facilitates expression of the pickle root phenotype in roots, whereas addition of 4.5×10^{-6} M 2,4-dichlorophenoxyacetic acid allows generation of embryogenic cell lines in the SAM.

It still remains to be determined when *PKL* acts to repress gene expression. The transcript level of *LEC1* is *PKL*-dependent and is elevated in *pkl* seedlings between 24 and 36 h after imbibition (Ogas et al., 1997; Rider et al., 2003). This observation suggests that *PKL* acts before this time to repress transcription of genes. We have now observed that transcript levels of *PKL* peak during imbibition before completion of germination (Fig. 1A). The timing of the increase in *PKL* transcript level is consistent with the hypothesis that PKL acts during this time to mediate repression of embryonic identity, presumably through the repression of transcription of genes such as *LEC1*. Further testing of this hypothesis will require identification of that time at which PKL binds to its presumptive targets by using chromatin immunoprecipitation or a related biochemical approach.

PKL, GA, and Repression of Embryonic Identity

We have shown that *PKL* is necessary for wild-type GA-dependent responses in the shoot. We also have shown that *PKL* acts throughout the germinating seedling to repress expression of embryonic traits. Expression of these traits in *pkl* seedlings is likely to be a consequence of the failure to repress expression of the *LEC* genes during germination (Rider et al., 2003). We propose that the dual roles of *PKL* in repression of embryonic identity and in promoting GA-dependent shoot responses are two aspects of the same activity; GA-dependent repression of the transcriptional activity of target genes via a CHD3 chromatin remodeling factor. Thus, we propose that that *PKL* mediates GA-dependent repression of some seed-specific genes during germination, including

the repression of genes such as *LEC1* that promote embryonic identity. We also propose that there is a *PKL*-independent pathway by which GA acts to repress expression of embryonic traits because GA can act in the absence of *PKL* to repress expression of the pickle root phenotype (Ogas et al., 1997).

A corollary of this hypothesis is that GA acts via *PKL* to repress transcription of certain genes in the adult plant as well. It is well known that GA promotes various developmental transitions in the plant. Such transitions are likely to involve transcriptional repression of various genes in addition to activation of others. Based on the characterization of the expression of genes that are inappropriately expressed during germination of *pkl* seeds (Rider et al., 2003), our prediction is that stage-specific restriction of developmental regulators is lost during other stages of *pkl* shoot development as well. Thus, the phenotype of *pkl* adult plants is a result of the failure to restrict genes to their normal developmental window. An implicit assumption of this hypothesis is that there are other GA response pathways that function in *pkl* plants but in an inappropriate developmental context as a result of the failure to repress various regulators. It remains to be determined what contribution, if any, is made to the *pkl* shoot phenotype by the seed-specific genes that exhibit elevated expression during germination of *pkl* seeds. Transcripts for *LEC1* and *LEC2* are not elevated in *pkl* leaves, but the *FUS3* transcript is elevated almost 6-fold (Rider et al., 2003). Overexpression of *FUS* under the control of the cauliflower mosaic virus 35S promoter, however, was not reported to generate a GA-deficient phenotype (Luerssen et al., 1998).

Because *pkl* plants exhibit a phenotype that is strikingly similar to a defect in the ability to respond to GA, we have assumed in our model that *PKL* activity is in some way responsive to GA. It is important to note, however, that no data have been presented that shows a direct link between PKL activity and GA. As a consequence, an alternative hypothesis is that PKL functions in a GA-independent manner during germination and during subsequent shoot development. Thus, repression of *LEC1* may be a *PKL*-dependent but GA-independent event. Similarly, *PKL* may be necessary to provide a proper developmental context for GA response pathways in the shoot, for example by repressing expression of one or more factors that would otherwise inhibit GA-dependent responses. It is important to note that this hypothesis does not presuppose that the normal role of these *PKL*-repressed factors is to act as an inhibitor of GA response when they are expressed in their proper developmental context. For example, perhaps the reduced elongation of *pkl* hypocotyls is because of inappropriate expression of cell wall factors that are intrinsically less able to promote cell wall expansion. Further experiments will be necessary to distinguish between these two hypotheses regarding the relationship between GA and *PKL* by determining if *PKL* expression and/or activity are GA dependent.

There is little evidence to indicate whether GA- and *PKL*-dependent response pathways analogous to those that act during germination are functioning in shoot development after germination. *PKL* has been demonstrated to play a role in repression of meristematic activity in the shoot (Eshed et al., 1999; Ori et al., 2000). It is unknown whether or not this activity is GA-dependent. It is intriguing to note, however, that genes that promote meristematic activity also down-regulate expression of genes involved in GA biosynthesis and that up-regulation of GA signaling results in decreased meristematic activity of mutationally compromised meristems (Hay et al., 2002). Thus, *PKL* and GA both act as negative regulators with respect to expression of meristematic activity in the shoot, an observation that is consistent with the hypothesis that PKL activity may be GA dependent in this context as well.

The proposed role for PKL as a hormone-dependent chromatin remodeling factor that mediates developmental transitions is not without parallel in animal systems. A CHD3 gene has been shown to play a strikingly similar role to *PKL* in *Caenorhabditis elegans* development (Unhavaithaya et al., 2002). Germline and somatic cells are segregated early during embryogenesis and express dramatically different differentiation programs. In worms that lack the CHD3 protein LET-418/Mi-2, somatic cells inappropriately continue to express germline traits. There is no evidence in this circumstance that the repressive activity is hormone dependent. In human (*Homo sapiens*) breast epithelial cells, however, the action of a CHD3 complex has been demonstrated to be hormone dependent for at least one locus (Fujita et al., 2003). The transcriptional repressor Snail acts as a master regulator of epithelial to mesenchymal transitions. The CHD3-containing Mi-2/NuRD complex mediates repression of Snail in an estrogen-dependent manner. Thus, the basic role of CHD3 proteins appears well conserved in both plants and animals. We anticipate that continued characterization of *PKL*-mediated events in Arabidopsis is likely to shed further light on how CHD3 proteins mediate transitions between developmental stage-specific transcription programs in eukaryotes.

MATERIALS AND METHODS

Plant Material and Growth Conditions

pkl-1 (Ogas et al., 1997), *pkl-3*, and *spy-3* (Jacobsen and Olszewski, 1993) are in the Col background. *ga1-3* (Koorneef et al., 1983), *gai* (Koorneef et al., 1985), and *pt* (Vizir et al., 1995) are in the L*er* background. Plants and plant explants were grown on synthetic media as previously described (Ogas et al., 1997). The pickle root phenotype was scored based on inspection under the dissection microscope. For hypocotyl dose response curves, seeds were plated and then incubated for 5 d at 4°C in the dark. Plates were then placed in a vertical position in a Percival CU36L5 incubator (Percival Scientific Inc., Perry, IA). Pictures of the plants were taken at 7 d after radicle emergence, and the hypocotyl length was determined using NIH Image software (Na-

tional Institutes of Health, Bethesda, MD). Plants that were examined for GA-dependent shoot phenotypes were grown in a walk-in chamber with 16 h of illumination (110–150 μE m^{-2} s^{-1}) at 22°C. Plants were sprayed with an aqueous solution containing 100 μM GA_3 and 0.05% (v/v) Tween 20 or with a mock solution containing 0.05% (v/v) Tween 20 twice a week. Flowering was scored as the number of days until the first flower opened.

GUS and Fat Red Staining

GUS staining was done as described by Hemerly et al. (1993). Staining with Fat red 7B was carried out as described previously (Ogas et al., 1997).

Determination of Embryogenic Potential of *pkl* and Wild-Type Tissue

Explants were isolated from plants that were grown on one-half-strength Murashige and Skoog medium under 8 h of light for 2 weeks. Explants of hypocotyl, cotyledon, and root parts, swollen or normal, were then excised and placed on Murashige and Skoog agar medium at 24°C. To assay for embryogenic ability, explants were transferred into liquid Murashige and Skoog medium and shaken at 110 rpm at 24°C. In 1 to 3 weeks, embryos will appear from cultures containing embryogenic explants. Embryogenic cell lines were established from the SAM of seedlings as described previously (Mordhorst et al., 1998). The number of cells in the L1 layer of the SAM as a measurement of the SAM size was analyzed in whole-mount preparations of mature embryos as described previously (Mordhorst et al., 1998).

Statistical Analysis of GA-Dependent Traits

The effect of genotype and GA on the response variables flowering time and height was analyzed using ANOVA for a completely random design in which each of the four treatment classes (WT + GA_3, WT + mock solution, *pkl* + GA_3, and *pkl* + mock solution) contained five pots of four plants each. Pots were randomly arranged in the growth chamber. The model included the effect of genotype, GA treatment, and the interaction between genotype and GA treatment. A significant interaction in this simple design means that the response surface of WT plants to GA_3 differs from the response surface of *pkl* plants to GA_3 for variable tested. The effect of genotypes *GA1 pkl-1* and *ga1-3 pkl-1* on hypocotyl response to exogenous GA was analyzed with a general linear model procedure, a generalized form of the ANOVA that can handle unbalanced data. In both analyses, tests of the response surface indicated that a simple linear model best explained the observed responses. Thus, a significant interaction between genotype and GA treatment means that the slope of the line for one genotype is significantly different from the slope of the line for the other genotype. The analyses were performed using SAS Proprietary Software Release 8.2 (SAS Institute Inc., Cary, NC).

Quantification of GAs

Plants were initially grown in 9-h photoperiods until large rosettes had developed. For 4 d before harvesting the rosettes, the 15-h dark period was replaced with light from incandescent bulbs at approximately 10 μmol m^{-2} s^{-1}. The entire rosettes (minus senescing yellow old leaves) were harvested and used for GA analysis. GAs were extracted, purified, and quantified as previously described (Talon et al., 1990a).

RNA Isolation and Quantitative RT-PCR Analysis

RNA isolation and quantitative RT-PCR analysis was carried out as described previously using the ABI Prism 7000 Sequence Detection System (Applied Biosystems, Foster City, CA; Rider et al., 2003). The 18s ribosomal RNA forward and reverse primers were 5′TCCTAGTAAGCGCGAGTCATCA3′ and 5′GAACACTTCACCGGATCAT3′, respectively. Primers used for the *PKL* transcript were 5′CTCATTCCGCATTTGGTAATTG 3′ and 5′GCCCATGTGGCAAACTCTCT 3′. The GA4 forward primer sequence was 5′CACCGCGCTCGGGTTA3′, and the reverse primer sequence was 5′CAGATTGCGGACCCCAAA3′. The GA5 forward primer sequence was 5′CCCTTTCTTTCCGGTTTTGC3′, and the reverse primer sequence was 5′CAACGCATCGCAGAAGTAATCT3′. Optimal final concentrations for primers used for 18s, *PKL*, and *GA4* were 900 nM for the forward primer and 900 nM for the reverse primer. Primer concentrations for *GA5* were 900 and 300 nM for the forward and reverse primers, respectively.

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LITERATURE CITED

Baud S, Boutin JP, Miquel M, Lepiniec L, Rochat C (2002) An integrated overview of seed development in *Arabidopsis thaliana* ecotype WS. Plant Physiol Biochem **40:** 151–160

Bewley JD, Black M (1994) Seeds: Physiology of Development and Germination, Ed 2. Plenum Press, New York

Blazquez MA, Green R, Nilsson O, Sussman MR, Weigel D (1998) Gibberellins promote flowering of Arabidopsis by activating the LEAFY promoter. Plant Cell **10:** 791–800

Blazquez MA, Weigel D (2000) Integration of floral inductive signals in Arabidopsis. Nature **404:** 889–892

Brundrett MC, Kendrick B, Peterson CA (1991) Efficient lipid staining in plant material with Sudan red 7B or fluoral yellow 088 in polyethylene glycol-glycerol. Biotechnol Histochem **66:** 111–116

Chiang HH, Hwang I, Goodman HM (1995) Isolation of the Arabidopsis GA4 locus. Plant Cell **7:** 195–201

Cowling RJ, Kamiya Y, Seto H, Harberd NP (1998) Gibberellin dose-response regulation of GA4 gene transcript levels in Arabidopsis. Plant Physiol **117:** 1195–1203

Davies PJ, editor (1995) Plant Hormones: Physiology, Biochemistry and Molecular Biology. Kluwer Academic Publishers, Dordrecht, The Netherlands

Eshed Y, Baum SF, Bowman JL (1999) Distinct mechanisms promote polarity establishment in carpels of Arabidopsis. Cell **99:** 199–209

Fujita N, Jaye DL, Kajita M, Geigerman C, Moreno CS, Wade PA (2003) MTA3, a Mi-2/NuRD complex subunit, regulates an invasive growth pathway in breast cancer. Cell **113:** 207–219

Gallardo K, Job C, Groot SP, Puype M, Demol H, Vandekerckhove J, Job D (2001) Proteomic analysis of Arabidopsis seed germination and priming. Plant Physiol **126:** 835–848

Gallardo K, Job C, Groot SP, Puype M, Demol H, Vandekerckhove J, Job D (2002) Proteomics of Arabidopsis seed germination: a comparative study of wild-type and gibberellin-deficient seeds. Plant Physiol **129:** 823–837

Girke T, Todd J, Ruuska S, White J, Benning C, Ohlrogge J (2000) Microarray analysis of developing Arabidopsis seeds. Plant Physiol **124:** 1570–1581

Goldberg RB, de Paiva G, Yadegari R (1994) Plant embryogenesis: zygote to seed. Science **266:** 605–614

Hay A, Kaur H, Phillips A, Hedden P, Hake S, Tsiantis M (2002) The gibberellin pathway mediates KNOTTED1-type homeobox function in plants with different body plans. Curr Biol **12:** 1557–1565

Helliwell CA, Chin-Atkins AN, Wilson IW, Chapple R, Dennis ES, Chaudhury A (2001) The Arabidopsis AMP1 gene encodes a putative glutamate carboxypeptidase. Plant Cell **13:** 2115–2125

Hemerly AS, Ferreira P, de Almeida Engler J, Van Montagu M, Engler G, Inze D (1993) cdc2a expression in Arabidopsis is linked with competence for cell division. Plant Cell **5:** 1711–1723

Hilhorst HWM, Karssen CM (1992) Seed dormancy and germination: the role of abscisic acid and gibberellins and the importance of hormone mutants. Plant Growth Reg **11:** 225–238

Holdsworth M, Kurup S, McKibbin R (1999) Molecular and genetic mechanisms regulating the transition from embryo development to germination. Trends Plant Sci **4:** 275–280

Jacobsen SE, Binkowski KA, Olszewski NE (1996) SPINDLY, a tetratricopeptide repeat protein involved in gibberellin signal transduction in Arabidopsis. Proc Natl Acad Sci U S A **93:** 9292–9296

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

Kende H, Zeevaart JAD (1997) The five "classical" plant hormones. Plant Cell **9:** 1197–1210

Koorneef M, Elgersma A, Hanhart CJ, van Loenen-Martinet EP, van Rijn L, Zeevaart JAD (1985) A gibberellin insensitive mutant of *Arabidopsis thaliana*. Physiol Plant **65:** 33–39

Koornneef M, Bentsink L, Hilhorst H (2002) Seed dormancy and germination. Curr Opin Plant Biol **5:** 33–36

Koorneef M, van Eden J, Hanhart CJ, de Jongh AMM (1983) Genetic fine-structure of the *GA-1* locus in the higher plant *Arabidopsis thaliana* (L.) Heynh. Genet Res Camb **41:** 57–68

Lotan T, Ohto M, Yee KM, West MA, Lo R, Kwong RW, Yamagishi K, Fischer RL, Goldberg RB, Harada JJ (1998) Arabidopsis LEAFY COTYLEDON1 is sufficient to induce embryo development in vegetative cells. Cell **93:** 1195–1205

Luerssen H, Kirik V, Herrmann P, Misera S (1998) FUSCA3 encodes a protein with a conserved VP1/AB13-like B3 domain which is of functional importance for the regulation of seed maturation in *Arabidopsis thaliana*. Plant J **15:** 755–764

Meinke DW (1992) A homeotic mutant of *Arabidopsis thaliana* with leafy cotyledons. Science **258:** 1647–1650

Meinke DW, Franzmann LH, Nickle TC, Yeung EC Leafy cotyledon mutants of Arabidopsis. Plant Cell **6:** 1049–1064, 1994

Mordhorst AP, Hartog MV, El Tamer MK, Laux T, de Vries SC (2002) Somatic embryogenesis from Arabidopsis shoot apical meristem mutants. Planta **214:** 829–836

Mordhorst AP, Voerman KJ, Hartog MV, Meijer EA, van Went J, Koornneef M, de Vries SC (1998) Somatic embryogenesis in *Arabidopsis thaliana* is facilitated by mutations in genes repressing meristematic cell divisions. Genetics **149:** 549–563

Mouradov A, Cremer F, Coupland G (2002) Control of flowering time: interacting pathways as a basis for diversity. Plant Cell Suppl **14:** S111–130

Ogas J, Cheng J-C, Sung ZR, Somerville C (1997) Cellular differentiation regulated by gibberellin in the *Arabidopsis thaliana pickle* mutant. Science **277:** 91–94

Ogas J, Kaufmann S, Henderson J, Somerville C (1999) PICKLE is a CHD3 chromatin-remodeling factor that regulates the transition from embryonic to vegetative development in Arabidopsis. Proc Natl Acad Sci USA **96:** 13839–13844

Okamuro JK, den Boer BG, Lotys-Prass C, Szeto W, Jofuku KD (1996) Flowers into shoots: photo and hormonal control of a meristem identity switch in Arabidopsis. Proc Natl Acad Sci USA **93:** 13831–13836

Ori N, Eshed Y, Chuck G, Bowman JL, Hake S (2000) Mechanisms that control knox gene expression in the Arabidopsis shoot. Development **127:** 5523–5532

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

Peng J, Richards DE, Moritz T, Cano-Delgado A, Harberd NP (1999) Extragenic suppressors of the Arabidopsis gai mutation alter the dose-response relationship of diverse gibberellin responses. Plant Physiol **119:** 1199–1208

Phillips AL, Ward DA, Uknes S, Appleford NE, Lange T, Huttly AK, Gaskin P, Graebe JE, Hedden P (1995) Isolation and expression of three gibberellin 20-oxidase cDNA clones from Arabidopsis. Plant Physiol **108:** 1049–1057

Rider SD, Henderson JT, Jerome RE, Edenberg HJ, Romero-Severson J, Ogas J (2003) Coordinate repression of regulators of embryonic identity by PICKLE during germination in Arabidopsis. Plant J **35:** 33–43

Ruuska SA, Girke T, Benning C, Ohlrogge JB (2002) Contrapuntal networks of gene expression during Arabidopsis seed filling. Plant Cell **14:** 1191–1206

Talon M, Koornneef M, Zeevaart JA (1990a) Endogenous gibberellins in *Arabidopsis thaliana* and possible steps blocked in the biosynthetic pathways of the semidwarf ga4 and ga5 mutants. Proc Natl Acad Sci USA **87:** 7983–7987

Talon M, Koornneef M, Zeevaart JAD (1990b) Accumulation of C19-gibberellins in the gibberellin-insensitive dwarf mutant *gai* of *Arabidopsis thaliana* (L.) Hehyn. Planta **182:** 501–505

Tong JK, Hassig CA, Schnitzler GR, Kingston RE, Schreiber SL (1998) Chromatin deacetylation by an ATP-dependent nucleosome remodelling complex. Nature **395:** 917–921

Unhavaithaya Y, Shin TH, Miliaras N, Lee J, Oyama T, Mello CC (2002) MEP-1 and a homolog of the NURD complex component Mi-2 act together to maintain germline-soma distinctions in *C. elegans*. Cell **111:** 991–1002

Vizir I, Thorlby G, Briarty G, Mulligan B (1995) Positional cloning of the primordia timing gene in Arabidopsis. *In* 6th International Conference on Arabidopsis Research.

Wade PA, Jones PL, Vermaak D, Wolffe AP (1998) A multiple subunit Mi-2 histone deacetylase from *Xenopus laevis* cofractionates with an associated Snf2 superfamily ATPase. Curr Biol **8:** 843–846

West MAL, Yee KM, Danao J, Zimmerman JL, Fischer RL, Goldberg RB, Harada JJ (1994) LEAFY COTYLEDON1 is an essential regulator of late embryogenesis and cotyledon identity in Arabidopsis. Plant Cell **6:** 1731–1745

Xu YL, Li L, Gage DA, Zeevaart JA (1999) Feedback regulation of GA5 expression and metabolic engineering of gibberellin levels in Arabidopsis. Plant Cell **11:** 927–936

Xu YL, Li L, Wu K, Peeters AJ, Gage DA, Zeevaart JA (1995) The GA5 locus of *Arabidopsis thaliana* encodes a multifunctional gibberellin 20-oxidase: molecular cloning and functional expression. Proc Natl Acad Sci USA **92:** 6640–6644

Xue Y, Wong J, Moreno GT, Young MK, Cote J, Wang W (1998) NURD, a novel complex with both ATP-dependent chromatin-remodeling and histone deacetylase activities. Mol Cell **2:** 851–861

Zhang Y, LeRoy G, Seelig HP, Lane WS, Reinberg D (1998) The dermatomyositis-specific autoantigen Mi2 is a component of a complex containing histone deacetylase and nucleosome remodeling activities. Cell **95:** 279–289