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28. Fluorescence in situ hybridization assays of sister chromatid cohesion: Yeast strains were grown in YPD and arrested with either α-factor (25 μg/ml) or nocodazole (20 μg/ml). Fixation and hybridization were carried out essentially as described (*19*) with the modifications described (*2*). Slides were mounted with antifade solution containing DAPI (1 mg/ml r-phenylenediamine, 1 μM DAPI, 1× phosphate-buffered saline in 90% glycerol) and viewed with a Nikon epifluorescence microscope. Images were captured digitally with a Princeton Instruments charge-coupled device camera and IP Lab Spectrum software.
29. Cells containing the *GFP-lacI* fusion were grown overnight in SC-histidine at 30°C. Cells were diluted to an absorbance at 600 nm (A_{600}) of 0.2 and allowed to double once more. Cells were spun and resuspended in the same volume of YPD plus nocodazole (20 μg/ml) or α-factor (25 μg/ml). After 4 hours, cells were fixed by adding 0.1 volumes of 37% formaldehyde, incubated for 5 min, and processed as described (*21*). Slides were viewed with a Nikon epifluorescence microscope with a 100× oil immersion lens and a *GFP* LP filter from Chroma Technology (Brattleboro, VT).
30. We thank S. Linn for providing the DNA pol I neutralizing antibody; L. Aravind and E. Koonin for pointing out the relation between *TRF4* and the β-polymerase superfamily; D. Auble and Q. Chen for advice about biochemistry; D. Koshland, A. Murray, B. Futcher, and G. Sherlock for strains and plasmids; and D. Auble, N. Levin, R. Li, D. Pellman, and M. Smith for comments on the manuscript. This work is supported by grants from the NIH and the Human Frontiers Science Program to M.F.C.

11 May 2000; accepted 16 June 2000

Cell-Cell Signaling and Movement by the Floral Transcription Factors LEAFY and APETALA1

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LEAFY (*LFY*) and *APETALA1* (*AP1*) encode unrelated transcription factors that activate overlapping sets of homeotic genes in *Arabidopsis* flowers. Sector analysis and targeted expression in transgenic plants were used to study whether *LFY* and *AP1* can participate in cell-cell signaling between and within different layers of the floral meristem. *LFY* signaled equally well from all layers and had substantial long-range action within layers. Nonautonomous action of *LFY* was accompanied by movement of the protein to adjacent cells, where it directly activated homeotic target genes. In contrast, *AP1* had only limited nonautonomous effects, apparently mediated by downstream genes because activation of early target genes by *AP1* was cell-autonomous.

Shoots and flowers are derived from collections of stem cells called meristems, which are stratified into distinct cell layers. In many plants, restrictions in the plane of cell division in the two outer layers lead to the generation of three cell lineages—the epidermal layer (L1), the subepidermal layer (L2), and the internal layer (L3)—thus allowing the generation of genetically mosaic shoots and flowers. Mosaic studies have shown that some floral transcription factors can signal from layer to layer, although signaling within layers always appeared to be largely absent (*1–6*).

Although cell-cell communication initiated by transcription factors is not unusual, plant cells differ from animal cells in that they are connected by plasma membrane–lined channels called plasmodesmata, which provide cytoplasmic continuity between adjacent cells. On the basis of the precedence of intercellular trafficking of viral proteins, it has been proposed that cell-cell communication by trafficking of transcription factors is a widespread phenomenon in plants (*7*). Two transcription factors, KN1 in maize and DEF in *Antirrhinum*, have indeed been shown to move to cells in which their RNAs are not found (*4, 8, 9*). However, because direct target genes have not been conclusively identified for either factor, the biological activity of the exported proteins could not be assayed, although DEF movement at late stages of development correlated with some nonautonomous phenotypic effects during early stages (*4*).

To investigate transcription factor trafficking in *Arabidopsis* flowers, we used two complementary approaches to compare the cellular autonomy of LFY and AP1, two unrelated transcription factors that activate overlapping sets of target genes (*10, 11*). In the first approach, we used FLP recombinase to create genetically mosaic plants with sectors marked by excision of a β-glucuronidase (GUS) gene. Activation of *FLP* under the control of a heat shock promoter (*HSP::FLP*) (*12*) resulted in *35S::AP1*$^+$ *GUS*$^-$ sectors in a *35S::AP1*$^-$ *GUS*$^+$ *ap1-1* background (*13*). In *ap1-1* flowers, first-whorl sepals are replaced by bracts in the axils of which secondary flowers arise, whereas second-whorl petals are typically absent (*14*). Analysis of mosaic shoots from heat-shocked *ap1-1 HSP::FLP FLP.AP1* plants revealed that the recombined allele had to be present in all layers for full rescue and that clones expressing *35S::AP1* only in L3 were indistinguishable from *ap1-1* mutants (*13*).

Clones expressing *35S::AP1* only in L1 produced first-whorl organs with L1 cells typical of wild-type sepals, but L2 and L3 cells more typical of *ap1* bracts (Fig. 1D). Second-whorl organs were restored, and these had petal identity in L1 but not in the internal layers (Fig. 1E). Conversely, expression of *35S::AP1* in L2 and L3 produced first-whorl organs with sepal anatomy in the internal layers, but a bract-like L1 (Fig. 1F). In the second whorl, organs with petal shape were produced, but L1 typically lacked petal identity (*13*). None of the L1, L2, or L3 clones suppressed the formation of secondary flowers (*13*). Mericlinal sectors, in which *35S::AP1*$^+$ and *35S::AP1*$^-$ cells abutted in the same layer, showed complete autonomy of *AP1* within layers (Fig. 1F). In summary, these genetic mosaics revealed that *AP1* acts largely cell-autonomously to control cellular identity, but nonautonomously to promote outgrowth of second-whorl organs.

A strategy similar to that for *AP1* was used to generate *35S::LFY* sectors in a *lfy-12* mutant background (*13*). Mosaic plants were obvious because they produced flowers with

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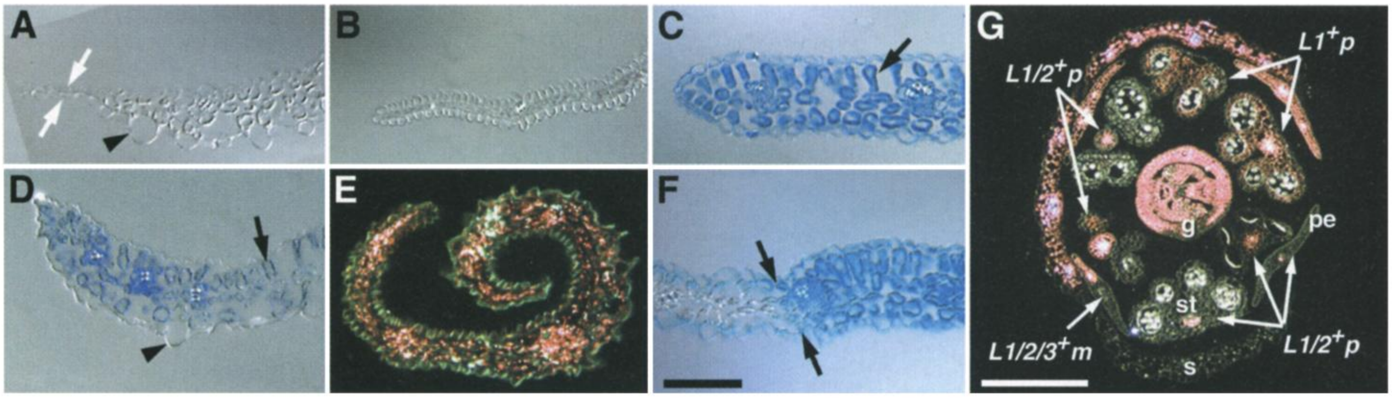

Fig. 1. *AP1* and *LFY* sectors. The first six panels are cross sections of floral organs; adaxial side is up. (**A**) Wild-type sepal, with adaxial curvature, no internal layers at the margin (arrows), large cells in the abaxial epidermis (arrowhead), and isodiametric internal cells. (**B**) Wild-type petal with distinct adaxial and abaxial L1 of conical cells. (**C**) *ap1-1 FLP.AP1* first-whorl bract, stained for GUS activity, and showing blunt margin, uniform epidermis of small cells, and subepidermal columnar cells (arrow). The next three panels show sectioned organs from mosaic *ap1-1 HSP::FLP FLP.AP1* plants. (**D**) First-whorl organ with a *35S::AP1* L1. The epidermis has large cells typical of the wild type (arrowhead). Internal layers are *ap1*-like (arrow). (**E**) Dark-field image of second-whorl organ with *35S::AP1* L1 consisting of petal-typical conical cells. The internal cells, which are *ap1-1* mutant (GUS-positive tissue appears orange), differ from those of wild-type petals, although their exact identity is unclear. (**F**) First-whorl organ with a mericlinal L2-L3 clone of *35S::AP1* cells. Sepal-like cells abut bract-like cells at the clone border between arrows. (**G**) Dark-field image of cross section through a GUS-stained flower from a *lfy-12 HSP::FLP FLP.LFY* plant. Two *35S::LFY* clones—one in the stamens (st) on the top, and one in the sepal (s), petals (pe), and stamens below—are indicated (g, gynoecium). Layer composition of organs is indicated (m, mericlinal sector; p, periclinal sector). Scale bars, 68 μm (A to F), 250 μm (G).

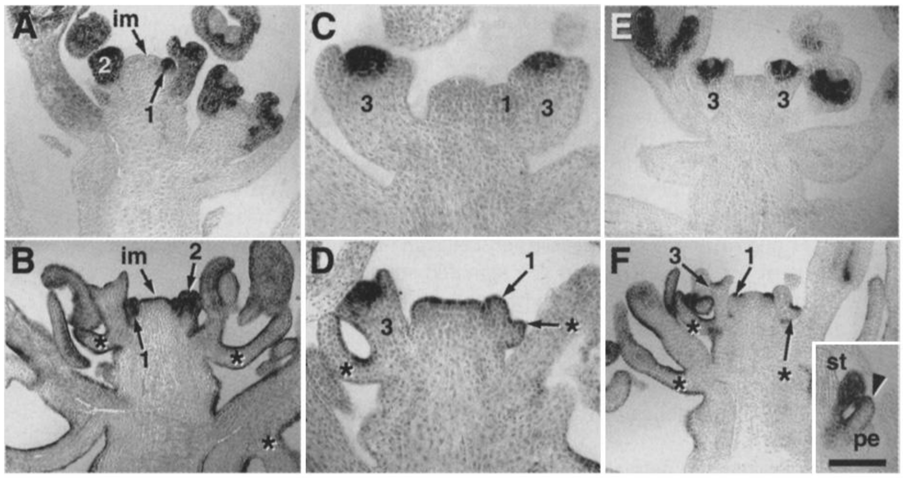

Fig. 2. RNA expression in *ML1::AP1* plants. Top row, *ap1-1* (left panel) or wild type (two right panels); bottom row, *ap1-1 ML1::AP1*. (**A** and **B**) In *ap1-1 ML1::AP1* plants, *AP1* is expressed in addition to its endogenous domain throughout L1, including the inflorescence meristem (im), pedicel organs (asterisks), and stem. (**C** and **D**) *AG* is ectopically expressed in L1 of *ap1-1 ML1::AP1*. (**E** and **F**) *AP3* is ectopically expressed in L1 of *ap1-1 ML1::AP1*. The inset shows L1-specific *AP3* expression in a rescued petal (pe) primordium of a stage 8 flower, which contrasts with *AP3* expression throughout all layers of the adjacent stamen (st) primordium, whose formation is *AP1*-independent. Scale bar, 100 μm, except 50 μm in (C) and 70 μm for inset in (F). Numbers indicate floral stages (*21*).

petals and stamens on *lfy* shoots (*13*), which normally produce only shoot-like flowers that lack petals and stamens (*15*). Some mosaic plants showed conversion of lateral branches into solitary rosette flowers as well as terminal flowers on the primary shoot, similar to *35S::LFY* plants (*13, 16*). Flowers were mosaic in many different ways, but often contained phenotypically wild-type organs that were genotypically completely *lfy* mutant. Sectors that occupied less than half of a meristem could reorganize it into a normal flower, demonstrating nonautonomous behavior of LFY both across and within layers (Fig. 1G) (*13*).

Genetic chimeras cannot be sexually propagated, and because of their sporadic nature, it is often impossible to analyze a particular chimeric arrangement at different developmental stages. To overcome these limitations, we generated molecular mosaics by expressing *AP1* and *LFY* under the control of the L1-specific *AtML1* promoter (*13*) in *ap1* and *lfy* mutants (Figs. 2B and 3B). Although most *ap1 ML1::AP1* lines had phenotypes similar to those of L1 genetic mosaics, a minority showed more extensive rescue of the mutant phenotype, suggesting that higher levels of *AP1* in L1 had limited nonautonomous effects on the cellular identity of internal layers. These lines also had gain-of-function phenotypes that included bract-like organs on the abaxial base of pedicels (*13*). In contrast to *ap1*, *lfy* mutants were fully rescued by *ML1::LFY*. Most *lfy ML1::LFY* lines had phenotypically wild-type flowers; about one-quarter of these lines also had gain-of-function phenotypes similar to those of *35S::LFY* plants (*13*).

To understand the molecular mechanisms underlying the phenotypes of *ap1 ML1::AP1* and *lfy ML1::LFY*, we analyzed the RNA expression patterns of the target genes *AP3* and *AG*. Early expression of *AP3* and *AG* is not markedly changed in *ap1* mutants, because *AP1* is only a redundant activator of these genes (*10, 11*). However, in strong *ap1 ML1::AP1* lines, *AG* was ectopically expressed in L1 of the shoot meristem and pedicel organ primordia and occasionally prematurely in incipient flowers (Fig. 2D). *AP3* was also ectopically expressed in L1 of stems and later-arising flowers of strong *ap1-1 ML1::AP1* lines. *AP3* RNA was found only in L1 of restored petals, indicating cell-autonomous activation of *AP3* within the normal expression domain of *AP1* (Fig. 2F). Thus, *AP1* activates *AG* and *AP3* cell-autonomously.

AP3 and *AG* expression, although much reduced in *lfy* flowers (*10*), was restored throughout all layers in *lfy ML1::LFY* plants (Fig. 3, D and F), indicating nonautonomous activation of *AP3* and *AG* by *LFY*. Because it has been proposed that such nonautonomous effects might be mediated by protein trafficking, we analyzed

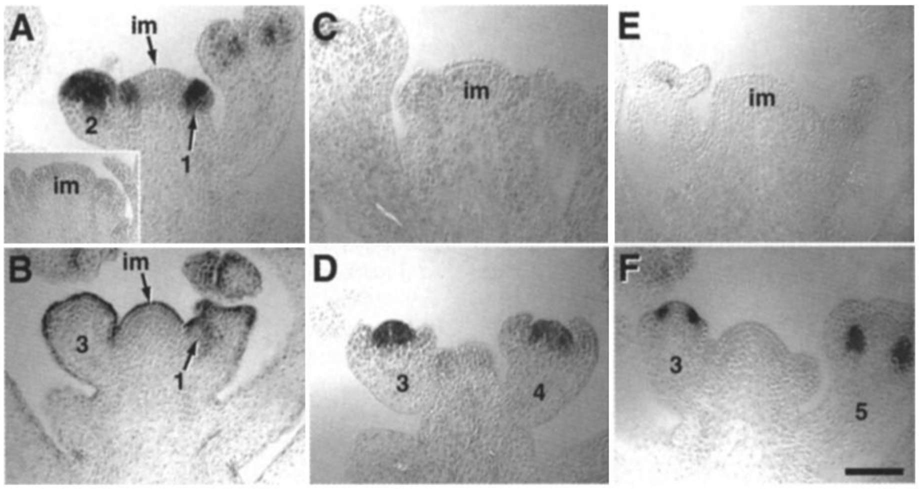

Fig. 3. RNA expression in *ML1::LFY* plants. Top row, wild type (left panel) or *lfy-12* (two right panels); bottom row, *lfy ML1::LFY*. (**A**) Inset shows absence of *LFY* RNA in the *lfy-30* deletion allele. (**B**) L1-specific expression of *LFY* in *lfy-30 ML1::LFY*. (**C** and **D**) *AG* expression is restored in all layers of *lfy-12 ML1::LFY*. (**E** and **F**) *AP3* expression is restored in all layers of *lfy-12 ML1::LFY*, but is initially shallower than in the wild type (compare to Fig. 2E). Scale bar, 50 μm, except 30 μm for inset in (A).

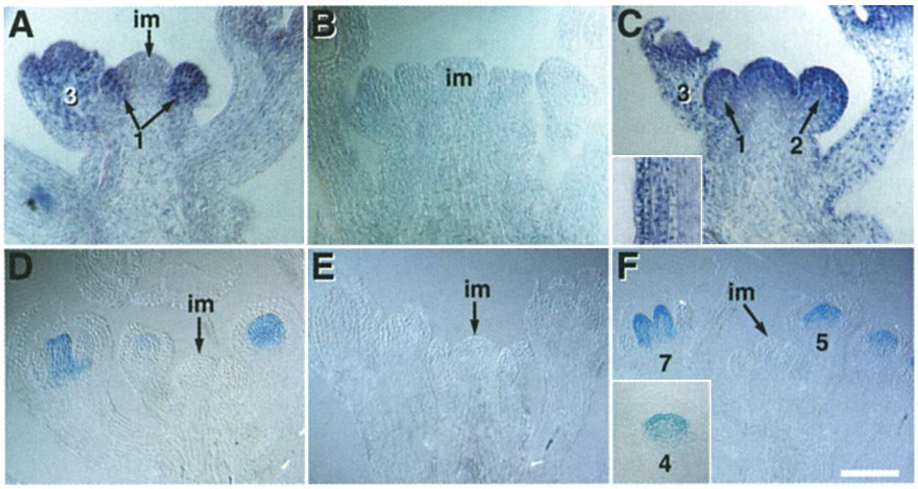

Fig. 4. Expression of LFY protein and a LFY-dependent reporter in *ML1::LFY*. (**A**) In the wild type, LFY is detected in the nuclei of all cells of young floral buds. (**B**) No LFY protein is detected in *lfy-12* mutants. (**C**) In this *lfy-12 ML1::LFY* inflorescence, a gradient of LFY protein is observed, with the highest levels in L1 and L2 and lower levels in internal layers. This gradient is also apparent in the stem shown in the inset. (**D** and **E**) KB18 GUS reporter is active in the *AG* domain in the wild type, but inactive in *lfy-12*. (**F**) KB18 GUS activity is restored in *lfy-12 ML1::LFY*, often in a gradient, with the highest levels in the outer cell layers (inset). Scale bars, 60 μm (A to C), 85 μm (D to F), and 42 μm [inset in (F)].

the expression of LFY protein in these plants. In contrast to *LFY* RNA (Fig. 3B), we detected LFY protein in all layers of *lfy ML1::LFY* plants (Fig. 4C). We also studied the expression of the KB18 *AG::GUS* reporter in *lfy ML1::LFY*, because KB18 activity, in contrast to that of endogenous *AG*, is completely LFY-dependent (*17*). The activity of KB18, which contains two essential LFY binding sites, was restored in all layers of *lfy ML1::LFY* flowers; this result confirmed that LFY protein that had moved to adjacent cells was active as a DNA-binding transcription factor.

Because our experiments used a reporter whose expression requires that it be directly bound by LFY, we were able to show not only that LFY antigen was found in cells where its RNA was not detected, but also that LFY protein was active in these cells. We have also documented substantial long-range action of an exported transcription factor, because expression of *LFY* in less than half of a meristem was sufficient to reorganize the entire meristem. Additionally, our studies have highlighted the importance of discriminating between nonautonomous effects at the level of mature phenotypes versus the level of early target genes. We found that L1-restricted AP1 could restore petal formation even though it activated the petal identity gene *AP3*, which is likely to be a direct AP1 target (*18*), only in L1 of rescued petals.

The finding of LFY movement raises the question of what its role in normal development is. Given that the patterns of *LFY* RNA and LFY protein in the wild type are similar, it is possible that movement of LFY protein provides only a redundant mechanism to ensure complete conversion of a meristem into a flower. Indeed, shoot-flower chimeras are rare in the wild type but are frequently observed in *lfy* mutants (*19*). On the other hand, nonautonomous effects of *LFY* and its ortholog *FLO* have been reported. For example, *FLO* is required for activation of *CEN* in the shoot meristem (*20*), whereas *LFY* is required to prevent ectopic activation of *AG* in the stem (*10*), although it is not known whether these effects are direct. To determine the requirement of LFY movement in wild-type plants, it will be necessary to examine the effects of disabling LFY movement during normal flower development.

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22. We thank N. Kilby, A. Mandel, G. Davies, and J. Murray for material; N. Kilby, C. Ferrándiz, M. Blázquez, and L. Smith for technical advice; E. Mendoza for excellent technical assistance; and M. Blázquez, M. Busch, and J. Lohmann for discussion and reading of the manuscript. Supported by an LSRF-DOE fellowship (A.S.) and grants from NIH (M.F.Y.), NSF (D.W. and M.F.Y.), and the U.S. Department of Energy (D.W.).

14 April 2000; accepted 14 June 2000