Hypomorphic Alleles Reveal FCA-Independent Roles for FY in the Regulation of FLOWERING LOCUS C

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The autonomous floral promotion pathway plays a key role in the regulation of flowering in rapid-cycling Arabidopsis (Arabidopsis thaliana) by providing constitutive repression of the floral inhibitor FLOWERING LOCUS C (FLC). As a result, autonomous pathway mutants contain elevated levels of FLC and are late flowering. Winter annual Arabidopsis, in contrast, contain functional alleles of FRIGIDA (FRI), which acts epistatically to the autonomous pathway to up-regulate FLC and delay flowering. To further explore the relationship between FRI and the autonomous pathway, we placed autonomous pathway mutants in a FRI-containing background. Unexpectedly, we found that a hypomorphic allele of the autonomous pathway gene fy (fy null alleles are embryo lethal) displayed background-specific effects on FLC expression and flowering time; in a rapid-cycling background fy mutants contained elevated levels of FLC and were late flowering, whereas in a winter annual background fy decreased FLC levels and partially suppressed the late-flowering phenotype conferred by FRI. Because FY has been shown to have homology to polyadenylation factors, we examined polyadenylation site selection in FLC transcripts. In wild type, two polyadenylation sites were detected and used at similar levels. In fy mutant backgrounds, however, the ratio of products was shifted to favor the distally polyadenylated form. FY has previously been shown to physically interact with another member of the autonomous pathway, FCA. Interestingly, we found that fy can partially suppress FLC expression in an fca null background and promote proximal polyadenylation site selection usage in the absence of FCA. Taken together, these results indicate novel and FCA-independent roles for FY in the regulation of FLC.

The timing of flowering is a crucial decision and directly affects the probability of successful reproduction. In Arabidopsis (Arabidopsis thaliana), flowering is regulated by pathways that are responsive to both environmental and developmental cues (Michaels, 2009). Several of these pathways converge at the floral repressor FLOWERING LOCUS C (FLC; Michaels and Amasino, 1999; Sheldon et al., 1999; Crevillon and Dean, 2010). In rapid-cycling accessions, a group of genes known collectively as the autonomous pathway acts to promote flowering by repressing FLC expression. Therefore mutations in autonomous pathway genes result in high levels of FLC and delayed flowering. In contrast to rapid-cycling strains, many naturally occurring accessions of Arabidopsis are late flowering unless flowering is promoted by a prolonged period of cold treatment known as vernalization. These winter annual accessions contain functional alleles of the FRIGIDA (FRI) gene, which act epistatically to the autonomous pathway to up-regulate FLC mRNA levels and delay flowering (most rapid-cycling accessions contain null mutations in FRI; Johanson et al., 2000). Vernalization can promote flowering in FRI-containing strains or autonomous pathway mutants by epigenetically suppressing FLC through a mechanism involving repressive histone modifications at the FLC locus (Bastow et al., 2004; Sung and Amasino, 2004).

Currently, little is known about the mechanism by which the autonomous pathway represses FLC expression; however, the known/predicted functions of the autonomous pathway genes suggest a link between RNA and chromatin structure. The most extensively characterized members of the autonomous pathway include three RNA-binding proteins (FCA, FPA, and FLOWERING LOCUS K [FLK]; Macknight et al., 1997; Meier et al., 2001; Schomburg et al., 2001; Lim et al., 2004; Mockler et al., 2004), a homolog of the yeast polyadenylation factor Pfs2p (FY; Simpson et al., 2003), a putative transcription factor that contains a divergent homeodomain (LUMINIDEPENDENS [LD]; Lee et al., 1994a), and two chromatin remodeling proteins (FLOWERING LOCUS D [FLD] and FVE; He et al., 2004).
In an effort to better understand the relationship between FRI and the autonomous pathway, FRI was introduced into autonomous pathway mutant backgrounds (Table I). If FRI activates FLC by preventing the repression of FLC by the autonomous pathway, then one would predict that the flowering time of a FRI autonomous pathway mutant line would be similar to that of the autonomous pathway mutant alone. If, however, FRI acts through a separate antagonistic pathway, then lines containing both FRI and an autonomous pathway mutant should flower significantly later than either FRI or the autonomous pathway mutant. When grown without vernalization, the autonomous pathway mutants used in this study vary significantly in their late-flowering phenotypes. fca-δ, fld-3, fpa-7, and ld-1 mutants show a strong late-flowering phenotype, flowering with >65 leaves, whereas foe-4, flk-4, and fy-5 mutants show a weaker phenotype (<35 leaves; Fig. 1A). With the exception of FRI fy-5 (discussed below), FRI autonomous pathway lines flowered similarly to the later-flowering parent (Fig. 1A). The vernalization response in the FRI autonomous pathway lines was similar to that of the FRI or the autonomous pathway mutant parental lines (Fig. 1A). Only a line containing FRI and the photo-period pathway mutant constans (co) showed an attenuated vernalization response (Fig. 1A). This is expected, as the late-flowering phenotype of co mutants is not due to elevated FLC expression (Michaels, 2009). These results indicate that FRI is not able to further delay flowering in autonomous pathway mutant backgrounds. This suggests that the repression of flowering by FRI and the promotion of flowering by the autonomous pathway may occur through a common mechanism.

The late-flowering phenotype of both FRI and autonomous pathway mutants are due to elevated levels of the floral repressor FLC (Michaels and Amasino, 2001). Therefore we also investigated the relationship between FLC expression and flowering time in the FRI autonomous pathway lines. The level of FLC transcript was strongly correlated with flowering time (Fig. 2, A and B; Supplemental Table S1). As expected, levels of the floral integrators SOC1 and FT, which are negatively regulated by FLC, are inversely correlated with FLC expression (Fig. 2B). The tight correlation between FLC expression and flowering time suggests that, similar to FRI or autonomous pathway mutants, the late-flowering phenotype of FRI autonomous pathway mutant lines is due to FLC. To confirm this hypothesis, we created FRI autonomous pathway mutants in an flc-3 mutant background. As predicted, the late
flowering phenotype of FRI autonomous pathway mutant lines is eliminated by the flc null mutation (Fig. 2C).

**fy-5 Shows Background-Specific Effects on Flowering Time and FLC Expression**

In general, FRI autonomous pathway mutant lines flowered similarly to the latest flowering parent (Fig. 1A). A notable exception to this trend was FRI fy-5. Alone, fy-5 shows a modest, but significant ($P < 0.0001$), late-flowering phenotype, flowering approximately nine leaves later than Columbia (Col; Fig. 1A). Unexpectedly, FRI fy-5 plants flowering significantly earlier than FRI ($P < 0.01$), forming approximately 30 fewer leaves (Fig. 1). To determine if these variable effects are due to changes in FLC levels, we examined FLC expression in Col and FRI backgrounds ± fy-5. Consistent with the effects on flowering time, fy-5 caused a 90% increase in FLC transcript in the Col background, in comparison to a 28% decrease in the FRI background (Fig. 2A; Supplemental Table S1). Thus, depending on genetic background, fy-5 can increase or decrease FLC expression and flowering time.

Given the partial suppression of FRI by fy-5, we also investigated the effect of fy-5 mutations in other autonomous pathway mutant backgrounds. Interestingly, the double mutants could be grouped into three classes based on flowering time. The first class, which includes fld and ld, shows an additive delay in flowering with fy-5. fld-3 fy-5 and ld-1 fy-5 plants flowered approximately 10 leaves later than the fld-3 and ld-1 single mutants, which is similar to the delay in flowering caused by fy-5 in Col (Fig. 3A). The second class, which includes fkl and fve, showed a synergistic delay in flowering. fkl-4 fy-5 and fve-4 fy-5 flowered much later (47 and 31 additional leaves, respectively) than the fkl-4 and fve-4 single mutants (Figs. 3, A and B; Koornneef et al., 1998). fca belongs to the third class and, similar to FRI, the late-flowering phenotype of fca-9 is partially suppressed by fy-5 (Fig. 3, A and C).

This result is particularly significant because it demonstrates that FY can influence flowering time in the absence of FCA. As with autonomous pathway mutants containing FRI, the flowering time of fy-5 autonomous pathway double mutants is well correlated with FLC expression (Fig. 2, D–F).

Because null alleles of fy are embryo lethal, hypomorphic alleles must be used for flowering time analysis. In prior work, a variety of lesions have been shown to cause delayed flowering; fy-1 contains a base change that blocks splicing of intron 15, fy-2 contains a T-DNA insertion in exon 16, and fy-3 contains a G to S substitution in the WD 40 domain (Fig. 3D; Simpson et al., 2003; Henderson et al., 2005). To determine if the observed interactions between fy and FRI/the autonomous pathway are specific to fy-5 or are more general, we created double mutants between fy-2 and FRI/autonomous pathway mutants. fy-2 was chosen because it has the strongest late-flowering phenotype of the described fy alleles (Henderson et al., 2005). Overall, the results were similar to those obtained with fy-5 (Fig. 3A); fkl and fve showed a greater delay in flowering when combined with fy-2 than fld-3 and ld-1. Although FRI fy-2 and fca-9 fy-2 did not flower earlier than FRI or fca-9 alone, it was nonetheless interesting that fy-2, which delays flowering by approximately 40 leaves in Col, had no effect on flowering in the FRI or fca mutant backgrounds (Fig. 3A).

**FVE and FLK Show Genetic Redundancy in the Repression of FLC**

As shown above, fy alleles show a stronger late-flowering phenotype in fkl-4 and fve-4 backgrounds than other autonomous pathway backgrounds (Fig. 3A). This is particularly interesting given that the late-flowering phenotype of fkl-4 and fve-4 single mutants is weaker than that of fld-3, fca-9, fpa-7, or ld-1 (Figs. 1A and 3A). Because of the relatively weak late-flowering phenotype of fve-4 and fkl-4, we wondered if FVE and
FLK might be partially functionally redundant in repressing FLC. Because the protein sequences of FVE and FLK are unrelated, it seems unlikely that the two proteins would have similar biochemical activities. It is possible, however, that they function in redundant pathways that repress FLC. Consistent with this model, we found that fve-4 flk-4 double mutants are much later flowering than either single mutant (Fig. 3E). In fact, the flowering time of fve-4 flk-4 is similar to, or later than, FRI or strong autonomous pathway mutants (e.g. fld, fca, fpa, or ld; Figs. 1A and 3, A and E). Thus the three earliest flowering autonomous pathway mutants (fy, fve, and flk) all show strong synergistic delays in flowering time as double mutants. These results suggest that FY, FVE, and FLK have partially redundant roles in the repression of FLC.

**fpa fy-5 Double Mutants Are Viable**

Previous double mutant analysis, performed in the Landsberg erecta background, failed to recover double mutants between fpa and fy-1, suggesting that fpa fy mutants are inviable (Koornneef et al., 1998). To de-
pathway double mutants containing fpa (fpa ld, fpa fld, and fpa fce) have also shown reduced viability and pleiotropic developmental defects (Veley and Michaels, 2008).

Because the fy-5 allele has a weaker flowering time phenotype than fy-2, we speculated that an fpa fy-5 plant might be viable. Indeed, an F2 population between fpa-7 and fy-5 yielded viable double mutants (Fig. 4, A and B). The growth rate of fpa-7 fy-5 plants was similar to Col, fpa-7, and fy-5 (Fig. 4C); however, the double mutants showed other developmental abnormalities. Most notably, fpa-7 fy-5 plants exhibited reduced apical dominance during vegetative development (Fig. 4, A and B). When grown under long days, the majority of double mutant plants underwent senescence without flowering. It should be noted, however, that fpa-7 fy-5 mutants typically underwent senescence before fpa-7 plants flowered (Fig. 4B), thus the lack of flowering cannot be attributed solely to a stronger late-flowering phenotype. In fact, FLC levels were similar in fpa-7 and fpa-7 fy-5 (Fig. 4D), suggesting that, if fpa-7 fy-5 plants lived long enough, they would flower similarly to fpa-7. To test this hypothesis, we vernalized fpa-7 fy-5 plants for varying lengths of time to accelerate flowering so that it would take place prior to senescence. Double mutants vernalized for 7 or more days did indeed flower similarly to the fpa-7 single mutant (Fig. 4D). Thus, with regards to flowering time, the interaction between fy and fpa appears to be similar to that between fy and fld or ld.

FY Can Affect Flowering Time and FLC Levels Independently of FCA

A number of experiments indicate that FCA and FY act together in the promotion of flowering. fca fy double mutants (in the Landsberg erecta background) do not flower later than fca single mutants, suggesting they function in a common pathway (Koornneef et al., 1998). More recently, the physical interaction between FY and FCA has also been shown to be important for the regulation of FLC. In vitro, the WW protein interaction domain of FCA interacts with the PPLPP domains of FY (Simpson et al., 2003; Henderson et al., 2005) and, in vivo, the repression of FLC by FCA overexpression (35S::FCA) is suppressed by mutations in one of the PPLPP domains of FY (Manzano et al., 2009). Interestingly, our data indicates that FY can also affect flowering time in the absence of FCA. Consistent with previous genetic data (Koornneef et al., 1998), the fca-9 fy-2 does not flower later than the fca-9 single mutant (Fig. 2B). The fy-5 allele, however, causes a reduction in FLC expression and earlier flowering in the fca mutant background (Figs. 2, D and F, and 3, A and C). This indicates that fy-5 has effects on FLC expression and flowering time that do not require the presence of FCA. Of course, this interpretation is predicated on a total lack of functional protein in the fca mutant. fca-9 contains a frameshift mutation in the 37th codon (full-length FCA gamma contains 505...
amino acids) and is therefore unlikely to produce a functional protein. To confirm that \( fy^{-5} \) confers early flowering in the absence of \( FCA \), we obtained a second \( fca \) allele (SALK_057540) that contains a T-DNA insertion. Similar to \( fca^{-9} \), the \( fca \) T-DNA allele \( fy^{-5} \) double mutant flowers significantly earlier than \( fca \) alone (Fig. 5A), confirming that \( fy^{-5} \) can promote flowering and repress \( FLC \) in the absence of \( FCA \).

\( fy^{-5} \) Is Likely to Be a Hypomorphic Allele

In addition to its effect on flowering time, \( fy^{-5} \) mutants showed abnormal floral development including alteration of floral organ number (Fig. 5B), reduced petal development (Fig. 5, C and D), and incomplete closure of the sepals around the developing floral buds (Fig. 5, F and G). Some of these phenotypes, such as reduced petal development and incomplete sepal closure, were more severe in \( fca^{-9} fy^{-5} \) than in \( fy^{-5} \) alone (Fig. 5E). The organization and appearance of epidermal sepal cells were also abnormal in \( fy^{-5} \) plants (Fig. 5, H and I). Cellular organization was more random in \( fy^{-5} \) and the cell surface showed a denser and more random arrangement of ridges (Fig. 5, H and I). Interestingly, a similar change in cell surface morphol-

![Figure 4](image_url)

**Figure 4.** \( fpa \) \( fy^{-5} \) double mutants are viable. A and B, \( fpa \) and \( fpa \) \( fy^{-5} \) plants grown under long days. \( fpa \) \( fy^{-5} \) plants show a loss in apical dominance and usually senesce without flowering in the absence of vernalization. C, Growth rate of Col (circles), \( fy \) (triangles), \( fpa \) (squares), and \( fpa \) \( fy \) (X) plants grown under long days. D, Bars represent the total number of rosette leaves formed prior to flowering under long days for Col (white), \( fy^{-5} \) (black), \( fpa \) (gray), and \( fpa \) \( fy^{-5} \) (crosshatched). V indicates days of cold treatment. Error bars indicate 1 SD. [See online article for color version of this figure.]

![Figure 5](image_url)

**Figure 5.** Effect of \( fy^{-5} \) on flowering time and floral development. A, Bars represent the total number of rosette leaves formed prior to flowering for the indicated genotypes alone (white) or with \( fy^{-5} \) (black). Error bars indicate 1 SD. B, Distribution of sepal (white bars) and petal (black bars) numbers in wild-type and \( fy^{-5} \) plants. C to E, Inflorescences of Col (C), \( fy^{-5} \) (D), and \( fca \) \( fy^{-5} \) (E). F and G, Scanning electron micrographs of developing flower buds in Col (F) or \( fy^{-5} \) (G). Scale bars represent 100 \( \mu m \). H and I, Scanning electron micrographs of sepal epidermis in Col (H) or \( fy^{-5} \) (I). Scale bars represent 10 \( \mu m \). All plants were grown under long days. [See online article for color version of this figure.]
ogy has been previously observed in other pleiotropic autonomous pathway double mutants (Veley and Michaels, 2008).

The interpretation of the phenotypes of fy mutant plants is complicated by the fact that fy null alleles are lethal (Henderson et al., 2005). Both fy-2 and fy-5 contain truncations of the 3’ end of the FY gene (Henderson et al., 2005; Fig. 3D). Truncations could affect the activity of FY in multiple ways, including a partial loss of function (e.g. a hypomorphic allele) or the creation of a protein with a novel function (e.g. a neomorphic allele). In an attempt to distinguish between these two possibilities, we created a genomic clone of FY, including the endogenous promoter sequences, that was truncated at a position near the T-DNA insertion in fy-5 and transformed the construct into Col and fy-5. If the truncation creates a neomorphic allele, it may act dominantly to delay flowering, whereas if the truncation produces a hypomorphic allele, it would be predicted to show no phenotype. Consistent with the later model, the truncated FY construct had no effect on flowering time in Col or fy-5 backgrounds (Fig. 6A). We also transformed a full-length FY genomic clone under control of its own promoter into fy-5. The majority of the T1 plants flowered earlier than fy-5, with many flowering similarly to Col (Fig. 6B). The fact that a wild-type FY genomic clone can rescue the late-flowering phenotype of the fy-5 mutant also suggests that the late-flowering phenotype of fy-5 is due to a partial loss of FY function and not due to a novel function imparted by the truncation. Finally, we transformed the full-length genomic FY construct into flk-4 fy-5 and fve-4 fy-5 double mutants, which flowered much later than flk-4 and fve-4 single mutants, and fca-9 fy-5 double mutants, which flower earlier than fca-9 (Fig. 3A). Consistent with the results in Col, the FY genomic clone restored earlier flowering in flk-4 fy-5 and fve-4 fy-5 and later flowering in fca-9 fy-5 (Fig. 6B). These experiments show that the flowering time effects of the fy-5 allele are recessive (Fig. 6, A and B). Nonetheless, it is interesting to note that FY transcript levels were significantly elevated in fy-5 (Fig. 6C). These results support the hypothesis that fy-5 is likely a hypomorphic allele and that, depending on genetic background, FY can act to either promote or repress FLC expression.

**FY Promotes Proximal Site Polyadenylation in FLC Sense Transcripts Independently of FCA**

Previous work has shown that FY promotes usage of a proximal polyadenylation site preference in FLC antisense transcripts (Liu et al., 2010). In the course of sequencing FLC cDNA clones, we noticed multiple polyadenylation sites. We therefore wondered if FY might also play a role in polyadenylation site selection in FLC sense transcripts. We performed 3′ RACE from Col and found two major products, a proximally polyadenylated form (191 bp 3′ of stop codon) and a distally polyadenylated form (250 bp 3′ of stop codon; Fig. 7, A and B). The two forms were present in approximately equal amounts (Fig. 7B). To determine if polyadenylation site selection might play a role in the regulation of FLC, we examined distal/proximal ratios in lines containing FRI or autonomous pathway mutations. In FRI, fca-9, fdl-3, flk-4, fpa-7, fve-4, or ld-1 backgrounds, distal/proximal ratios were similar to Col (Fig. 7B), thus in these backgrounds, increased FLC expression does not correlate with altered polyadenylation site selection. Interestingly, however, both fy-2 and fy-5 showed a significant decrease in the proximally polyadenylated form of FLC (Fig. 7B). Thus FY acts to promote polyadenylation at the proximal site in FLC sense transcripts.

One of the striking findings of our work is the result that, depending on the genetic background, fy muta-
functions can either increase or decrease FLC expression. We were therefore curious to determine if the effect of fy on polyadenylation site selection would be indicative of its effect on FLC expression. We determined the effect of fy-2 and fy-5 on distal/proximal ratios in a Col background (where fy-5 mutations increase FLC and delay flowering) and in fca-9 and FRI backgrounds (where fy-5 mutations reduce FLC expression and accelerate flowering). Interestingly, fy mutations had similar effects in all three genetic backgrounds (Fig. 7C). In Col, fca-9, or FRI, both fy-2 and fy-5 mutations caused an increase in usage of the distal polyadenylation site. It has recently been shown that antisense transcripts play a role in the regulation of FLC (Swiezewski et al., 2009). This raises the possibility that fy-2 and fy-5 may differ in their effect on FLC antisense RNA; however, no significant differences in FLC antisense transcripts were observed between fy-2 and fy-5 lines (Fig. 7D). Thus, despite the fact that fy-5 and fy-2 have significantly different effects on flowering time (Figs. 1–3), their effects on FLC polyadenylation are indistinguishable. Thus differential polyadenylation of FLC transcripts cannot explain the differences in FY allele behavior. We also investigated whether the proximal and distal polyadenylation variants might differ in their stability or nuclear export. When treated with the transcription inhibitor actinomycin D, no change was observed in distal/proximal ratio (Fig. 7E), suggesting that both forms have similar levels of turnover. Similarly, the ratio of the two forms of FLC transcript was similar in total RNA or nuclear RNA (Fig. 7F).

Taken together with published findings, our work indicates that FY plays both FCA-dependent and FCA-independent roles in the regulation of FLC expression. Previous work has shown that an FY FCA complex influences the alternative splicing of the FCA transcript. Because only the gamma form of FCA gives rise to a functional repressor of FLC, the role of FY in FCA splicing is critical in setting basal FLC levels. Here we show that FY can also affect FLC expression and polyadenylation site selection in the absence of FCA. Although analyses using weak alleles must be interpreted with caution, the genetic interactions between fy-5 and FRI or other autonomous pathway mutations are intriguing nonetheless. The observation that fy-5 alleles cause increased FLC expression in some genetic backgrounds and decreased expression in others also supports the model that FY plays multiple roles in the regulation of FLC. The characterization of fy-5 may also shed light on the evolution of FY function. Natural variation in the region of FY truncated in the fy-5 allele has been implicated in the regulation of flowering time in response to light quality (Adams et al., 2009). In the future it will be interesting to determine the molecular interactions that underlie the various epistatic relationships between FY, other members of the autonomous pathway, and FRI.

MATERIALS AND METHODS

Plant Materials and Growth Conditions

FRI-Col (Lee et al., 1994b), fca-9 (Bezerra et al., 2004), fld-3 (He et al., 2003), flk-4 (SALK_112850; Veley and Michaels, 2008), fpa-7 (Michaels and Amasino, 2001), fse-4 (Michaels and Amasino, 2001), fy-2 (Simpson et al., 2003), id-1 (Redei, 1962), and flc-3 (Michaels and Amasino, 1999) are in the Col genetic.
background and have been described previously: fp-5 (SALK_005697) and fca (SALK_057540) were obtained from the Arabidopsis Biological Resource Center. Plants were grown at 22°C under cool-white fluorescent lights with a light intensity of 100 µmJ m⁻² s⁻¹. Long days consisted of 16 h of light followed by 8 h of darkness. For vernalization, seedlings were sown on agar-solidified medium (2.2 g L⁻¹ Murashige and Skoog salts, 1% Suc, and 0.8% agar) and were kept at 4°C for the indicated periods of time.

RNA Expression Analysis

Total RNA isolation, reverse transcription (RT), semi-quantitative RT-PCR analysis (Michaels et al., 2004), and quantitative RT-PCR analysis (Mockler et al., 2004) were performed as described previously. Detection of the FLC antisense transcript was performed as described (Swiezedewski et al., 2009). Primer sequences are shown in Supplemental Table S2.

3′ RACE

M13-oligo(dT) primer (Supplemental Table S2) was used for RT. A 5-fold dilution of RT products was used as a template for 3′ RACE. Touchdown (TD) PCR was carried out using an M13 forward primer and an FLC-specific primer, 550-FLC-F (Supplemental Table S2). TD PCR conditions were: 94°C for 3 min; followed by 29 cycles of 94°C for 1 min, 56°C (with a per-cycle reduction of 0.5°C) for 2 min, 72°C (with an elevation per cycle 0.2°C) for 1 min; then nine cycles of 94°C for 1 min, 55°C for 2 min, and 72°C for 2 min; and concluded with 1 min of extension at 72°C for 7 min. TD PCR products were then diluted 5-fold for a second round of nested PCR using the M13 forward primer and a FLC 3′ UTR-specific primer, 1121-FLC-3. Nested PCR conditions were: 95°C for 5 min; followed by 27 cycles of 95°C for 30 s, 60°C for 30 s, 72°C for 2 min; and then final extension at 72°C for 7 min. PCR products were resolved on 2.5% agarose gel and quantified using ImageJ (Abramoff et al., 2004).

RNA Stability Assay

RNA stability assay was performed as described previously (Mockler et al., 2004). Briefly, 10-d-old FRI-Col seedlings were placed in tubes and immersed with transcription inhibitor solution containing 1 µM PIPES buffer (pH 7.0), 1 mM spermidine, 0.5 µM actinomycin D (Sigma). For the control group, FRI-Col seedlings were treated with the same buffer without actinomycin D. Samples were collected at the indicated time points up to 8 h.

Microscopy

Scanning electron microscopy was performed as described previously (Jacob et al., 2007).

Sequence data from this article can be found in EMBL/GenBank under the following accession numbers: FCA, AT1g16280; FLD, AT3g10390; FLK, AT3g104610; FPA, AT2g3410; FVE, AT2g19520; FY, AT5g13480; LD, AT4g02560; FRI, AT4g00650; CO, AT1g15840; FT, AT1g165480; SOC1, AT2g45660; FLC, AT5g10140; and UBQ, AT4g05320.

Supplemental Data

The following materials are available in the online version of this article.

Supplemental Table S1. FLC mRNA quantification.

Supplemental Table S2. PCR and quantitative PCR primers used in this study.

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