PICKLE is a CHD3 chromatin-remodeling factor that regulates the transition from embryonic to vegetative development in *Arabidopsis*

Joe Ogas*†, Scott Kaufmann‡, Jim Henderson*, and Chris Somerville‡

*Department of Biochemistry, Purdue University, West Lafayette, IN 47907-1153; and ‡Carnegie Institution of Washington, Department of Plant Biology, 260 Panama Street, Stanford, CA 94305

The life cycle of angiosperms is punctuated by a dormant phase that separates embryonic and postembryonic development of the sporophyte. In the *pickle* (*pkl*) mutant of *Arabidopsis*, embryonic traits are expressed after germination. The penetrance of the *pkl* phenotype is strongly enhanced by inhibitors of gibberellin biosynthesis. Map-based cloning of the *PKL* locus revealed that it encodes a CHD3 protein. CHD3 proteins have been implicated as chromatin-remodeling factors involved in repression of transcription. PKL is necessary for repression of *LEC1*, a gene implicated as a critical activator of embryo development. We propose that PKL is a component of a gibberellin-modulated developmental switch that functions during germination to prevent reexpression of the embryonic developmental state.

During the final stages of embryo development in angiosperms, the embryo accumulates massive amounts of nutrient storage reserves and then undergoes programmed desiccation and transition to dormancy (1). The embryo may remain dormant for extended periods of time. The quiescent embryo emerges from dormancy and undergoes postembryonic vegetative development in response to one or more endogenous and exogenous cues that may vary from one species to another. The regulatory processes that control the transition from the late stages of embryo development to vegetative growth and development are poorly characterized.

The ability of the growth regulator gibberellin (GA) to promote germination of seeds of numerous plant species has been established through the use of chemical inhibitors of GA biosynthesis and the characterization of mutants defective in GA biosynthesis (2). Very little is known about the mechanism by which GA promotes germination. Genes that undergo GA-dependent transcription are known, and the ability of GA to regulate transcription of genes in the aleurone layer of germinating cereal grains has been characterized extensively (2). However, a receptor for GA has not been identified. GA plays other well characterized roles in plant growth and development in addition to its role in germination, including promotion of elongation and regulation of the transition to flowering (3, 4).

pkl mutants are defective in repressing embryonic differentiation characteristics after germination (5). Primary roots of *pkl* plants sometimes fail to develop normally after germination and, instead, express embryonic differentiation characteristics. These embryonic differentiation characteristics include expression of genes for seed storage proteins and accumulation of large amounts of neutral lipids. Primary roots that express embryonic differentiation characteristics are called "pickle roots" based on the visual appearance of the distal end of the primary root, which is swollen and greenish. Penetrance of the pickle root phenotype is strongly increased when endogenous GA levels are reduced by treatment with inhibitors of GA biosynthesis. Although the pickle root phenotype is evident only several days after germination, the phenotype is determined during the first 36 h of imbibition, a period when germination of the seed is not evident.

Thus, we infer that PKL acts within this time frame to promote the transition from embryonic to postembryonic development.

We describe herein the cloning and characterization of the *PKL* locus. *PKL* encodes a putative CHD3 protein, a chromatin-remodeling factor that is conserved in eukaryotes and is proposed to act as a negative regulator of transcription (6–10). *LEC1*, a seed-specific transcription factor that promotes embryonic identity (11, 12), is derepressed in pickle roots. Furthermore, *LEC1* is derepressed in *pkl* seeds during the first 36 h of imbibition. We propose that PKL is a component of a GA-modulated determination pathway that functions to establish repression of embryonic identity during germination.

Materials and Methods

Plant Material and Media. The *pkl-1* mutation was isolated from an ethyl methanesulfonate-mutagenized population of the Col ecotype (5). The *pkl-7*, *pkl-8*, and *pkl-9* alleles were isolated from a fast neutron-mutagenized population of the Col ecotype that was obtained from Lehle Seeds (www.arabidopsis.com; catalogue no. M2F-01A-04). Plants were grown as described (5).

Cloning of PKL. *pkl-1* plants of the Col ecotype were crossed to plants of the Landsberg *erecta* type to generate a mapping population, and 300 F$_2$ progeny expressing the pickle root phenotype were isolated. DNA was isolated from these progeny as described (13). The simple sequence length polymorphisms (SSLP) markers used are described at genome.bio.upenn.edu/ SSLP_info/SSLP.html, and the PCR analysis of the markers was done as described (14). The amplified fragment length polymorphism (AFLP) analysis was performed as described (13). The AFLP primers used for mapping analysis were as follows: the basic *Eco*RI primer was 5′-AGA CTG CGT ACC AAT TCx y-3′ (where x and y indicate base pairs added for specificity), and the basic *Mse*I primer was 5′-GAT GAG TCC TGA GTA Axy z-3′ (where x, y, and z indicate base pairs added for specificity). E11M48 denotes the primer pair *Eco*RI-AA and *Mse*I-CAC; E11M49 denotes the primer pair *Eco*RI-AA and *Mse*I-CAG; E14M59 denotes the primer pair *Eco*RI-AT and *Mse*I-CTA (15).

To identify polymorphisms in the fast neutron-derived alleles of *PKL*, Southern blots were performed by using genomic DNA from plants and digoxigenin-labeled probes that were generated from yeast artificial chromosome (YAC) DNA by using AFLP technology. DNA from YAC CIC8H12 was prepared as described (16). Approximately 50 ng of CIC8H12 DNA was used

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in a restriction and ligation reaction as described (carnegiedpb-.stanford.edu/methods/aflp.html), with the following differences: the DNA was digested only with *Mse*I, and only the *Mse*I adaptor was ligated on to the DNA. This mixture (5 μl) was then used in a 100-μl digoxigenin-labeling PCR (Roche Molecular Biochemicals, catalogue no. 1 636 090) with 100 pmol each of 6 *Mse*I-xy primers (where x and y indicate base pairs added for specificity). The entire PCR was then used to probe a Southern blot as described in the Dig User's Guide (Roche Molecular Biochemicals, catalogue no. 1 438 425). Random combinations of 6 *Mse*I-xy primers were used to screen for polymorphisms in the fast neutron-derived alleles. Polymorphisms were revealed when the following six primers were used: xy = CT, GG, GC, AG, TG, AT.

Bacterial artificial chromosome (BAC) filters representing the *Arabidopsis* genome were obtained from the Arabidopsis Biological Resource Center at Ohio State University (stock no. CD4-25F). Southern blots were performed as described in the Dig User's Guide. BAC DNA was isolated by using a midiprep kit and protocol from Qiagen (Chatsworth, CA; catalogue no. 12143). Approximately 5 ng of T3H2 DNA was used to generate a digoxigenin-labeled AFLP probe as described above for CIC8H12. DNA sequence was determined by using an ABI 310 (Applied Biosystems).

Complementation of *pkl* Mutant. A *Bst*BI–*Nco*I 11.9-kilobase (kb) genomic fragment that spanned the predicted *CHD* gene was subcloned into the plant transformation vector pCambia 3300 (CSIRO, Canberra, Australia) by using the *Bst*XI and *Xba*I sites to generate pJO634. *pkl-1* and *pkl-7* plants were transformed with both empty vector and pJO634 by using an *in planta* transformation protocol with the *Agrobacterium tumefaciens* strain GV3101 (17).

PKL cDNA sequence. Reverse transcription–PCR (RT-PCR) was performed on total RNA isolated from wild-type Columbia by using the Access RT-PCR kit from Promega (catalogue no. A1250). Overlapping fragments of *PKL* cDNA were cloned, and the entire *PKL* cDNA was sequenced at least twice. The sequence of the *PKL* cDNA is deposited in GenBank (accession no. AF185577).

Ribonuclease Protection Assays. Ribonuclease protection assays were performed by using the RPA III kit from Ambion (Austin, TX; catalogue no. 1414). To generate a *PKL*-specific probe, a DNA fragment was generated via RT-PCR by using the primers JOpr244 (TGT TGA GCC AGT TAT TCA CGA) and JOpr247 (ACC TTT CCA TCA ATT CGC TCG) and subcloned by using the pGEM-T vector system (Promega, catalogue no. A3600) in an orientation such that the T7 promoter would produce an antisense transcript. This plasmid was called pJO657. To generate a *LEC1*-specific probe, a DNA fragment was generated via PCR by using the primers JOpr272 (CCG CTC GAG GAA ACC TCC AGG TTC ATC GTG) and JOpr256 (TCT TTC ACC TCA ACC ATA CCC), digested with *Xho*I and *Kpn*I, and subcloned into pBluescript II SK (−) cut with *Xho*I and *Kpn*I to produce pJO660. To generate a *ROC3*-specific probe, a DNA fragment was generated via PCR by using the primers JOpr276 (AAG TCT ACT TCG ACA TGA CCG) and JOpr277 (CTT CCA GAG TCA GAT CCA ACC) and subcloned by using the pGEM-T vector system in an orientation such that the T7 promoter would produce an antisense transcript. This plasmid was called pJO662. To generate ^{32}P-labeled RNA probes for ribonuclease protection assay analysis, the T7 Maxiscript kit was used (Ambion, catalogue no. 1312) with pJO657, pJO660, and pJO662 digested with *Not*I. The full-length transcripts were gel-purified to reduce background. For each ribonuclease pro-
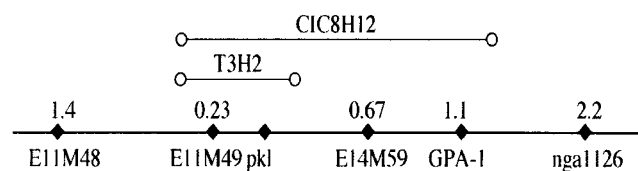

Fig. 1. Genetic map of the region surrounding *PKL*. The name of a marker is indicated below the line, whereas the distance in centimorgans of the locus from *pkl* is indicated above the line. The extent of YAC and BAC clones covering the region is illustrated.

tection assay, approximately 2×10^4 cpm of probe was added to 10 μg of total RNA (18).

Results

Cloning of PKL and Complementation of the pkl Mutant. Fast neutron-derived alleles of *PKL* were identified to facilitate the cloning of *PKL* by map-based methods. Fast neutron mutagenesis generates mutations that consist of chromosomal deletions at a high frequency (19). Approximately 50,000 fast neutron-mutagenized M_2 seed were screened for the pickle root phenotype in the presence of 10^{-8} M uniconazole-P, a GA biosynthetic inhibitor (20) that increases penetrance of the pickle root phenotype (5). Three independent *pkl* mutants were identified and used as described below.

The *pkl* mutation was genetically mapped relative to previously mapped polymorphisms between the Col and Ler ecotypes of *Arabidopsis*. Plants carrying the *pkl-1* allele in the Col ecotype were crossed to wild-type Ler plants, and 300 F_2 progeny expressing the pickle root phenotype were isolated. DNA from the 300 *pkl* F_2 progeny was used to localize the *pkl-1* mutation by interval mapping with SSLP markers (13). The *pkl* mutation mapped to chromosome 2 near the nga1126 marker (Fig. 1). Based on the analysis of 231 F_2 progeny, the *pkl-1* mutation mapped to within 1.1 centimorgans of the SSLP marker GPA-1, which had been anchored on the physical map of chromosome 2 (21). Further analysis of the 231 F_2 progeny revealed that the AFLP markers E11M48 and E14M59 (15) flanked *pkl-1* and were tightly linked (Fig. 1).

Based on the position of *pkl* on the physical map of chromosome 2 (21), YAC CIC8H12 was selected for further analysis. PCR analysis revealed that CIC8H12 contained the flanking markers E11M49 and E14M59 (Fig. 1), indicating that CIC8H12 spanned the *PKL* locus (data not shown). Five pools of random probes were generated from CIC8H12 by a PCR-based method. These random probe mixtures were then used to probe Southern blots of genomic DNA isolated from wild-type plants and the three *pkl* lines generated by fast neutron mutagenesis. One of the probes revealed polymorphic bands associated with two of the three fast neutron alleles (data not shown).

To isolate a BAC clone that spanned the *pkl* locus, the AFLP marker E11M49, which mapped 0.23 centimorgans from *pkl*, was cloned and then used to probe BAC filters covering the *Arabidopsis* genome (22). BAC T3H2 hybridized to E11M49 and contained restriction fragments corresponding to those that were polymorphic in the fast neutron lines. A random probe mixture generated from T3H2 by PCR identified the same polymorphic bands in the fast neutron lines as did the probe mixture from CIC8H12 (data not shown).

The nature of the lesions in the fast neutron lines was characterized in greater detail by using specific probes generated from T3H2. Various DNA fragments from T3H2 were subcloned and used as probes on Southern blots of *Arabidopsis* genomic DNA. When probed with a 10-kb *Sal*I fragment, polymorphic bands were observed in *pkl-7* and *pkl-9* (Fig. 2). The extent of the
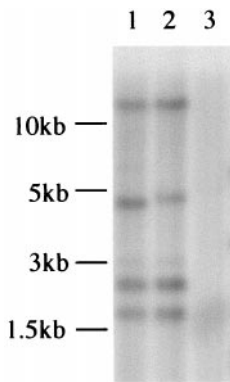
Fig. 2. Polymorphisms associated with two fast neutron-derived alleles of *PKL*. Southern blot of *PKL* (lane 1), *pkl-7* (lane 2), and *pkl-9* (lane 3) genomic DNA digested with *Xba*I and probed with the *Sal*I fragment indicated in Fig. 3. Migration of size standards is indicated to the left.

alterations in the genomic DNA in *pkl-7* and *pkl-9* was deduced (Fig. 3). The mutation in *pkl-7* is caused by either a translocation or an insertion, whereas the mutation in *pkl-9* is caused by a large deletion.

Sequencing of 17 kb of wild-type genomic DNA surrounding the site of the *pkl-7* polymorphism indicated that only one gene is disrupted in both the *pkl-7* and *pkl-9* mutants. The 17-kb region contains all or part of four genes, as indicated in Fig. 3. These four genes have sequence similarity to a cytochrome P450 monooxygenase, a clpB protease, a CHD family member, and a 2-component regulator (J.O., unpublished work). Only the gene coding for the CHD family member was disrupted in both the *pkl-7* and *pkl-9* mutants (Fig. 3).

Complementation analyses confirmed that *PKL* corresponds to the CHD3 gene. A binary vector, pJO634, carrying an 11.9-kb BstBI–*Nco*I genomic fragment that spanned the predicted *CHD* gene (Fig. 3) was constructed and transformed into *pkl* plants. *pkl* plants transformed with pJO634 were complemented for all *pkl*-related phenotypes (Fig. 4), whereas *pkl* plants transformed with the vector alone were not (data not shown). Segregation analyses done on two independent lines transformed with pJO634 confirmed that the ability to suppress the *pkl* mutant phenotype cosegregated with the transgene (data not shown).

PKL Is a CHD3 Homolog. RT-PCR was used to clone cDNA fragments representing the entire predicted *PKL* ORF. Subsequently, a BAC that spanned the *PKL* locus F13D4 (accession
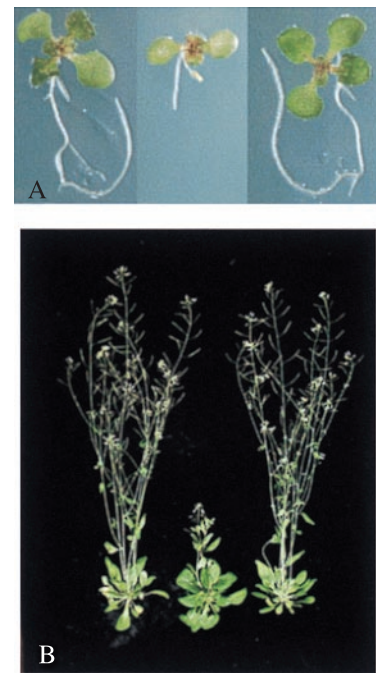

Fig. 4. Complementation of *pkl* phenotype. Complementation of *pkl-1* seedling (*A*) and mature *pkl-1* plant (*B*) phenotype with vector carrying *PKL*. For each panel, the plant on the left is *PKL*; the plant in the center is *pkl-1*; and the plant on the right is *pkl-1* transformed with pJO634, which carries the *PKL* gene. The seedlings (*A*) were grown in the presence of 10^{-8} M uniconazole-P in continuous light. The mature plants (*B*) were grown under 18-h illumination.

no. AL031369) was sequenced by another group as part of the ongoing effort to sequence the *Arabidopsis* genome. The sequences were identical, with the exception that some of the splice sites that were used to generate the *PKL* transcript were different from those predicted by the computer algorithm (the *PKL* cDNA sequence is deposited in GenBank, accession AF185577). Analysis of the *PKL* ORF revealed *PKL* codes for a predicted CHD3 homolog that is 1,384 amino acids in length. A search of the GenBank database revealed that genomic sequence for another *Arabidopsis* CHD3 homolog that is located on chromosome V (accession no. AAC79140) has also been obtained by the genome project. Also, an *Arabidopsis* CHD1 homolog is located on chromosome IV (accession no. CAB40760). We refer to this other CHD3 homolog as *PICKLE RELATED 1* (*PKR1*) and the CHD1 homolog as *PICKLE RELATED 2* (*PKR2*).
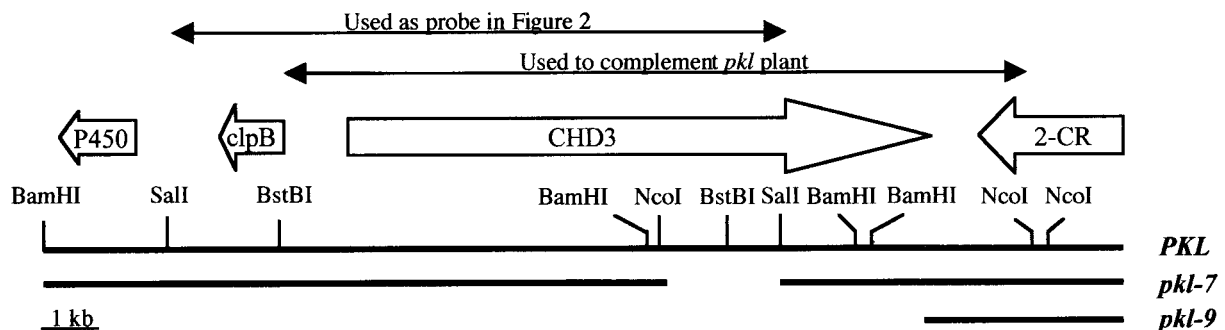

Fig. 3. Disruption of a *CHD* gene in the *pkl-7* and *pkl-9* mutants. A restriction map that highlights various features of the *PKL* locus is shown. The relative position of four ORFs is indicated as well as the region of genomic DNA that was found not to be altered in the fast neutron-derived PKL alleles *pkl-7* and *pkl-9*. The portion of genomic DNA that was used as a probe as described in the legend to Fig. 2 is indicated in addition to the fragment that was used to complement the *pkl* mutant.
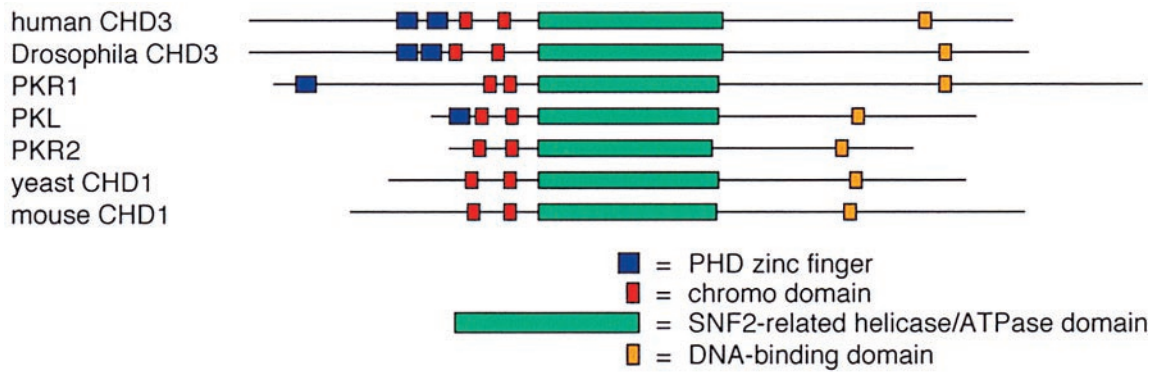
Fig. 5. PKL is a CHD3 protein. This schematic diagram illustrates the location of domains of sequence homology found in PKL and other CHD proteins from *Arabidopsis* and other species. CHD3 proteins contain PHD zinc fingers, whereas CHD1 proteins do not.

PKL, *PKR1*, and *PKR2* contain all of the sequence domains expected of CHD proteins (6, 23). CHD proteins are defined by three domains of sequence similarity: a chromo (chromatin organization modifier) domain, a SNF2-related helicase/ATPase domain, and a DNA-binding domain. CHD3 proteins are distinguished from CHD1 proteins by the presence of another domain, a PHD zinc finger (6). Fig. 5 is a schematic of the various domains found in PKL, PKR1, PKR2, and related CHD proteins. Only one PHD zinc finger is found in *PKL* and *PKR1*, whereas two PHD zinc fingers are typically found in CHD3 proteins from other species. Based on the domains of homology identified, we have classified *PKL* and *PKR1* as CHD3 family members and *PKR2* as a CHD1 family member.

The PKL Transcript Is Ubiquitously Expressed. To determine where *PKL* is normally expressed, we analyzed *PKL* transcript levels. The *PKL* transcript was not detected by Northern analysis of poly(A)$^+$ mRNA of rosette leaves. This failure to detect the *PKL* transcript may be due to technical difficulties associated with preparation of long transcripts from plant tissues (24). Therefore, ribonuclease protection assays were used to quantitate *PKL* mRNA (Fig. 6). At this level of resolution, the *PKL* transcript was present at approximately equal levels in all tissues examined: roots, shoots, inflorescences, and siliques (Fig. 6, lanes 1–4, respectively). This ubiquitous expression pattern is consistent with the pleiotropic shoot and root phenotypes of *pkl* plants. The *PKL* transcript was not detected when the ribonuclease protec-
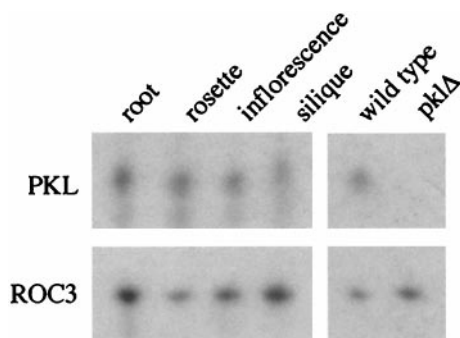
tion assay was performed on RNA isolated from a plant carrying a deletion allele of *PKL*, *pkl-9* (Fig. 6, lanes 5 and 6).

LEC1 Is Expressed in Pickle Roots. Pickle roots are primary roots of adult plants that express embryonic differentiation traits such as expression of storage protein genes and accumulation of storage lipids (5). These and other embryo-specific traits are thought to be under control of the *LEC1* gene, which has been proposed to be a critical regulator of embryonic identity (11, 12). Therefore, we investigated the possibility that the *LEC1* transcript, which normally is expressed only in seeds, was expressed in pickle roots. Ribonuclease protection assays were performed by using total RNA isolated from wild-type roots and pickle roots with a *LEC1* probe and a cyclophilin probe as a control (Fig. 7). The *LEC1* transcript was detected in siliques (Fig. 7, lane 2) but not in rosette leaves (Fig. 7, lane 1). The *LEC1* transcript was not detected in wild-type roots (Fig. 7, lane 3), but expression of *LEC1* was clearly detected in pickle roots (lane 4).

Because expression of *LEC1* is sufficient to induce expression of embryonic differentiation traits in seedlings (12), the presence of the *LEC1* transcript in pickle roots suggested that *LEC1* may play a key role in promoting expression of the pickle root phenotype. Penetrance of the pickle root phenotype in *pkl* seedlings is increased by treatment of seed with uniconazole-P before germination. If the level of the *LEC1* transcript is the limiting factor in determining the penetrance of the pickle root phenotype, then the *LEC1* transcript would be predicted to be expressed in a uniconazole-P-dependent manner in imbibed *pkl* seeds.

We found that the *LEC1* transcript was present in imbibed *pkl* seeds before germination (Fig. 8). Ribonuclease protection

Fig. 6. The *PKL* transcript is expressed throughout the plant. Ribonuclease protection assays were performed to determine the level of the *PKL* transcript in the root, rosette, inflorescence, and siliques of *Arabidopsis*. To show that the probe used was specific for *PKL*, a ribonuclease protection assay with the same probe was performed with RNA isolated from a wild-type plant and a plant carrying a deletion allele of *PKL*, *pkl-9* (*Right*). A probe for the cyclophilin transcript *ROC3* was used as a positive control.
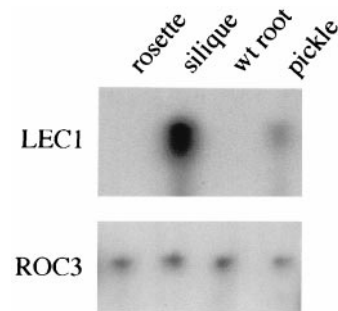

Fig. 7. LEC1 is expressed in pickle roots. Ribonuclease protection assays were used to determine the level of the *LEC1* transcript in the rosette, silique, and root of wild-type plants as well as in the pickle root of *pkl-1* plants. A probe for the cyclophilin transcript *ROC3* was used as a positive control.
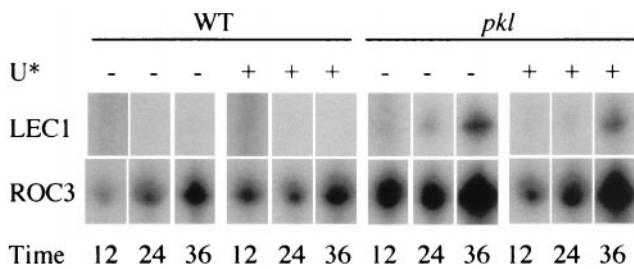
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Fig. 8. LEC1 is expressed in germinating *pkl* seeds. Ribonuclease protection assays were used to determine the level of the *LEC1* transcripts in wild-type and *pkl-1* seeds at 12, 24, and 36 h after imbibition in the absence (−) or presence (+) of uniconazole-P (U*). A probe for the cyclophilin transcript *ROC3* was used as a positive control.

assays were performed by using total RNA isolated from wild-type seed (Fig. 8, lanes 1–6) and *pkl* seed (Fig. 8, lanes 7–12) with a *LEC1* probe and a cyclophilin probe as a control. Seeds were imbibed in the absence or presence of uniconazole-P for 12, 24, or 36 h. The *LEC1* transcript is clearly present in *pkl* seeds at 24 and 36 h. However, the level of the *LEC1* transcript was not elevated in *pkl* seed treated with uniconazole-P.

Discussion

In wild-type *Arabidopsis*, many of the developmental pathways that contribute to embryo formation are not expressed in adult tissues. In *pkl* mutants, at least some aspects of this stage-specific control are lost; embryonic developmental programs such as expression of seed storage protein genes and genes involved in storage lipid deposition are expressed after germination (5). Also, vegetative tissues have an abnormal capacity to produce somatic embryos spontaneously. Thus, *PKL* is apparently necessary to repress embryonic identity and contributes to the transition from embryonic to postembryonic development.

The identification of *PKL* as a gene encoding a CHD3 protein suggests that *PKL* mediates its effects on developmental identity through regulation of chromatin architecture. CHD genes have been identified in numerous eukaryotes, and the corresponding proteins are proposed to function as chromatin-remodeling factors. The name "CHD" is derived from the three domains of sequence homology found in CHD proteins (6, 23), a chromo (chromatin organization modifier) domain, a SNF2-related helicase/ATPase domain, and a DNA-binding domain, each of which is thought to confer a distinct chromatin-related activity (6, 25–27). CHD proteins are separated into two classes, CHD1 and CHD3, based on the presence of yet another domain of sequence homology typically found in proteins with chromatin-related activity, the PHD zinc finger (28). CHD3-related proteins possess a PHD zinc finger, whereas CHD1-related proteins do not (6).

Experimental observations to date strongly suggest a role for CHD3 proteins in repression of transcription. CHD3 proteins from *Xenopus* and human have been shown to be a component of a complex that contains histone deacetylase as a subunit (8–10). Deacetylation of histones is correlated with transcriptional inactivation (29). Thus, by virtue of CHD3 proteins being a component of a histone deacetylase complex, they would be predicted to function as repressors of transcription. In a mutant of *Drosophila* that lacks the CHD3-related gene *dMi-2*, this prediction is borne out; a mutation in the *dMi-2* gene results in increased derepression of homeotic genes when combined with other mutations that affect repression of homeotic genes (7).

Based on data presented herein and previously, we propose that *PKL* also functions as a repressor of transcription. In *pkl* mutants, embryo-specific genes are expressed inappropriately

after germination (5). One possible cause of such a phenotype would be inappropriate expression of a general activator of the embryo-specific genes. *LEC1* codes for a seed-specific transcription factor and is a critical activator of the embryonic developmental program (12). We have shown that *LEC1* is inappropriately expressed after germination in *pkl* tissue expressing embryonic differentiation characteristics after germination.

Because expression of *LEC1* after germination is sufficient to cause expression of embryonic differentiation characteristics (12), one possible model to explain expression of embryonic identity after germination in *pkl* seedlings is that *PKL* is necessary for repression of *LEC1*. We found that *LEC1* is expressed in *pkl* seeds before germination (Fig. 8), but the level of the *LEC1* transcript is not increased in the presence of uniconazole-P. This result is consistent with a direct role for *PKL* in repression of *LEC1* and with a substantive role for *LEC1* in generation of the pickle root phenotype. However, this result is not consistent with a role for *LEC1* as a rate-determining factor governing penetrance of the pickle root phenotype. In fact, this result strongly suggests that there is a separate factor that promotes expression of embryonic genes; this factor is in some way repressed by GA.

We have not yet genetically tested the requirement for *LEC1* in generating the pickle root phenotype by examining the penetrance of the pickle root phenotype in a *lec1 pkl* double mutant. In reality, analysis of such a double mutant is likely to prove confounding. In a *lec1* mutant, embryo-development is perturbed, and seedling-specific differentiation programs are expressed prematurely. *lec1* seeds are desiccation intolerant and must be rescued before they dry to obtain viable plants. In such a developmental context, in which the embryo-specific developmental program is not robustly established, it is not clear that loss of a factor such as *PKL* that represses embryo-specific genes would have phenotypic consequences. Analysis of a transgenic *pkl* plant carrying a conditional allele of *LEC1* might help to address these concerns.

The cloning of the *PKL* gene has not yet clarified the role of GA in modulating the penetrance of the *pkl* phenotype. We have observed previously that the pickle root penetrance is greatly enhanced by inhibition of GA biosynthesis. This observation implies that both *PKL* and a factor whose activity is GA-dependent are necessary for repression of the embryonic state. In conjunction with previous observations concerning GA, our results imply that GA plays two roles in germinating seeds of *Arabidopsis*. One well established role is that GA triggers metabolic activity and activates postembryonic developmental processes. In addition, our results indicate that GA plays a role in repression of embryonic developmental processes. Thus, we propose that GA acts as both a differentiation factor (promotion of the postembryonic state) and a determination factor (repression of the embryonic state) during germination.

A class of mutations, originally named *gymnos (gym)* but subsequently found to be allelic to *pkl* (and renamed as *pkl* alleles), has recently been identified in a screen for enhancers of the *crabs claw (crc)* mutation (30). *pkl crc* double mutants develop ectopic ovules along the abaxial replum. The interpretation of the *pkl crc* phenotype by Eshed *et al.* (30) is that *PKL* normally acts to restrict primordium formation at the margins of carpels by repressing genes that promote meristematic activities. This model of PKL action is generally consistent with our proposed role for PKL in repression of embryonic identity. Indeed, it might be argued that *CRC* plays an analogous role to the GA-modulated factor that we have invoked to explain the GA-dependence of the pickle root phenotype.

pkl plants have numerous phenotypes that are reminiscent of mutants with defects in GA synthesis or signal transduction. The rosette leaves are dark green with shortened petioles; time to

flowering is increased; apical dominance is reduced; anther dehiscence is delayed; and *pkl* shoots accumulate bioactive GAs (ref. 5 and J.O., S.K., J.H., and C.S., unpublished work). In addition, combining the *pkl* mutation with a *gai* mutation, which also perturbs GA signal transduction (31, 32), gives rise to synergistic phenotypes (5). Based on these observations, we hypothesize that GA promotes transitions from differentiation state A to differentiation state B by activating expression of genes necessary for state B and by repressing expression of genes necessary for state A via a *PKL*-dependent pathway. This model does not preclude the possibility that *PKL* activity may be stimulated by factors other than GA.

Cloning of a gene necessary for repression of embryonic identity has lead to the proposition that a chromatin-remodeling factor mediates a GA-modulated developmental transition in *Arabidopsis*. We anticipate that further characterization of *PKL* and identification of proteins that either regulate or are targets of *PKL* will shed light on the mechanism of GA signal transduction and the role of GA in regulating differentiation and development in *Arabidopsis*. It remains to be determined whether CHD proteins in animal systems will play an analogous role in hormone-mediated developmental events.

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