ified by phosphorylation, as previously shown for HD1-A (*17*) and for HD2 in this report. A major advance in our understanding the function of histone acetylation was the identification of a mammalian deacetylase as a conserved homolog of the yeast transcriptional regulator *RPD3* (*5*). Since then, *RPD3*-homologous HDs have been identified in a variety of organisms (*6*, *18–20*). The identification of a maize *RPD3* homolog (*21*) and an HD that is not homologous to *RPD3* confirms the biochemical heterogeneity of maize HDs. Because HD2 is tightly chromatin-bound, located in the nucleolus, and shares homology to other nucleolar proteins, it may be involved in regulation of ribosomal chromatin structure and function by deacetylating nucleolar core histones. It is possible that enzymes for histone acetylation that are specific for ribosomal genes exist because these genes function differently in comparison to polymerase II (Pol II) genes. Ribosomal RNA (rRNA) genes are characterized by a distinct localization, a specific subset of transcriptional regulators, and a specific RNA polymerase (*22*). Moreover, rRNA is the most abundant transcript of the cell. With respect to acidic regions, HD2 shares homologies to NOR (nucleolus organizer region)-associated proteins. Proteins of this group, like RNA Pol I, nucleolin, UBF-transcription factors, and other Ag-NOR-proteins, function in rDNA transcription and are bound to ribosomal chromatin, regardless of the actual transcriptional activity. In addition, some of these proteins, like HD2, are modulated by phosphorylation (*23*).

We have previously demonstrated that HC toxin of the maize pathogen *Cochliobolus carbonum* and related cyclic tetrapeptides inhibit HDs and cause hyperacetylation of histones in susceptible, but not in resistant, maize strains (*24*). Our interpretation was that the inhibition of histone deacetylation interfered with the induction of plant defense genes. Hence, inhibition of deacetylation by HC toxin may lead to a rather general inhibition of host rDNA transcription, owing to inhibition of nucleolar HD2. Recently, the cyclic tetrapeptide apicidin was shown to inhibit protozoal HD (*25*). The antiparasitic effect was explained by the effect of HD in transcriptional control but may also be due to inhibition of rDNA replication or transcription by targeting the nucleolar HD.

Our results show that apart from *RPD3*-type HD, another nucleolar deacetylase exists. This finding confirms the multiplicity of HDs in maize and other organisms at a molecular level. The divergent functions of acetylation in nuclear processes may be reflected in multiple enzymes that differ in specificity, molecular targets, and expression in certain developmental stages.

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Cellular Differentiation Regulated by Gibberellin in the *Arabidopsis thaliana pickle* Mutant

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The plant growth regulator gibberellin (GA) has a profound effect on shoot development and promotes developmental transitions such as flowering. Little is known about any analogous effect GA might have on root development. In a screen for mutants, *Arabidopsis* plants carrying a mutation designated *pickle* (*pkl*) were isolated in which the primary root meristem retained characteristics of embryonic tissue. Expression of this aberrant differentiation state was suppressed by GA. Root tissue from plants carrying the *pkl* mutation spontaneously regenerated new embryos and plants.

Gibberellin is required for seed germination and plays a variety of roles during growth and development after germination. GA-deficient plants exhibit defects in germination, time lag to flowering, stem elongation, apical dominance, maintenance of floral meristem identity, and trichome distribution (*1*). All of these phenotypes primarily affect the shoot. Gibberellin also affects root development by promoting cell elongation (*2*), but there is essentially no information on the effect of GA on fate determination in roots. We report here a mutant of *Arabidopsis* that is defective in a GA signaling pathway that promotes the transition of the primary root from an embryonic to an adult differentiation state.

During a screen for *Arabidopsis* mutants exhibiting abnormal root development, we identified a class of mutants in which the primary root, after a period of apparently normal growth, would thicken and become opaque and green (Fig. 1, A and B). Lateral and adventitious roots did not express this phenotype. Because of the visual appearance of the altered primary roots, we refer to this root phenotype as "pickle." Genetic analysis revealed that the mutant phenotype was due to a mutation at a single recessive locus located near position 48.4 on chromosome 2, which we named *PICKLE* (*PKL*) (*3*). Eight independent mutant alleles of the *PKL* locus have been identified by screening ~20,000 M2 plants from mutagenized populations.

Unusual cell proliferation was observed when roots were removed from mutant plants and placed on synthetic mineral me-

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dium without plant hormones (*4*). The excised pickle roots, at a frequency of 10 to 30%, produced callus-like growths and generated globular- and torpedo-stage embryo-like structures (Fig. 1C). Under identical conditions, excised wild-type roots exhibited neither callus growth nor any event resembling somatic embryogenesis (*5*). In the absence of any experimental manipulation, pickle root callus produced phenotypically normal *pkl* plants at a frequency of about 1%.

Because of the ability of excised pickle roots to generate structures resembling somatic embryos, we investigated the possibility that pickle roots express embryonic characteristics before removal from the plant. *Arabidopsis* embryos accumulate large amounts of triacylglycerols as storage reserves to support early seedling growth (*6*). When infiltrated with a dye that specifically stains neutral lipids (*7*), the portion of the pickle roots that had differentiated abnormally were intensely stained red (Fig. 1D), indicating the presence of large quantities of triacylglycerols (*8*). Analysis of the fatty acid composition of extracted pickle root triacylglycerols by gas chromatography revealed that the fatty acid composition differed from that found normally in roots but was indistinguishable from that of seeds (*9*). Transmission electron microscopy of sections of pickle root tips revealed the presence of densely packed oil bodies reminiscent of seed oil bodies (Fig. 1, E and F) as well as large starch granules normally not present in either roots or seeds. In addition, transcripts for the oleosin (*10*) and 2S1 storage protein (*11*) genes, which are normally expressed only in seeds or pollen, accumulated in pickle roots (Fig. 2). On the basis of the unique differentiation characteristics of pickle roots, we infer that the primary roots of *pkl* plants either retain or resume some degree of their embryonic differentiation status after germination.

An important clue in determining how the *pkl* mutation might result in aberrant primary root differentiation was provided by the observation that expression of the pickle phenotype is suppressed by GA. When seeds were germinated and grown in continuous light on synthetic media plates (*4*), expression of the pickle phenotype by homozygous *pkl* plants exhibited low penetrance, typically 1 to 10% depending on the batch of seed. Addition of 10 nM uniconazole-P (*12*), a GA biosynthetic inhibitor, increased penetrance of the pickle phenotype to greater than 80% (Table 1). Addition of 10 μM GA_4 completely suppressed the ability of 100 nM uniconazole to increase penetrance of the pickle phenotype, indicating that uniconazole is acting by inhibiting GA biosynthesis. Gibberellin also suppressed the pickle root phenotype in *pkl1-1 ga1-3* plants (Table 2), in which the amount of GA in the plants was determined by the amount of GA exogenously supplied because of the inability of plants carrying the *ga1-3* mutation to synthesize GA (*13*, *14*).

To determine the developmental stage at which the differentiation state of the root was most responsive to uniconazole treatment, *pkl* seedlings were transferred to or from uniconazole-containing media at different times during the first 96 hours after imbibition (Fig. 3). Thirty-six hours of either regimen was largely sufficient to determine the fate of the primary root meristem; germinating *pkl* seeds on uniconazole plates for only 24 hours and then transferring them to plates without uniconazole was sufficient to substantially induce the pickle phenotype. Conversely, shifting *pkl* seeds to uniconazole plates after 36 hours on plates without uniconazole was largely ineffective in inducing expression of the pickle pheno-

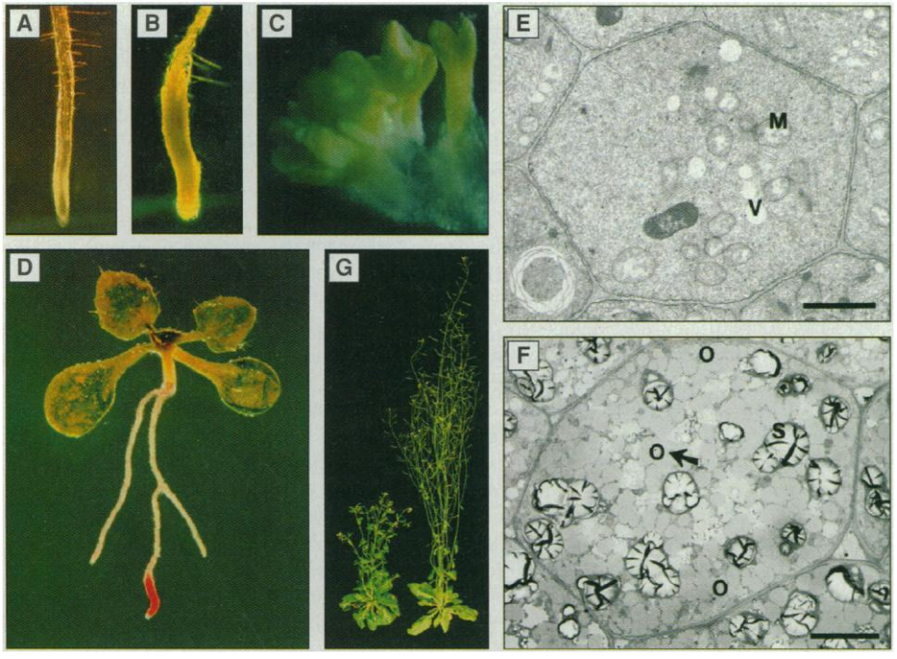

Fig. 1. Phenotypes of Columbia wild-type and *pkl* plants. All comparisons of wild-type and pickle plants are presented at the same magnification unless otherwise noted. Wild-type (**A**) and pickle (**B**) primary roots from 10-day-old seedlings. (**C**) Structures resembling somatic embryos initiated from pickle callus on basal MS media (*4*). (**D**) A 10-day-old *pkl* seedling stained with Fat Red 7B (*7*). Transmission electron micrograph (*17*) of wild-type (**E**) and pickle (**F**) primary root cells illustrating the presence of oil bodies and starch granules in pickle root cortical cells. Scale bars, 0.5 μm in (E), and 2 μm in (F). M, mitochondrion; S, starch granule; V, vacuole; and O, oil body. (**G**) Forty-six-day-old wild-type (right) and *pkl* (left) plants grown for 16 hours under illumination (130 μE m^{-2} s^{-1}, where E is the energy of 1 mol of photons).

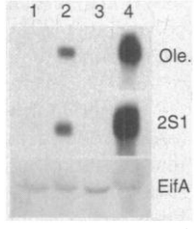

Fig. 2. RNA blot analysis. About 15 μg of total RNA (*18*) was isolated from wild-type leaves (lane 1), wild-type siliques (lane 2), wild-type roots (lane 3), and pickle roots (lane 4). The top panel shows transcripts detected by an oleosin (Ole.) Complementary DNA probe, the middle panel shows transcripts detected by a 2S1 seed storage protein cDNA probe, and the bottom panel shows transcripts detected by an Eif-4A (*19*) cDNA probe as a loading control.

Table 1. Effect of uniconazole-P (Un-P) and GA on penetrance of the pickle phenotype. For each treatment, 144 seeds were incubated at 22°C in continuous light (60 μE m^{-2} s^{-1}) on synthetic media (*4*) containing uniconazole-P or GA_4 at the indicated concentrations. Germination and the pickle phenotype were scored at 10 days. Expression of the pickle phenotype was calculated as a percentage of total seeds and as a percentage of seeds that germinated.

Un-P (nM)	GA_4 (μM)	Germinated (%)	Pickle (% of total)	Pickle (% of germinated)
0	0	99.3	0.7	0.7
0.1	0	98.6	7.6	7.7
0.2	0	98.6	13.9	14.1
0.5	0	100.0	25.7	25.7
1	0	100.0	56.3	56.3
2	0	99.3	62.5	62.9
5	0	97.2	75.0	77.1
10	0	96.5	82.6	85.6
100	0	83.3	72.3	87.5
100	10	100.0	0	0
0	10	100.0	0	0

type. At 24 hours after imbibition, the *pkl* seed coat has split, but the radicle has yet to emerge. Thus, at 24 hours germination is not complete, but the GA-dependent fate of the *pkl* root meristem may already have been determined. Germination is complete by 36 hours, by which time the fate of the root has been determined. Consequently, GA acts concurrently to promote establishment of adult root fate and germination of *pkl* seedlings.

The *pkl* plants exhibit several shoot phenotypes that are reminiscent of other *Arabidopsis* mutants deficient in GA biosynthesis (*ga1* through *ga5*) or GA signaling (*gai*) (*1*, *15*). *pkl* plants have dark green leaves with short petioles, exhibit delayed bolting and reduced apical dominance, and are reduced in stature (Fig. 1G). All eight defective alleles of the *PKL* locus result in expression of these phenotypes with 100% penetrance.

Analysis of a *pkl-1 ga1-3* double mutant indicated that it is unlikely that *PKL* codes for a GA biosynthetic enzyme. Because it inactivates the first step of the GA biosynthetic pathway (*13*, *14*), the *ga1-3* mutation is expected to be epistatic to any other GA biosynthetic mutant. However, *pkl-1 ga1-3* plants exhibited more exacerbated GA-deficient shoot phenotypes than either *pkl* or *ga1* plants. In particular, the *pkl-1 ga1-3* plants had rosettes that were 30% smaller than *ga1-3* plants and were at least 50% smaller in stature.

To address the possibility that *PKL* affects GA responsiveness, we examined the effect of the *pkl* mutation in a *gai* line. A *pkl-1 gai* plant exhibited severe GA-deficient phenotypes that were much more extreme than those exhibited by *pkl* or *gai* plants. A particularly dramatic effect was seen on flowering time: in continuous light, *pkl* plants and *gai* plants flowered 1 day and 3 days, respectively, later than wild-type plants, whereas *pkl gai* plants flowered 33 days later than wild-type plants on average. The synergistic effect of combining the *pkl* and *gai* mutations suggests that *PKL*, like *GAI*, may play a role in GA signal transduction.

At present, the simplest hypothesis to explain our findings is that *pkl* plants are defective in a GA signaling pathway. This *PKL*-dependent GA signaling pathway is ubiquitously used by the plant, resulting in *pkl* plants that express shoot phenotypes that are reminiscent of other GA-deficient plants. In addition, this *PKL*-dependent GA signaling pathway promotes the transition of root cells from an embryonic to an adult state during germination. The observation that the pickle phenotype is of low penetrance and is suppressed by GA indicates that there is yet another GA signaling pathway in addition to the *PKL*-dependent pathway governing root differentiation during germination. This additional pathway is unlikely to be *GAI*-dependent, because *pkl-1 gai* plants do not exhibit increased penetrance of the pickle phenotype compared with *pkl-1 GAI* plants.

The properties of the *pkl* mutant suggest that GA may play a greater role in determination of root fate in *Arabidopsis* than previously appreciated. In addition, the properties of the *pkl* mutant indicate that germination and differentiation during germination are genetically separable, GA-regulated events. This separation implies that there is more than one GA response pathway governing the transformation of a dormant seed into an actively growing seedling.

The *pkl* mutation in *Arabidopsis* indicates a distinction of the primary root from the secondary roots. We speculate that regulation of *PKL* expression may be necessary for generation of the specialized primary roots present in other members of the Brassicaceae, such as turnips and radish. In addition, it is notable that the plant permits expression of embryonic characteristics, specifically oil, in a portion of its root system. Very few plant species are known that accumulate significant amounts of oil in roots (*16*). Characterization of *PKL* and related gene products may eventually lead to the ability to produce commercially useful amounts of oil in root crops.

Table 2. Effect of GA on penetrance of the pickle phenotype in *pkl-1 ga1-3* seedlings. For each treatment, 72 seeds were incubated at 22°C in continuous light (60 μE m^{-2} s^{-1}) on synthetic media (*4*) containing GA_4 at the indicated concentrations. Germination and the pickle phenotype were scored at 10 days. Expression of the pickle phenotype was calculated as a percentage of total seeds and as a percentage of seeds that germinated.

GA_4 (M)	Germinated (%)	Pickle (% of total)	Pickle (% of germinated)
10^{-9}	14.0	9.7	69.0
10^{-8}	78.0	18.0	23.0
10^{-7}	95.9	6.9	7.2
10^{-6}	100.0	0	0
10^{-5}	100.0	1.4	1.4

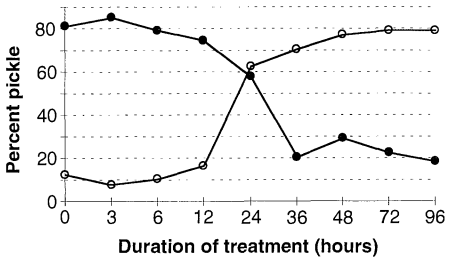

Fig. 3. Effect of time of application of uniconazole-P on penetrance of the pickle phenotype. *pkl* seeds were imbibed in water and immediately plated on media (*4*) containing 10^{-8} M uniconazole and then shifted at different times to media without uniconazole-P (open circles), or the seeds were plated on media not containing uniconazole-P and then shifted at the indicated times to media containing 10^{-8} M uniconazole-P (closed circles). The *x* axis indicates the times at which seeds were shifted. Eleven days after imbibition, the seedlings were scored for the pickle phenotype. The *y* axis indicates the percentage of seedlings expressing the pickle phenotype for each data point. Forty-eight seedlings were scored at each point on the graph. The experiment was carried out at 22°C in continuous light (60 μE m^{-2} s^{-1}).

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Evolution of a Strain of CJD That Induces BSE-Like Plaques

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Bovine spongiform encephalopathy (BSE) has become a public health issue because a recently evolved BSE agent has infected people, yielding an unusual form of Creutzfeld-Jakob disease (CJD). A new CJD agent that provokes similar amyloid plaques and cerebellar pathology was serially propagated. First-passage rats showed obvious clinical signs and activated microglia but had negligible PrP-res (the more protease-resistant form of host PrP) or cerebellar lesions. Microglia and astrocytes may participate in strain selection because the agent evolved, stabilized, and reproducibly provoked BSE-like disease in subsequent passages. Early vacuolar change involving activated microglia and astrocytes preceded significant PrP-res accumulation by more than 50 days. These studies reveal several inflammatory host reactions to an exogenous agent.

The recent epidemic of bovine spongiform encephalopathy (BSE) has brought increased attention to its human counterpart, Creutzfeldt-Jakob disease (CJD), as well as to scrapie, an endemic infection of sheep. Since the first recognized case of BSE in 1985, it is likely that more than one million cows have become infected by dietary exposure to scrapie-contaminated food (*1*). Domestic cats and various zoo animals have been similarly infected. Additional experimental propagation of the cow-derived agent in many species, including pigs, rodents, and primates (*2*, *3*), suggests that the new BSE agent has acquired an enhanced ability to evade host defenses. In 1996, the BSE agent was linked to a variant disease in younger people (*4*) that was reproduced in primates by BSE inoculation (*3*). Because human CJD infections can be undetectable for more than 20 years, it is likely that we will see more BSE-linked cases (*5*, *6*). Thus, there is an emerging public health issue that requires a greater understanding of agent strains and their specific interactions with the host.

Many different infectious strains of the scrapie and CJD agents have been propagated in inbred mice encoding the same PrP amino acid sequence (*7*, *8*). The main reasons why human "BSE" is considered to be caused by a new agent strain are based on (i) the youth of most victims (less than 30 years old), (ii) the prolonged clinical course, (iii) severe involvement of the cerebellum, and (iv) the presence of many large plaques. Sporadic CJD, which accounts for ~90% of CJD worldwide and is infectious in a variety of species (*9*, *10*), rarely shows cerebellar pathology or widespread plaques. Only two other human transmissible encephalopathies with a long clinical phase display cerebellar lesions and a plaque-rich phenotype. These are kuru, an infection transmitted by ritual cannibalism (*9*), and Gerstmann-Straussler-Sheinker disease (GSS), a group of rare "familial" forms of CJD (*11*).

One explanation for these different phenotypes focuses on mutations in host PrP. It has been suggested that a combination of mutations causes PrP to fold into an infectious conformation that encrypts and propagates strain-specific information (*12*). However, people infected with "BSE" do not have any of the plaque-associated PrP mutations such as Phe^{102} to Leu^{102} (*13*). Alternatively, a newly mutated exogenous virus may provoke unusual phenotypic responses in the host, including different forms of PrP-res (*8*). Additional host responses could signify recognition of the foreign agent hidden within cells. Because strong lymphocyte responses are evaded, it is often stated that these infections lack any inflammatory component (*9*, *14*). We found that inflammatory cells can participate in the evolution of a new infectious strain. This strain causes a disease with notable similarity to both "familial" GSS and human "BSE" and evokes inflammatory reactions at early stages of infection.

To change an established CJD strain into one that could produce plaques and cerebellar lesions, we inoculated alternate species without manipulating normal host PrP sequences. Unusual inflammatory responses in microglia or astrocytes (or both) were used to evaluate host recognition. Such responses could subject the agent to more rigorous selective pressures favoring a mutated plaque-evoking strain. We therefore challenged several types of inbred mice, Chinese hamsters, and rats with a strain of CJD (designated SY) that was highly selected by serial passage 5 times in guinea pigs and then 24 times in Syrian hamsters (*15*). SY was successfully transmitted to all three species and had a relatively prolonged incubation period even after serial passage (>350 days). Only the rats showed remarkable pathology.

In the first passage (P1), only two of six rats (killed at 672 and 732 days) showed obvious spongiform changes (*16*). The 672-day rat exhibited clear clinical signs (startle myoclonus in response to noise, hunched posture, and terminal lack of feeding and grooming). The progression to terminal disease was rapid, lasting only 8 days. The 732-day rat showed no clinical signs, but both animals had a similar distribution of vacuoles and these were located predominantly in the cerebral cortex and to a lesser extent in CA1 and CA2 of the hippocampus. Only nonspecific aging changes were seen in the cerebellum. PrP-res was not detected in histological sections from the clinically ill rat, although very rare PrP deposits were found near the inoculation site in the nonclinical rat. The most unusual finding in both rats was a marked increase in reactive microglia in the cerebrum, even in regions with minimal vacu-

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