FIDDLEHEAD, a gene required to suppress epidermal cell interactions in *Arabidopsis*, encodes a putative lipid biosynthetic enzyme

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In plants, the outer epidermal cell wall and cuticle presents a semipermeable barrier that maintains the external integrity of the plant and regulates the passage of various classes of molecules into and out of the organism. During vegetative development, the epidermal cells remain relatively inert, failing to respond to wounding or grafting. During reproductive development and fertilization, however, the epidermis is developmentally more labile and participates in two types of contact-mediated cell interactions: organ fusion and pollen hydration. Here we describe the isolation and characterization of one gene whose product normally functions in blocking both types of epidermal cell interactions during vegetative development: the *FIDDLEHEAD* gene. As suggested by previous biochemical analyses, the gene encodes a protein that is probably involved in the synthesis of long-chain lipids found in the cuticle and shows similarity to a large class of genes encoding proteins related to β-ketoacyl-CoA synthases and chalcone synthases. *In situ* hybridization reveals an epidermal pattern of expression consistent with a role for this protein in the synthesis of lipid components that are thought to localize extracellularly and probably modify the properties of the cuticle.

Land plants are exposed to a variety of environmental fluctuations, including changes in humidity, light intensity, and temperature. In addition to these environmental challenges, plants must also cope with attack by herbivores and pathogens. On an organ level, structures such as thorns and epidermal hairs offer some protection. At the cellular level, however, it is the epidermal cell wall and cuticle that present the first line of defense. Although the function of the epidermis is largely a protective one, there are exceptional cases during development in which epidermal cells must express a more flexible developmental potential. In higher plants, these instances usually occur during floral development and involve interactions between epidermal derivatives and other epidermal cells on neighboring primordia or the male gametophyte (1). Because contact between interacting cells is established after cells are at or near developmental maturity, the initial signaling events must take place across an intact cell wall and cuticle. The biochemical and molecular composition of this extracytoplasmic compartment can therefore directly impinge on signaling by either enhancing or attenuating the transit of signal molecules.

The plant extracellular matrix is known to play a key role in a number of developmental processes. It has been demonstrated directly, for example, that the cell wall provides the fate-determining factors that operate during asymmetric cell division in *Fucus* embryogenesis (2, 3). Similarly, in carrots, soluble molecules that crossreact with the monoclonal antibody JIM8 and are released from the wall of cultured cells can stimulate somatic embryo development (4). In roots, factors sequestered in the cortical cell extracellular matrix are thought to provide positional information that specifies the site of root hair development in *Arabidopsis* (5). Organ fusion, on the other hand, is thought to be regulated in part by simple changes in the permeability characteristics of the cuticle (1, 6). Biochemical analyses on *fiddlehead* (*fdh*) mutants, for example, suggests that a change in the lipid composition of the cuticle is one way to achieve a change in the relative permeability to small molecules, thereby permitting exchange of the developmental signals required for organ adhesion (7).

Much of our understanding of the developmental process of postgenital organ fusion stems from work done on carpel fusion in *Catharanthus roseus* (reviewed in ref. 8). Results from these investigations indicate that the epidermal cell interactions taking place during the early stages of the fusion process are specific and involve reciprocal recognition events. Although molecular details remain to be elucidated, it is known that in *C. roseus*, small water-soluble morphogenetic factors are exchanged between interacting partners (9). In response to these signals, the *C. roseus* epidermal cells not only adhere but also take on new, nonepidermal cell fates. Analysis of the *Arabidopsis* fusion mutant *fdh* (10, 11) suggests that properties of the cuticle are altered such that the cuticle becomes much more permeable to small molecules (7), some of which presumably act to promote fusion. Genetic analysis of a larger collection of *Arabidopsis* fusion mutants indicates that perturbation of at least seven other genes can achieve changes in permeability that may similarly permit signals to be exchanged between fusion competent and contacting cells (6). The situation is no less complex in maize, with two very distinct types of mutants leading to epidermal fusion events. Mutations in *CRINKLY4* (*CR4*) result in an apparent loss of cell fate determination in epidermal cells (as well as subepidermal cell types) and this leads to adhesion events taking place between organs (12). Mutations in *ADHERENT1* or *ADHERENT2* lead to interorgan fusion by a mechanism that is distinct from that of *cr4* mutants, but is at least superficially similar to that seen in *fdh* mutants (13–15). In addition, there are a large number of induced mutations with adherent phenotypes in maizeDB whose detailed phenotypes have yet to be described. With the exception of the *cr4* mutants, loss of epidermal cell fate has not been demonstrated in any of the organ fusion mutants studied.

Another contact-mediated developmental process that involves an epidermal partner is the interaction that occurs between pollen and stigma. In *Arabidopsis*, some of the surface determinants important in the initial recognition process have been identified genetically. Four genes, *CER1*, *CER3*, *CER6*, and *CER10*, which contribute to the synthesis and/or deposition of epicuticular waxes on the surface of the *Arabidopsis* shoot (16),

Abbreviations: *fdh*, *fiddlehead* mutant; *FDH*, *FIDDLEHEAD* gene.

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have also been shown to be required for early events in pollen recognition (17, 18). Plants having mutations in any one of these genes, in addition to manifesting a waxless phenotype, produce pollen grains with a tryphine layer that is deficient in specific classes of long-chain lipids. Without these classes of lipids, contact-mediated pollen hydration does not take place. Two of the four genes, *CER1* and *CER3*, have been cloned (19, 20). Based on sequence homology, the *CER1* gene is proposed to encode a membrane-localized decarbonylase (19), whereas the *CER3* gene is thought to be localized to the nucleus and may play a regulatory role in lipid biosynthesis (20). Interestingly, one of these genes, *CER10*, also results in ectopic expression of the contact-mediated organ fusion program (6). Additional support for the idea that lipids are key factors in promoting the early events in the pollen-stigma interaction in *Arabidopsis* has come from work showing that the exogenous application of unsaturated triacylglycerides is sufficient to promote pollen tube growth on *Arabidopsis* leaves (21).

In plants homozygous for representative mutant alleles of seven of the nine known *Arabidopsis* organ fusion loci, ectopic pollen hydration and germination on nonreproductive structures can also be demonstrated (6). In this subset of mutants, the specificity of the interaction with pollen mimics that seen on the wild-type stigma (6), suggesting that the process of organ fusion and pollen hydration share some mechanistic overlap (1). Here we describe the isolation and characterization of the *FIDDLEHEAD* (*FDH*) gene, which plays a role in regulating both epidermal fusion and epidermal interactions with pollen. Sequence data indicate that the *FDH* gene encodes a protein related to β-ketoacyl-CoA synthase that is likely to be involved in the synthesis of long-chain lipid molecules. These findings are consistent with our biochemical studies that suggest such a deficiency in the mutant (7) and highlight a role for lipids both in pollen recognition and epidermal cell adhesion.

Materials and Methods

Plant Growth and Manipulation. Plants were grown as described in Lolle *et al.* (6).

A transgenic *Arabidopsis* line (GT1658) manifesting organ fusion was identified in a collection of $\approx$ 4,000 *Ds*-based gene trap lines generated as described (22). An additional *fdh* mutant allele (*fdh-9*) not described previously was obtained from the laboratory of John Bowman (University of California, Davis). This mutation arose in a T-DNA-transformed population and was originally designated JB26. The other *fdh* alleles used in this study were derived from ethyl methanesulfonate-mutagenized lines described (6). Complementation analyses were performed by crossing heterozygous GT1658 plants to heterozygous *fdh-1* plants. F_1 plants were scored for segregation of the mutant phenotype.

The conditional male sterile line, TH154, was used as a female parent and crossed with a transgenic line containing an immobilized *Ac* element as a source of transposase (22). F_2 lines homozygous for the male sterile mutation and the *Ac* transposase (TH154-*Ac*) were tested for transposase activity by using the streptomycin-resistance assay described by Bancroft *et al.* (23). Seeds for the transgenic line containing the streptomycin construct were obtained from the *Arabidopsis* Biological Resource Center (ABRC stocks CS1645 and CS1646; Columbus, OH).

Genetic Mapping, Complementation, and Reversion Analysis. F_2 plants generated by crossing GT1658 heterozygotes with Columbia were used to map the GT1658 mutation by using PCR-based molecular markers as described (6). TH154-*Ac* was also crossed to GT1658 heterozygotes, the F_1 plants were allowed to self-pollinate, and F_2 progeny were collected. F_2 mutant plants showing segregation for a fusion phenotype and containing the *Ac* transposase were screened for wild-type sectors, and in cases where sectors extended into an inflorescence, seed was collected. The F_3 progeny derived from revertant sectors were planted and scored for segregation of the fusion phenotype.

Isolation and Characterization of GT1658-Flanking DNA. Inflorescence tissue from GT1658 plants showing the fusion phenotype was homogenized in extraction buffer according to the protocol of Edwards *et al.* (24). DNA sequences flanking the *Ds*-transgene were amplified by using the thermal asymmetric-interlaced–PCR protocol of Liu *et al.* (25) with modifications as described by Grossniklaus *et al.* (26). PCR products were ligated into a TA cloning vector (pCRII) from Invitrogen and transformed into *Escherichia coli*. Plasmids containing inserts of the correct size were subjected to DNA sequencing, which was done at the Harvard Medical School Sequencing Facility. Three sets of primers were designed that span the three exons comprising the *FDH* gene by using sequence data obtained from the genome database (1L: 5′-CTCTCACCAACCACCAAACC-3′; 1R: 5′-ACGTAACACATGCAAAGGCA-3′; 2L: 5′-CCGGTTCTCTCGGGTTTAAT-3′; 2R: 5′-GAAGAGAAGCTGCTCGGAGA-3′; 3L: 5′-TCCGAACTCATAAGGCTGCT-3′; 3R: 5′-CAGGACTCTAGCCACATCCA-3′). These primers were used to amplify DNA from plants homozygous for different *fdh* alleles and these PCR products were sequenced directly. DNA sequencing of *fdh* alleles was done at the Department of Molecular and Cellular Biology sequencing facility (Harvard University).

Expression Analysis. *In situ* RNA hybridization analysis was performed as described (27). PCR products corresponding to exon 1 of the *FDH* gene were amplified by using the primers described above and cloned into the pCR2.1–TOPO vector (Invitrogen). Probes for *in situ* hybridization were prepared by transcription of these cloned fragments by using the Boehringer Mannheim Transcription Kit. Hybridization of the sense strand was used as a control for nonspecific hybridization.

Total RNA was prepared from different tissues by using the Qiagen RNeasy Mini preparation kit (Chatsworth, CA). Reverse transcription–PCR (RT-PCR) was performed as described by Grossniklaus *et al.* (26) with M-MuLv reverse transcriptase (Roche Diagnostics). PCR reactions were then done on 2 μl of the reverse transcription product by using the 3L and 2R primers described previously. These primers span the short second intron found in the *FDH* gene. PCR products were size fractionated on agarose gels.

Results

Determining Gene Identity by Mapping and DNA Sequencing. A strong organ fusion phenotype is seen when any one of four *Arabidopsis* genes (*conehead*, *cod*; *deadhead*, *ded*; *fiddlehead*, *fdh*; or *thunderhead*, *thd*) are disrupted by mutation (6). Therefore, the preliminary identification of the affected gene in the line GT1658 was accomplished by mapping the mutation by using PCR-based molecular markers. The GT1658 mutant phenotype showed linkage with the SSLP marker nga168 (28) and strong linkage to the CAPS marker, GPA1 (29) found on chromosome 2, consistent with the linkage profile of the *FDH* gene (6). Complementation analysis verified that this mutation represented an allele of the *FDH* gene. This new allele was henceforth designated *fdh-8*. Amplification, isolation, and sequencing of the region flanking the transgene insert identified sequences showing homology to a region which spanned parts of chromosome 2 already sequenced as part of the *Arabidopsis* Genome Initiative (GenBank accession no. AC004484, BAC clone T1D16). Comparison of these sequences with those derived from the isolated flanking piece indicated that the *Ds* element had inserted into a

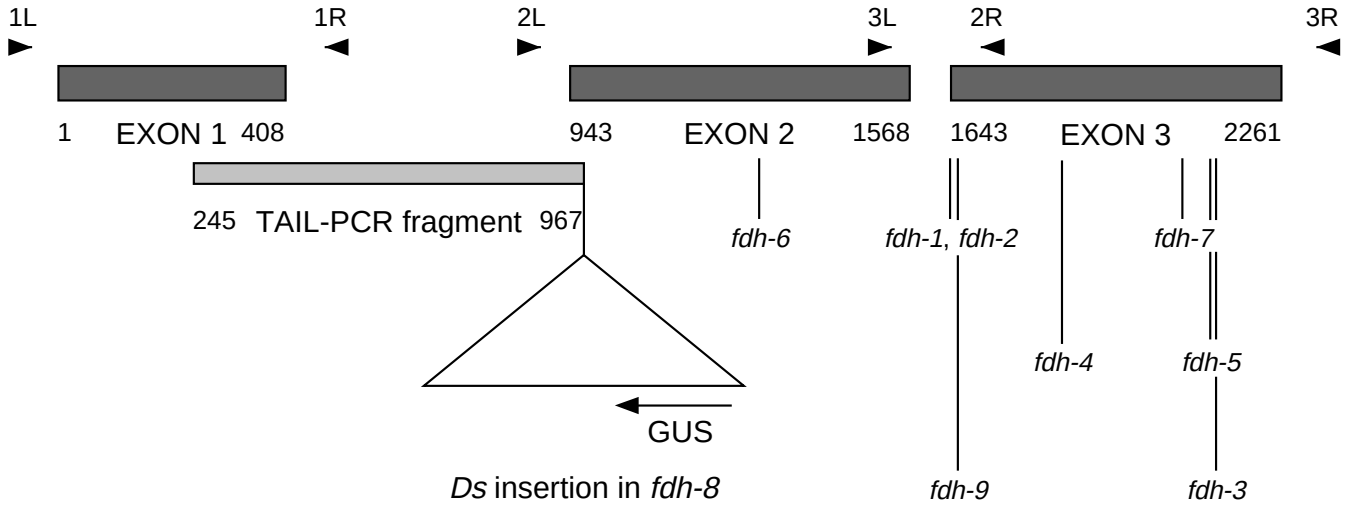

Fig. 1. Schematic representation of the *FDH* gene showing its three exons and two introns. Numbers indicate nucleotide positions relative to the start of translation. The positions of the lesions in *fdh-1* through *fdh-9* are indicated as well as the location of the thermal asymmetric-interlaced–PCR (TAIL-PCR) product. Orientation of the GUS transgene is inverted relative to *FDH*. The locations of PCR primers are indicated by arrowheads.

hypothetical coding sequence at the extreme 5′ end of the second exon (Fig. 1). The orientation of the *Ds* insertion is such that the GUS gene carried as a gene trap is inverted relative to the direction of *FDH* transcription and no β-glucuronidase activity was detected in GT1658. The genomic sequence data predicts that the *FDH* gene, not including the 5′ regulatory region, is just over 2 kb long and is made up of three exons with two introns (Fig. 1).

To verify that the hypothetical gene identified is indeed the *FDH* gene, all of the mutant *fdh* alleles were sequenced (Table 1). As summarized schematically in Fig. 1, mutations were detected in the DNA sequences of all eight *fdh* alleles other than the *Ds* insertion already described. All seven of the ethyl methanesulfonate-induced mutations are G-to-A transitions as would be expected from an alkylating agent, whereas *fdh-9*, which resulted from a T-DNA transformation (although it is not associated with a T-DNA insertion), is a G-to-T transversion. Three alleles (*fdh-4*, *fdh-5*, and *fdh-9*) have stop codons in exon 3, whereas two others (*fdh-1* and *fdh-2*) bear the same single-nucleotide change and result in the alteration of the splice acceptor sequence between intron 2 and exon 3. The remaining three alleles have sequences that lead to single amino-acid substitutions in the gene product. The lesion in *fdh-3* converts a Gly residue that is completely conserved in all sequences closely related to *FDH* (see below) into an Asp residue, whereas the lesion in *fdh-7* converts a completely conserved Glu residue into a Lys residue. The lesion found in *fdh-6* is the only one other than the *Ds* insertion in *fdh-8* to be found outside of exon 3 and converts a Gly residue which is conserved in both the β-ketoacyl-CoA synthase family and the chalcone synthase family into an Arg residue. In the chalcone synthase and stilbene synthase families, this Gly is immediately adjacent to a Cys residue that is required for enzymatic activity (30). Interestingly, no mutations were found in exon 1, which is the least conserved part of the sequence between different family members.

DNA Sequences Related to *FDH*. The use of two sequence analysis tools, BLAST (31) and GENEQUIZ (32), identified a large number of sequences with similarity to *FDH*. By using stringent criteria for similarity, there are at least eight sequences that are closely related to *FDH*, three hypothetical genes from the *Arabidopsis* genome project, four β-ketoacyl-CoA synthases from within the crucifer family (including *FAE1* from *Arabidopsis*), and a β-ketoacyl-CoA synthase from jojoba (*Simmondsia chinensis*). Comparison of these nine sequences reveals that although the four crucifer β-ketoacyl-CoA synthase genes show sequence conservation over their entire length, the other five sequences show conservation primarily in the regions corresponding to the second and third exons of the *FDH* gene, especially in two large blocks extending from residue 217 through 267 (33/51 identical residues and 46/51 conserved residues for all nine sequences) and 471 through 527 (34/57 identical residues and 41/57 con-

Table 1. Summary of *fiddlehead* mutant alleles

Allele	Genetic background	Mutagen	DNA lesion*		Ref.
1	Landsberg *erecta*	EMS	$G_{1642} \rightarrow A$	[splice site]	10
2	Landsberg *erecta*	EMS	$G_{1642} \rightarrow A$	[splice site]	6
3	Landsberg *erecta*	EMS	$G_{2149} \rightarrow A$	[Gly-514 → Asp]	6
4	Landsberg *erecta*	EMS	$C_{1776} \rightarrow T$	[Gln-390 → stop]	6
5	Landsberg *erecta*	EMS	$G_{2135} \rightarrow A$	[Trp-509 → stop]	6
6	Landsberg *erecta*	EMS	$G_{1300} \rightarrow A$	[Gly-256 → Arg]	6
7	Landsberg *erecta*	EMS	$G_{2097} \rightarrow A$	[Glu-497 → Lys]	6
8	Landsberg *erecta*	*Ac/Ds*	*Ds* insertion at 967		This study
9	Landsberg *erecta*	T-DNA	$G_{1665} \rightarrow T$	[Glu-353 → stop]	This study

EMS, ethyl methanesulfonate.

*The alteration to the DNA sequence is indicated together with the position in the nucleotide sequence relative to the start of the coding sequence. The resulting change in the RNA or protein is indicated in brackets.

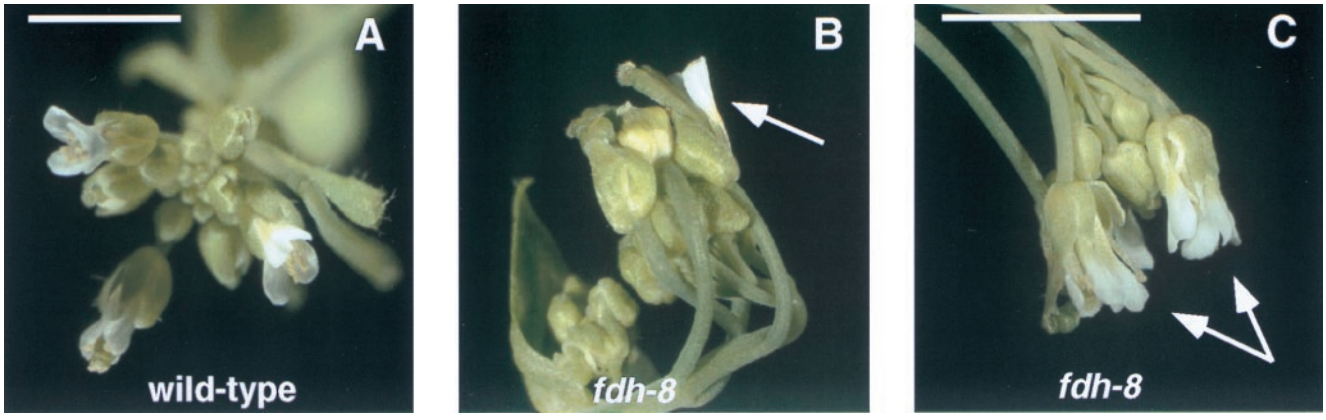

Fig. 2. Phenotype of *fdh-8* revertant sectors. Light micrographs of wild-type (*A*) and *fdh-8* mutants showing small (*B*) and larger (*C*) revertant sectors. In the presence of *Ac*, the *fdh-8* mutation is unstable because of excision of the *Ds* element. Arrows indicate emergent fully expanded petals that are diagnostic for loss of the fusion phenotype. *B* and *C* are at the same magnification. (Bars = 1 cm.)

served residues for all nine sequences) of the *FDH* protein sequence. The use of GENEQUIZ software revealed a much larger family of related sequences, including sequences encoding both chalcone and stilbene synthases. Sequence similarity in both of these other gene families was primarily limited to the two highly conserved blocks defined above.

Reversion of the *fdh-8* Allele. To demonstrate that the *fdh-8* allele was in fact caused by the *Ds* insertion, transposase was introduced in *trans* by crossing the *fdh-8* line with a conditional male sterile line homozygous for an immobilized *Ac* (22). Out of 105 F_2 plants that showed the mutant phenotype, 21 were identified that had wild-type sectors, indicating that the *fdh-8* mutation was unstable in the presence of *Ac* (Fig. 2). Because 25% of these plants would not have contained an *Ac* element, the frequency with which the *Ds* element moved in the presence of *Ac* is estimated to be 27% [21/(105 × 0.75)]. These sectored plants often produced wild-type floral branches which set seed. Seeds were collected from these sectors and progeny scored for the mutant phenotype. Only in 1 of 12 cases was a 3:1 segregation of the mutant phenotype observed in the progeny population, indicative of transmission of the reverted allele. In the remaining 11 cases, wild-type sectors produced progeny that were all phenotypically mutant.

Expression of the *FDH* Gene. The expression profile of *FDH* was determined by both reverse transcription–PCR analysis and *in situ* hybridization to tissue sections. Reverse transcription–PCR analysis was performed with RNA samples generated from roots and shoots and demonstrated that *FDH* expression is limited to the aboveground parts of the plant (Fig. 3). *In situ* RNA hybridization analysis showed that *FDH* mRNA localizes to epidermal cells. The strongest signal was detected in petal cells (Fig. 3*E*), although epidermal cells from leaf, stem, and floral organ sections also hybridize to the antisense *FDH* probe. In addition, *FDH* mRNA is detected in ovule primordia (Fig. 3*A*) but becomes restricted to the chalazal pole of the ovule at maturity (Fig. 3 *B* and *C*). No hybridization occurs to ovule tissue if the sense RNA strand is used (Fig. 3*D*). *FDH* mRNA is not detected in the septum of the ovary or in the stigmatic papillar cells. Interestingly, *FDH* mRNA can also be detected in phloem tissue (Fig. 3*F*).

Discussion

The cuticle layer is a heterogeneous matrix found on the outer surface of the epidermis and is the distinguishing feature that

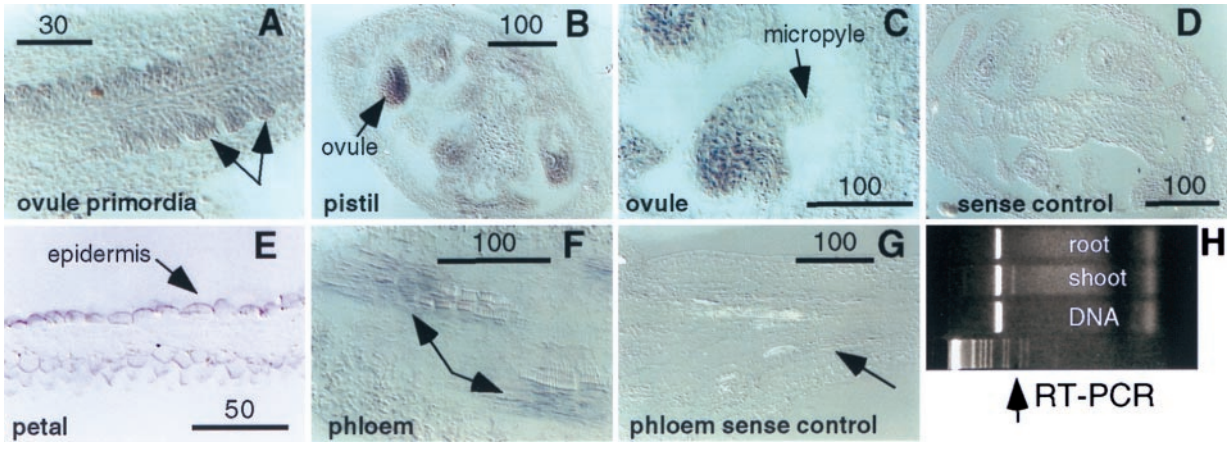

Fig. 3. Expression pattern of the *FDH* gene. (*A*–*G*) Light micrographs of wild-type floral tissues showing *in situ* RNA hybridization of the *FDH* antisense (*A*–*C*, *E*, and *F*) and sense (*D* and *G*) probes. Hybridization is detected in ovule primordia (arrows, *A*) as well as mature ovules (*B* and *C*). Petal epidermal cells (*E*) show a strong hybridization signal as does phloem tissue in vascular strands (arrows, *F*). Control sense strand hybridizations show no signal in the ovary (*D*) or vascular tissues (*G*). Reverse transcription–PCR confirms that *FDH* expression is limited to the shoot (*H*). Primers 3L and 2R (*Materials and Methods*) were used to specifically amplify a 256-bp genomic region that spans intron 2, which is 74 bp long. Maturation of the *FDH* mRNA results in the synthesis of a smaller 182-bp PCR product from the cDNA template (arrow). (Scale bars indicate magnification in μm.)

characterizes this cell type. The cuticle limits desiccation, exposure to harmful radiation, and offers the first line of defense against pathogens. It is also thought to represent the real barrier to diffusion of molecules into and out of the cell wall and protoplast (33). As such, the cuticle presents a unique problem in instances where cell interactions between epidermal cells are desirable. The normal function of the cuticle must be bypassed to facilitate cell–cell communication. Both genetic and molecular evidence now point to a mechanism in which the properties of the cuticle are modified to accommodate cell–cell interactions by altering its lipid composition.

During postgenital fusion, contact between two fusion-competent epidermal cells promotes a reciprocal recognition reaction which is followed by adhesion of the cell walls and cuticles (8). Contact-mediated adhesion requires the exchange of small diffusible molecules between the apposed cells. Epidermal adhesion culminates in the partial or complete union of two separate organs into a single distinct structure. At the cellular level, fused epidermal cells can either remain epidermal in character or redifferentiate into a subepidermal cell type (8). In either case, a response to contact takes place initially across an intact cuticle and without cytoplasmic coupling (34). Numerous genes are involved in this complex developmental pathway and evidence from the characterization of a large collection of *Arabidopsis* mutants manifesting a fusion phenotype suggests that surface characteristics of the contacting cells play an important role in this process (1, 6).

Some of the surface determinants mediating recognition between pollen and stigma in *Arabidopsis* are found in the outer tryphine layer which coats the pollen grain (17, 18). Both biochemical and genetic data indicate that long-chain lipids contained in the tryphine act as the recognition factors in this interaction. Deposition of compatible tryphine potentiates the local release of water from the stigmatic papillar cell, thereby effecting pollen hydration and subsequent growth. Two of the four *Arabidopsis* genes known to mediate pollen recognition, *CER1* and *CER3*, have been isolated (19, 20). *CER1* sequence data suggest that the gene product is a component of a lipid metabolic pathway, whereas *CER3* sequences show no homology to any known genes or gene products. Recently, it has been demonstrated that direct application of triacylglycerides to non-reproductive surfaces facilitates pollen growth (21), lending further support to the notion that in *Arabidopsis*, lipids are sufficient to modify the properties of the cuticle, allowing cellular interaction.

One gene known to maintain the developmental stability of the epidermis is the *Arabidopsis FDH* gene. Epidermal cells in *fdh* mutant plants become responsive to contact, resulting in organ adhesion and impaired growth (10). The role of the wild-type gene is therefore an inhibitory one, ensuring normal growth and development of organs that are in close apposition following their initiation at the meristem. We have isolated the *FDH* gene from a *Ds*-containing gene trap line (22) and confirmed the identity of the gene both by genetic reversion of the phenotype with *Ac* and DNA sequence analysis of known mutant *fdh* alleles. Analysis of the DNA sequence of the gene reveals that it encodes a protein most closely related to β-ketoacyl-CoA synthase, an enzyme that catalyzes the first step in the biosynthesis of very long-chain fatty acids in plants (35). The reaction catalyzed involves the condensation of a long-chain fatty acyl-CoA ester with malonyl-CoA to produce a β-ketoacyl-CoA ester two carbons longer than the original ester. The sequence of *FDH* also shows weaker similarity with chalcone synthase and stilbene synthase genes, both of which encode enzymes that catalyze similar condensation reactions involving malonyl-CoA, but involving different starter substrates in the condensation. Detailed examination of the *FDH* sequence reveals that it is most similar to all of these genes in the regions where they are most similar to each other, and that it is quite dissimilar from the bona fide β-ketoacyl-CoA synthase genes that have been isolated from *Arabidopsis* and other crucifers. Given this pattern of sequence similarity, together with biochemical data that suggests that *fdh* mutant plants accumulate at least one unusual lipid in their cuticles, we believe it is likely that the enzyme encoded by the *FDH* gene catalyzes a condensation reaction involving malonyl-CoA and a novel lipid substrate. Further biochemical experiments are being performed to determine the nature of this reaction.

An interesting question raised by searches of sequence databases concerns the function of the other genes with sequence similarity to *FDH*, *FAE1*, and *CHS*. The presently available *Arabidopsis* genome sequence contains at least five closely related sequences in this family. In addition to *FDH* and *FAE1*, one of these sequences corresponds to the recently characterized *CUT1* gene (36), which is required both for cuticular wax deposition and male fertility. It remains to be determined whether any of the other members of the family will correspond with other genes that have been shown to be responsible for suppressing organ fusion in *Arabidopsis* (6). Extrapolating on the ideas presented above, one might predict that each of these genes encodes an enzyme that carries out a unique condensation reaction. It will be interesting to see how diverse the biochemistry catalyzed by the family is. Given that *FDH* and *CUT1* both appear to be essential for synthesis or deposition of cuticular lipids, further characterization of these genes and their products also provides us with an opportunity to learn more about the molecular and cellular mechanics of how the cuticle is produced.

The distribution of the molecular lesions found in the various *fdh* mutant alleles is also interesting. None of the mutations occur in the first exon, only the *Ds* insertion and one point mutation lie in the second exon, with the remaining seven point mutations being found either in the third exon or at the splice acceptor sequence at the intron 2/exon 3 boundary. One potential explanation for this distribution is that mutations toward the 5′ end of the gene may result in a null phenotype, whereas the existing *fdh* alleles may represent genetically weaker mutations. We consider this explanation unlikely because three of the existing alleles (*fdh-1*, *fdh-2*, and *fdh-9*) result in the loss of the entire highly conserved portion of the protein encoded in exon 3, whereas a fourth allele (*fdh-6*) alters an amino acid residue that is thought to be required for activity in a related gene family. A more probable alternative explanation is that most mutations in the relatively poorly conserved portion of the protein encoded by exon 1 fail to produce an organ fusion phenotype and were therefore not recovered in our mutant screens.

Based on the phenotype of *fdh* mutant plants, one would predict that the gene product is primarily required in epidermal cells. The results of both genetic and molecular experiments support this view. The fact that most phenotypic reversion events fail to be transmitted to progeny indicates that the sectors containing the reversion events most commonly fail to include tissues derived from the L2 cell layer. This implies that genetic reversion in either the L1 (epidermal) layer or the L3 (internal to L2) layer is sufficient for phenotypic reversion of the organ fusion phenotype. Given the nature of the phenotype, it is more probable that these events are taking place in the L1 layer. *FDH* mRNA distribution as determined by reverse transcription–PCR and *in situ* hybridization is also consistent with this hypothesis, indicating that *FDH* is expressed predominantly in epidermal cells. The gene is not expressed in stigmatic papillar cells or in the septum of the ovary; however, these are both sites where contact-mediated epidermal interactions normally take place.

Although the most striking phenotypic consequence seen in *fdh* mutants is the impairment of normal expansive growth caused by adhesion of adjacent organs, dissection of the ovary reveals an additional effect on ovule development (6). Our

analysis of *FDH* expression is consistent with the observed abnormality in ovule development. Whether *fdh* mutant ovules develop a normal complement of tissues and cell types remains to be determined. Preliminary results suggest that at least some ovules are capable of being fertilized and developing into mature seed, indicating that normal development takes place in some instances.

FDH expression seen in phloem tissue is less readily explained. It is possible that phloem tissues require unique lipid classes that may facilitate transport or permeability requirements specific to these highly specialized cells. Alternatively, the distribution of the *FDH* mRNA may not coincide with the localization of the gene product itself and the *FDH* gene product may be active at some distance from this site. Immunolocalization of the *FDH* protein should resolve this issue.

The molecular identity of the *FDH* gene product and its expression pattern are consistent with our previous model that *FDH* acts by altering the permeability of the epidermal cuticle (7). Although the cuticle presumably formed as an adaptation to a nonaquatic environment, the epidermal surface of higher land plants must still offer an interactive and responsive interface for sexual reproduction, and in some specialized clades, for epidermally mediated postgenital fusion (37). Based on our findings, one way this may have been achieved was by biochemically modifying the cuticle such that under most conditions, epidermal cells are prevented from transmitting or receiving constitutively available signals, rather than by regulating the availability of the signals or the downstream signal transduction machinery. Alleviating this block requires only the modification of the permeability barrier that, based on genetic evidence, can be orchestrated in a variety of ways (6).

The permeability properties of cell wall and cuticle have an impact on plant–pathogen relations as well as on the efficacy of superficially applied man-made agents such as agrochemicals. It seems plausible that at least some pathogens capable of penetrating the cuticle do so by parasitizing processes normally used by the plant to promote transmission of signals across the cuticular barrier. Understanding how to modulate the properties of the cuticle may therefore assist in developing cultivars more resistant to specific classes of pathogens. On the other hand, the permeability of agrochemicals might be positively or negatively modified by selectively targeting endogenous molecular modifiers of the cuticle such as the putative long-chain lipids synthesized by the *FDH* gene pathway. Molecular genetic manipulation of the *FDH* gene, as well as other organ fusion genes, may prove to have a multitude of practical applications.

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