Genetic interactions of the Arabidopsis flowering time gene FCA, with genes regulating floral initiation

Tania Page1,†, Richard Macknight1,‡, Chang-Hsien Yang2 and Caroline Dean1,*

1Department of Molecular Genetics, John Innes Centre, Colney, Norwich NR4 7UH, UK, and
2Graduate Institute of Agricultural Biotechnology, National Chung Hsing University, Taichung, Taiwan 40227, Republic of China

Summary

The genes controlling the timing of the transition from vegetative to reproductive growth are likely candidates for regulators of genes initiating floral development. We have investigated the interaction of one particular gene controlling flowering time, FCA, with the meristem identity-genes TERMINAL FLOWER 1 (TFL1), APETALA 1 (AP1) and LEAFY (LFY) and the floral repression gene EMBRYONIC FLOWER 1 (EMF1). Double mutant combinations were generated and the phenotypes characterized. The influence of strong and intermediate 

Introduction

The control of the transition from vegetative to reproductive development is being extensively studied in Arabidopsis thaliana. Genes involved in controlling the timing of the floral transition have been identified through mutagenesis and analysis of natural variation (Koornneef et al., 1998b). Mutations that confer late flowering are considered to identify gene products whose wild-type function is to promote flowering. The flowering time of some of the late flowering mutants, including fca, fve, fy and ld is accelerated in response to a long period of cold temperature (vernalization) but it is further delayed in restrictive short day photoperiods. These genes are considered to act in the autonomous floral promotion pathway (Koornneef et al., 1991, 1998a; Martinez-Zapater and Somerville, 1990). The flowering time of other late flowering mutants, including co, gi, fwa, fd, fe, ft and fha is less responsive to both vernalization and photoperiod. These genes are considered to act in a genetic pathway controlling the long-day promotion of flowering (Koornneef et al., 1991, 1998a; Martinez-Zapater and Somerville, 1990).

A likely function of the genes controlling the timing of the floral transition is the regulation of genes involved in formation of a floral meristem (‘meristem-identity genes’). How this regulation might occur has been studied recently using different approaches. Analysis of double mutant combinations of the late flowering mutants fve, fpa, fwa and ft with the meristem-identity mutants leafy (lyf) and apetala1 (ap1) mutations led to the division of fwa and ft into a different functional group with respect to other flowering time genes (Ruiz-Garcia et al., 1997). fwa lyf and ft lyf double mutants did not produce any flower-like structures or AP1 RNA. FT and FWA were therefore proposed to act redundantly with LFY to activate AP1 and act as an intermediary step between some of the other floral promoters and floral meristem gene activation.

FWA and FT have also been found to control the competence to respond to LFY activity. Nilsson et al. (1998) analysed flowering time in 35S-LFY transgene lines carrying different late flowering mutations and GUS activity from a LFY-GUS transgene in the same set of late flowering mutant backgrounds (Nilsson et al., 1998). The data indicated that CO, GI, FCA, FVE and the genes involved in the gibberellin response pathway GA1 and GAI all play a role in LFY activation. Most of the genes also seem to be required for the function of LFY in that the flowering time of the late flowering mutant 35S-LFY line was intermediate between that of both parents. The situation was different, however, for fwa, fe and ft. The late flowering phenotype was epistatic to the early flowering conferred by the 35S-LFY transgene showing that FWA, FE and FT function in parallel with or downstream of LFY.

Activation of meristem-identity genes has also been studied by Simon et al. (1996) who used a glucocorticoid-inducible system to activate CO. Induction of CO was sufficient to induce flowering in SD and to initiate transcription of LFY and TFL1 as rapidly as when these genes are

Received 22 September 1998; revised 13 November 1998; accepted 20 November 1998.

*For correspondence (fax +44 1603 505725; e-mail caroline.dean@bbsrc.ac.uk).

†Present address: Horticulture Research International, Wellesbourne, Warwick CV35 9EF, UK.

‡Present address: Department of Biochemistry, University of Otago, Cumberland Street, Dunedin, New Zealand.
induced by LD photoperiods. AP1 transcription, however, was induced more slowly by CO compared to LD.

EMBRYONIC FLOWER (EMF)1 and EMF2 genes also regulate meristem-identity gene activation and are considered to function as a central regulator repressing the transition to flowering (Chenet et al., 1997; Sung et al., 1992). Strong emf1 alleles bypass vegetative development and form carpelloid structures immediately after germination. AP1 and AGAMOUS promoters are activated in germinating emf seedlings and constitutive expression of LFY in emf1 mutants increased the severity of the emf phenotype. A reciprocal negative regulation between the EMF genes and the meristem-identity genes has therefore been suggested (Chenet et al., 1997). The role of EMF as a component of the central floral repression pathway is supported by the observation that emf mutations are epistatic to gi and co (Yang et al., 1995).

In order to further dissect the relationship between meristem-identity and flowering time genes, we have analysed the interaction of FCA with genes involved in the formation of a floral meristem. Mutations in the FCA gene strongly delay the floral transition resulting in plants with much larger numbers of rosette leaves and coflorescences. The increased vegetative phase is fully reversed when fca mutant plants are vernalized (Koornneef et al., 1991). X-ray induced fca mutant sectors did not affect bolting or flowering, suggesting that FCA may act non-cell autonomously (Furner et al., 1996). FCA encodes an RNA-binding protein with a WW protein interaction domain and the FCA transcript is alternatively spliced with only one form encoding the entire FCA protein (Macknight et al., 1997). It appears, therefore, that FCA is a component of a post-transcriptional cascade regulating flowering time. A series of double mutants between fca and mutations affecting floral initiation have been made, using multiple combinations of different alleles wherever practicable. The phenotypes of the double mutants have been analysed with respect to flowering time and inflorescence phenotype.

Results

Effect of fca mutations on the floral transition

Mutations in the FCA gene significantly delay the transition from vegetative to floral development. The six mutant alleles (fca 1–6) that have previously been described (all in the Landsberg erecta (Ler) background) show a range of flowering times (Koornneef et al., 1991). Two of the Ler fca alleles, fca-1 (one of the strongest alleles) and fca-4 (an intermediate allele), were used in the double mutant analysis. In addition, fca-9 (a Columbia (Col) allele) was used. The availability of mutant alleles in both ecotypes meant that double mutants could be made without mixing backgrounds, thus avoiding any complications of modifier genes affecting the phenotype of the double mutant. In addition, observation of similar interactions between mutant alleles in different backgrounds strengthens the conclusions that can be made.

fca-1 and fca-4 (the Ler mutant alleles) and the wild-type Ler parent were grown in a controlled environment cabinet (used to grow the majority of the double mutants) in which the plants were exposed to a long-day light regime of 10 h high intensity light, followed by a 6 h low intensity day extension. fca-4 flowered with an average of 23 total (rosette + cauline) leaves compared to 29 for fca-1 and 7 for Ler (Table 1). Both mutations significantly increased the number of coflorescences (the lateral inflorescences arising from the main inflorescence, subtended by a cauline leaf) with fca-1 showing a stronger effect than fca-4. The number of floral nodes was also significantly increased with both mutants producing approximately 50 as compared to 23 in wild-type Ler plants. fca-9 flowered with approximately 37 rosette leaves and produced about eight coflorescence nodes under greenhouse conditions (Table 1).

The strong fca-1 allele carries a single nucleotide substitution (C to T) which introduces a stop codon within exon 13 (Macknight et al., 1997) and production of a truncated FCA protein. The intermediate fca-4 allele is the result of a large inversion that disrupts the FCA gene at the 3’ end of exon 4 (C. Lister and C. Dean, unpublished results). The 3’ fragment is expressed as a translational fusion in fca-4 as determined by the presence of a polypeptide of 84 kDa (16 kDa larger than predicted to be encoded from the 3’ FCA fragment) on Western blots probed with an antibody specific to the C-terminal half of the FCA protein (P Dijkwel and C. Dean, unpublished results). The resulting polypeptide, which is expressed at approximately the same level as wild-type FCA protein, would contain the second RNA-binding domain and the C-terminal region of the protein including the WW protein interaction domain (Macknight et al., 1997). In comparison with a mutation in another strong allele (fca-6) that terminates the open reading frame very close to the break point in fca-4, we deduce that production of this partial protein accounts for the intermediate flowering time phenotype.

fca-9 (Col) flowers much later than fca-1 (Ler) when grown side by side (C. Dean, unpublished results). The Columbia ecotype contains dominant alleles at FLC shown to enhance the late flowering phenotype of fca mutations (Sanda and Amasino, 1996) so it is difficult to directly compare the relative strengths of the fca-1 and fca-9 mutations. Our current analysis shows that fca-9 produces a truncated protein shorter than that in fca-1, indicating that fca-9 is a strong mutant allele (C. Lister and C. Dean, unpublished results).

Combination of fca and tfl1 mutations

Mutations in the TFL1 gene result in conversion of the indeterminate apical meristem into a determinate floral
grown in controlled environment rooms whereas wild-type and mutant alleles in Columbia were grown under greenhouse conditions.

The number of floral nodes on the main inflorescence of the double mutants was later than the respective fca parents. Coflorescence and cauline leaf number on the main inflorescence, not including secondary inflorescences subtended by rosette leaves.

The role of FCA in floral initiation as promoted by LFY was examined. LFY acts in combination with AP1 and other genes to promote floral meristem identity. In addition, LFY and the coflorescence number in the double mutant was significantly delayed. Where analysed, short day and the formation of the terminal flower was significantly delayed.

The flowering time, leaf number, coflorescence and floral node number of different Arabidopsis genotypes are shown in Table 1. *fca-1 tfl1–2* (Figure 1b) and *fca-4 tfl1–2* was later than in the *tfl1–2* mutant. Production of the terminal flower was significantly delayed and the coflorescence number in the double mutant was 18-fold greater than in *tfl1–1*. Where analysed, short day photoperiod effects on the *tfl1–2* phenotype were additive with those of *fca* mutations (data not shown).

All the *fca* alleles therefore showed a similar interaction with the different *tfl1* alleles. The late flowering phenotype was epistatic to the early flowering conferred by *tfl1* alleles and the formation of the terminal flower was significantly delayed.

**Combination of fca and ify mutations**

The flowering time, leaf number, coflorescence and floral node number of different Arabidopsis genotypes are shown in Table 1.

<table>
<thead>
<tr>
<th>Flowing time</th>
<th>Rosette leaves</th>
<th>Cauline leaves</th>
<th>Coflorescence no.</th>
<th>Floral nodes</th>
<th>No. of plants</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ler</td>
<td>22.9 ± 0.1</td>
<td>4.4 ± 0.3</td>
<td>2.4 ± 0.2</td>
<td>2.4 ± 0.2</td>
<td>22.9 ± 2.1</td>
</tr>
<tr>
<td>fca-1</td>
<td>47.5 ± 1.2</td>
<td>20.9 ± 0.8</td>
<td>7.8 ± 0.5</td>
<td>7.9 ± 0.5</td>
<td>51.9 ± 1.7</td>
</tr>
<tr>
<td>fca-4</td>
<td>42.9 ± 0.5</td>
<td>16.7 ± 0.5</td>
<td>6.2 ± 0.3</td>
<td>6.3 ± 0.3</td>
<td>48.5 ± 1.6</td>
</tr>
<tr>
<td>tfl1–2</td>
<td>21.1 ± 0.1</td>
<td>3.7 ± 0.2</td>
<td>2.1 ± 0.1</td>
<td>0.8 ± 0.4</td>
<td>5.0 ± 0.4</td>
</tr>
<tr>
<td>fca–1 tfl1–2</td>
<td>47.0 ± 0.9</td>
<td>24.0 ± 1.0b</td>
<td>8.9 ± 0.5</td>
<td>9.3 ± 0.5</td>
<td>33.9 ± 0.8</td>
</tr>
<tr>
<td>fca–4 tfl1–2</td>
<td>43.4 ± 0.9</td>
<td>19.0 ± 1.1</td>
<td>5.6 ± 0.4</td>
<td>5.6 ± 0.4</td>
<td>30.6 ± 1.5</td>
</tr>
<tr>
<td>ify–5</td>
<td>22.7 ± 0.2</td>
<td>5.5 ± 0.2a</td>
<td>5.6 ± 0.3</td>
<td>6.0 ± 0.4</td>
<td>26.3 ± 3.1</td>
</tr>
<tr>
<td>fca–1 ify–5</td>
<td>42.4 ± 0.8</td>
<td>22.9 ± 0.8a</td>
<td>17.0 ± 0.8</td>
<td>20.8 ± 0.8</td>
<td>39.5 ± 1.4</td>
</tr>
<tr>
<td>fca–4 ify–5</td>
<td>41.9 ± 1.3</td>
<td>18.0 ± 1.2</td>
<td>14.4 ± 1.2</td>
<td>21.2 ± 1.8</td>
<td>33.3 ± 1.8</td>
</tr>
<tr>
<td>ify–6</td>
<td>38.0 ± 1.1</td>
<td>6.3 ± 0.4a</td>
<td>7.8 ± 0.7</td>
<td>7.4 ± 0.6</td>
<td>13.0 ± 0.6</td>
</tr>
<tr>
<td>fca–1 ify–6</td>
<td>59.1 ± 1.5</td>
<td>23.0 ± 0.1b</td>
<td>42.5 ± 5.5</td>
<td>45.5 ± 4.5</td>
<td>28.5 ± 2.5</td>
</tr>
<tr>
<td>fca–4 ify–6</td>
<td>58.8 ± 2.3</td>
<td>24.5 ± 3.3a</td>
<td>42.9 ± 1.9</td>
<td>37.6 ± 1.9</td>
<td>21.6 ± 1.7</td>
</tr>
<tr>
<td>ap1–1</td>
<td>21.1 ± 0.1</td>
<td>3.2 ± 0.2a</td>
<td>2.5 ± 0.2</td>
<td>4.4 ± 0.2</td>
<td>25.0 ± 2.1</td>
</tr>
<tr>
<td>fca–1 ap1–1</td>
<td>47.1 ± 2.1</td>
<td>22.9 ± 2.1</td>
<td>8.6 ± 0.8</td>
<td>18.1 ± 2.8</td>
<td>34.6 ± 3.5</td>
</tr>
<tr>
<td>fca–4 ap1–1</td>
<td>51.0 ± 0.5</td>
<td>27.4 ± 0.4c</td>
<td>9.1 ± 0.6</td>
<td>22.4 ± 1.0</td>
<td>19.8 ± 1.9</td>
</tr>
<tr>
<td>Col</td>
<td>22.4 ± 2.1</td>
<td>11.2 ± 1.8</td>
<td>2.2 ± 0.4</td>
<td>2.2 ± 1.4</td>
<td>&gt; 35</td>
</tr>
<tr>
<td>fca–9</td>
<td>58.5 ± 7.8</td>
<td>37.6 ± 5.3</td>
<td>8.4 ± 1.3</td>
<td>8.4 ± 1.3</td>
<td>&gt; 35</td>
</tr>
<tr>
<td>tfl1–1</td>
<td>18.4 ± 1.2</td>
<td>5.3 ± 1.0d</td>
<td>0.3 ± 0.4</td>
<td>0.3 ± 0.4</td>
<td>3.2 ± 0.9</td>
</tr>
<tr>
<td>fca–9 tfl1–1</td>
<td>54.8 ± 10.3</td>
<td>35.3 ± 6.9</td>
<td>5.4 ± 0.9</td>
<td>5.4 ± 0.9</td>
<td>18.6 ± 7.4</td>
</tr>
<tr>
<td>ify–2</td>
<td>21.3 ± 1.9</td>
<td>8.8 ± 0.9</td>
<td>7.3 ± 1.1</td>
<td>7.3 ± 1.1</td>
<td>15</td>
</tr>
<tr>
<td>ify–10</td>
<td>23.3 ± 2.0</td>
<td>7.8 ± 1.1</td>
<td>7.3 ± 1.3</td>
<td>7.3 ± 1.3</td>
<td>15</td>
</tr>
<tr>
<td>fca–9 ify–2</td>
<td>56.7 ± 9.4</td>
<td>38.2 ± 7.1</td>
<td>40.9 ± 7.9</td>
<td>40.9 ± 7.9</td>
<td>15</td>
</tr>
<tr>
<td>fca–9 ify–10</td>
<td>62.2 ± 7.4</td>
<td>44.8 ± 6.4</td>
<td>40.5 ± 4.9</td>
<td>40.5 ± 4.9</td>
<td>15</td>
</tr>
<tr>
<td>ap1–3</td>
<td>21.6 ± 1.6</td>
<td>7.7 ± 1.1</td>
<td>2.4 ± 0.5</td>
<td>2.4 ± 0.5</td>
<td>15</td>
</tr>
<tr>
<td>fca–9 ap1–3</td>
<td>53.8 ± 7.9</td>
<td>33.1 ± 4.3</td>
<td>7.8 ± 1.4</td>
<td>7.8 ± 1.4</td>
<td>15</td>
</tr>
</tbody>
</table>

*Significantly different from Landsberg erecta parent (all significance tests done at 0.05 level). aSignificantly different from fca-1. bSignificantly different from fca-4. cSignificantly different from Columbia parent. Wild-type and mutant alleles in Landsberg erecta were grown in controlled environment rooms whereas wild-type and mutant alleles in Columbia were grown under greenhouse conditions.

dLateral inflorescences arising from the main inflorescence, not including secondary inflorescences subtended by rosette leaves.

Figure 1. Phenotypes of double mutant combinations.

(a) Wild-type Ler; (b) fca-1 tfl1–2 in LD; (c) fca-1 lfy-5 in LD; (d) fca-1 ap1–1 in LD; (e) apex of wild-type Ler plant in LD; (f) apex of fca-4 tfl1–2 in LD; (g) apex of fca-1 lfy-6 in LD; (h) apex of fca-1 ap1–1 in LD; (i) fca-9 emf1–1 25 d after germination; (j) fca-9 emf1–1, showing trichomes on the adaxial (upper) surface of the sessile leaves; (k) fca-9 emf1–1 40 d after germination; and (l) fca-9 emf1–2 25 d after germination.

functions in the formation of normal flowers as ify mutants form flower-like structures that lack petals and stamens (Huala and Sussex, 1992; Weigel et al., 1992). LFY is considered to act as a link between the timing of the floral transition and the initiation of individual flowers (Blazquez et al., 1997). Double mutant combinations of fca-1 lfy-5 (a weak allele), fca-1 lfy-6 (a strong allele), fca-4 lfy-5 and fca-4 lfy-6 were generated and analysed in the LD controlled growth cabinet. fca-9 in combination with lfy-2 and lfy-10 (both weak alleles) were analysed under greenhouse conditions (Table 1). Ify mutations increased rosette leaf number at flowering in the single and in all the double mutant combinations. The combination of lfy-6 with fca-4 resulted in a strong delay in flowering with lfy-6 fca-4 plants producing nine leaves more than the fca-4 parent. In many fca and ify combinations there was a large (fivefold) increase in the number of coflorescences formed (Figure 1c). Thus, production of nodes resulting in the typical ify flower-like structure was significantly delayed in fca mutants. Grown side by side in the LD cabinet, the phenotype of an fca-1 lfy-5 double mutant was indistinguishable from that of a co2 ify-5 double mutant (Simon and Coupland, 1996). One feature of the fca-1 lfy-6 double mutant was the lack of secondary inflorescences produced compared to fca-1 (Figure 1c). Where analysed, the effects of short day photoperiods were additive to the effects of fca mutations (data not shown).

The individual flowers of the ify fca double mutants closely resembled the flowers of the respective ify parent (Figure 1g) (Weigel et al., 1992). The flowers of the Ify 5 fca double mutants were fertile, unlike flowers of the strong allele Ify-6, showing that any enhancement of the Ify5 floral phenotype by fca mutations was only moderate. Grown under greenhouse conditions, fca-9 ify-2 and fca-9 ify-10
flowers appeared to show a slightly more extreme *lfy* phenotype than their respective *lfy* parent. Petals and stamens were absent, carpels were unfused and the plants were sterile.

In summary, *fca* alleles enhanced various aspects of the *lfy* phenotype, most notably a large increase in the number of shoot-like structures on the inflorescence. As this enhancement is seen with null *lfy* mutations, the effects of the *fca* mutations cannot be due to reduction of residual LFY activity. Combination of *fca* and *lfy* alleles caused the plants to flower even later, with the greatest delay caused by *lfy-6* in an *fca-4* background.

Combination of *fca* and *ap1* mutations

In order to analyse the interaction of *FCA* and *AP1*, different combinations of *fca* mutations with *ap1* alleles (Bowman et al., 1993; Mandel et al., 1992) were generated. The strong *ap1–1* allele was combined with *fca-1* and *fca-4*. The relatively weak allele, *ap1–3*, was combined with *fca-9*. In the LD cabinet conditions, the *ap1–1* mutant flowered earlier and with fewer rosette leaves than the *Ler* parent (in agreement with Schultz and Haughn, 1993). The *fca-1 ap1–1* double mutant plants flowered at a similar time to the *fca-1* parent, with a similar number of rosette leaves (Figure 1d), but the *fca-4 ap1–1* plants flowered considerably later than *fca-4* (27.4 versus 16.7 leaves). Under greenhouse conditions *ap1–3* flowered significantly earlier than the *Col* parent but the *fca-9 ap1–3* double mutant flowered with approximately the same number of leaves as *fca-9*.

The number of cauline leaves and floral nodes were unchanged in *ap1–1* in the LD conditions relative to *Ler*, but the number of coflorescences increased almost two-fold. The coflorescence number was approximately two- and 3.5-fold greater in the double mutants *fca-1 ap1–1*, *fca-4 ap1–1* compared to the respective *fca-1*, *fca-4* parents. The number of floral nodes was greatly reduced in *fca-4 ap1–1*.

The phenotypic abnormalities of the individual flowers were similar in the single and double mutants. The characteristic determinate branched flowers previously described for *ap1–1* mutations (Bowman et al., 1993) were present and these formed a dense mass of floral-like structures at the inflorescence apex in the late flowering backgrounds (Figure 1h). The fertility of the *fca-9 ap1–3* double mutant was slightly reduced under greenhouse conditions compared to its parents.

In summary, all *fca* alleles enhanced the *ap1* inflorescence phenotype but did not significantly affect floral development in the *ap1* flowers. As *ap1–1* is a strong allele this suggests that *FCA* functions to promote the formation of flowers in pathways that act redundantly with *AP1*. The early flowering phenotype of *ap1–1* mutations indicates that *AP1* functions as a repressor of the floral transition and the epistasis of the late flowering phenotype of *fca-1* over the early flowering of *ap1–1* suggests that it acts via inhibiting *FCA* function. In contrast to the early flowering conferred by *ap1–1* in an *Ler* background, *ap1–1* produced a significant delay in flowering time in an *fca-4* background.

Analysis of *FCA* RNA abundance and splicing in *tfl1–2* and *ap1–1* mutants

One interpretation of the epistasis with respect to flowering time of *fca* mutations in *fca tfl1* and *fca ap1* combinations is that *TFL1* and *AP1* negatively regulate *FCA* function. This could be through modulation of *FCA* expression or through a downstream antagonistic action. The *FCA* gene is alternatively spliced and four different *FCA* transcripts have been characterized (Macknight et al., 1997). Transgenic plants carrying fusions where the *FCA* gene is driven by the strong, constitutive 35S cauliflower mosaic virus promoter accumulate a short *FCA* transcript, termed transcript β, which does not encode a full-length functional *FCA* protein (Macknight et al., 1997). It is possible, therefore, that the splicing of the *FCA* gene could be regulated to limit *FCA* protein levels. We investigated whether the levels of *FCA* or the splicing of the *FCA* transcript were altered in *tfl1–2* or *ap1–1* mutants. RNase protection experiments were used to assay the two major *FCA* transcripts, transcript β which constitutes approximately 55% of the *FCA* message in wild-type plants and transcript γ, which constitutes approximately 35% of the transcripts and is the transcript that would encode the full-length *FCA* protein (Macknight et al., 1997). The level and ratio of the *FCA* transcripts was found to be the same in RNA from *ap1–1*, *tfl1–2* and *Ler* seedlings (Figure 2). Thus, the repressive effects of *AP1*...
Figure 3. Model showing possible interactions of FCA with meristem-identity genes. EMF1 is shown negatively regulating AP1 based on the results of Chen et al. (1997). Later functions of TFL1 are not included. This is based on the hypothesis that fca-4 produces a product that is active in only a subset of pathways in which wild-type FCA functions. The model needs to be tested by introduction of transgenes expressing truncated FCA proteins into different genetic backgrounds.

and TFL1 on FCA function that influence the timing of the floral transition do not appear to act through changes in the gross levels or splicing of the FCA message.

Combination of fca-9 with emf1 alleles

Mutations in the EMF1 gene result in flowering in the absence of an obvious vegetative phase (Sung et al., 1992). Plants carrying the weak allele emf1–1 produce just a small inflorescence with cauline leaves and one or two flowers with incomplete floral organ development. Plants carrying emf1–2 show a much stronger phenotype and produce only carpelloid structures, capped with stigmatic papillae. To assess the interaction of EMF1 and FCA, fca-9 emf1–1 and fca-9 emf1–2 double mutants were generated (only the Col fca allele was used to avoid mixing genetic backgrounds which was a complication in the previous analysis (Haung and Yang, 1998). fca-9 emf1–1 double mutants displayed the emf1 phenotype during early development, for example, small shoot size, sessile cotyledons and leaves, short hypocotyl and oval-shaped cotyledons. However, the majority of the fca-9 emf1–1 double mutants did not flower and produced significantly more sessile leaves (> 10) than the emf1–1 single mutant (Figure 1i–k) before senescing. Among those double mutants which did flower, the flowering time was delayed, the number of sessile leaves was higher (approximately 10), and several flower-like structures with multiple pistils were produced (Figure 1k). Thus, the early appearance of flowers in emf1–1 is dependent on FCA function. The sessile leaves carried trichomes on both surfaces, although the density was much higher on the adaxial (upper) surface (Figure 1j). The production of leaves with trichomes on both surfaces is a characteristic of leaves produced in the adult phase of vegetative development (Telfer et al., 1997). emf1–2 fca-9 double mutants showed the early emf1–2 phenotype with small, sessile cotyledons, although later in development the presence of the fca-9 allele caused a significant reduction in the extent of the carpelloid structures produced (Figure 1l). Thus, loss of FCA function delayed the formation of reproductive structures in both emf1 alleles.

Table 2. Effect of a 35S LFY transgene on flowering time of Ler, fca-1 and fca-4

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Total leaf number at flowering</th>
</tr>
</thead>
<tbody>
<tr>
<td>non-transgenic control</td>
<td>35S-LFY</td>
</tr>
<tr>
<td>Ler</td>
<td>11.6 ± 0.2</td>
</tr>
<tr>
<td>fca-1</td>
<td>39.8 ± 0.9</td>
</tr>
<tr>
<td>fca-4</td>
<td>24.6 ± 0.4</td>
</tr>
</tbody>
</table>

Values are means ± standard errors from populations of between 15 and 40 plants. Plants were grown in similar but not identical controlled environment conditions to those shown in Table 1, thus the relative flowering times are the same but the absolute values are different.

The effect of fca mutations on the acceleration of flowering conferred by 35S-LEAFY

The putative roles of FCA downstream of TFL1 and AP1, and the requirement for FCA for early flowering of emf1 mutants, prompted us to look at the effect of fca mutations on the acceleration of flowering conferred by a 35S-LFY transgene. Ectopic expression of LFY results in an acceleration of the transition to flowering and all lateral shoots being converted into flowers (Weigel and Nilsson, 1995). A transgenic line (in Ler and therefore avoiding any complications due to segregation of modifiers) carrying the 35S-LFY transgene was crossed to fca-1 and fca-4 and the leaf number at flowering scored in plants doubly homozygous for the mutation and the transgene (Table 2). The leaf number of fca-1 35S-LFY was intermediate with that of both parents (agreeing with data from Nilsson et al., 1998), whereas leaf number of fca-4 35S-LFY was not significantly different to fca-4. The fca-1 35S-LFY and fca-4 35S-LFY lines showed the conversion of axillary nodes into flowers, the shortening of the inflorescence, the terminal flower and the twisting and curling of the leaves typical of the parent 35S-LFY line. However, even in the
presence of the 35S-LFY transgene, fca mutations delay the floral transition at the apex and the two fca alleles show different interactions.

Discussion

The interaction of FCA with TFL1, LFY, AP1 and EMF1 has been analysed with respect to effects on inflorescence and flower formation and changes in flowering time. With respect to inflorescence formation, fca mutations showed non-additive interactions with tf1, Ify and ap1 mutations. Formation of a terminal flower in the fca tf1 combinations was significantly delayed, demonstrating that FCA function is required for the early transition of the apex into a determined floral meristem. fca mutations also enhanced the number of coflorescence nodes in Ify and ap1 backgrounds. This enhancement occurs in a background where LFY or AP1 activity is absent, suggesting that FCA is required for the activation or function of the genes that act redundantly with LFY or AP1 to affect the initiation of flowers. This interaction is very similar to that described for FVE and FPA (Ruiz-García et al., 1997). There was very little effect of fca mutations on flower structure.

Analysis of the flowering time of the different double mutant combinations yielded informative interactions. In Ler, AP1 appears to repress the floral transition as ap1–1 mutant plants (both in Ler and Col) flowered earlier and with fewer leaves than wild-type, in agreement with Schultz and Haughn (1993). In contrast, ap1–1 fca-1 combinations flowered as late as fca-1. Why ap1–1 mutants flower early is unclear as AP1 overexpression resulting from a 35S-AP1 transgene results in early flowering (Mandel and Yanofsky, 1995) and expression of AP1 appears to be an indicator of floral determination (Hempel et al., 1997). It is possible that AP1 is expressed at very low levels early in development and it is this activity that acts to repress flowering. Whatever the mechanism, the early flowering phenotype of ap1–1 requires FCA which suggests that AP1 may repress the floral transition through a repressive effect on FCA function.

The late flowering phenotype of fca was also found to be epistatic to the early flowering of tf1 alleles. As with AP1, this would indicate that TFL1 acts in the same genetic pathway as FCA and represses FCA function. Loss of TFL1 activity would result in earlier flowering due to decreased repression of FCA function. A similar interaction has been found between tf1–2 and mutant alleles of FVE and FPA (Ruiz-García et al., 1997). In contrast, the double mutant phenotype of tf1 and mutant alleles of FT, FWA and SIN1 is considered to be additive (Ray et al., 1996; Ruiz-García et al., 1997), the double mutant phenotype flowering slightly but significantly earlier than the late flowering parent. This then places FT, FWA and SIN1 in a different floral promotion pathway to FCA, FVE and FPA. These results are consistent with previous divisions of FCA, FVE and FPA as part of the autonomous floral promotion pathway, and FT and FWA as part of the long day promotion pathway (Koornneef et al., 1998a). How TFL1 functions in the autonomous promotion pathway remains to be established. The lack of detectable change in FCA transcript level and splicing suggests that the repression on FCA function is not through a direct effect on FCA transcription or splicing. FCA RNA has been detected throughout the plant and at different developmental stages (Macknight et al., 1997). TFL1 expression was detected in subapical meristematic cells and throughout the inflorescence stem (Bradley et al., 1997). The expression patterns could indicate that TFL1 function acts to antagonize the promotive effects of FCA in meristematic cells.

The early flowering of emf1 mutants was also affected by the lack of FCA function. However, in contrast to the epistasis observed with ap1 and tf1, emf1–1 fca-9 double mutants showed a phenotype that could be interpreted as additive to that of both parents. The early emf1 character-istics were present but many more leaf-like structures were produced before floral-like structures appeared in a proportion of the double mutants. This phenotype was more extreme than that found in emf1–1 fca-1 double mutants (Haung and Yang, 1998) where 5–6 sessile leaves were produced. The attenuated phenotype of emf1 fca-1 as compared to emf1 fca-9 is probably the result of the combination of modifiers inherited from the Ler and Col parents used in the cross. However, both cases show that FCA function is required for the early transition to reproductive development in emf1 mutants, suggesting that FCA operates in an independent pathway to EMF1. emf1–1 gi-3 and emf1–1 co double mutants showed the emf1–1 phenotype (Haung and Yang, 1998; Yang et al., 1995). In contrast, the combination of fwa and fld-2 with emf1 alleles produced plants with more sessile leaves than fca emf1 1 double mutants (Chou and Yang, 1998; Haung and Yang, 1998). EMF1 would appear, therefore, not to act in a central repression pathway to flowering but acts as a repressor within the long-day promotion pathway, with GI and CO functioning as upstream repressors of EMF1. The similar phenotype of emf1 in long and short day photoperiods would support placing EMF1 in the CO, GI pathway (Bai and Sung, 1995).

In some of the interactions, fca-1 and fca-9 showed different effects to fca-4. fca-4 ify-6 and fca-4 ap1–1 fwerenow significantly later than fca-4, whereas fca-1 ify-6, fca-1 ap1–1 and fca-9 ify-2, fca-9 ap1–3 flowered at about the same time as fca-1 and fca-9, respectively. LFY and AP1 function must therefore still be acting to promote flowering in fca-4. A 35S-LFY transgene caused accelerated flowering in fca-1 but not in fca-4 such that 35S-LFY fca-4 flowered at the same time as 35S-LFY fca-1. The molecular basis of the intermediate phenotype conferred by fca-4 is unknown. The fca-4 allele does not produce one of the FCA transcript
forms, δ or the protein isoform derived from it. In addition, a truncated FCA polypeptide is made consisting of a translational fusion to an unknown protein of the second of the two RNA-binding domains, the glutamine-rich C terminus and the WW protein interaction domain (C. Lister, P. Dijkwel and C. Dean, unpublished results). This chimaeric protein does not appear to create a gain of function or dominant negative allele as fca-4 is recessive. The fca-4 allele may produce an FCA protein which functions to activate the same targets as wild-type but with reduced activity. If these targets were LFY and AP1, this would lead to partial activation of LFY and AP1 explaining the lateness in fca-4 ify or ap1 combinations. However, this would not account for the late flowering conferred by fca-4 being epistatic to the early flowering from the 35S-LFY transgene. If FCA acted downstream of LFY and AP1, the late flowering of the strong fca-1 allele would be epistatic to the early flowering from the 35S-LFY transgene rather than additive. A third possibility is that FCA acts in a parallel pathway to LFY and AP1. However, it would then be hard to explain the different interactions of strong ify and ap1 mutations with the fca-1 and fca-4 alleles.

A model that can account for all the results on interactions affecting flowering time and inflorescence formation is one in which FCA functions in a parallel pathway to LFY and AP1 as well as in the activation of LFY and AP1 (Figure 3). Strong fca alleles, for example fca-1 and fca-9, would represent loss of both pathways, whereas the partial activity in fca-4 would represent loss of the parallel pathway only. Activation of LFY and AP1 would still occur in fca-4, which would explain why ify and ap1 mutations result in later flowering in an fca-4 background. The similar flowering times of 35S-LFY fca-4 and 35S LFY fca-1 genotypes would indicate that the loss of the parallel pathway could not be compensated for by overexpression of LFY. This model is heavily dependent on the interpretation of the results from the rather unusual allele, fca-4, and therefore needs to be tested and elaborated by introduction of transgenes expressing truncated FCA proteins into different genetic backgrounds. It does have the attraction that it may provide an explanation for the presence of the multiple transcripts and FCA protein isoforms in wild-type plants. Further analysis is required to determine if the multiple FCA isoforms produced in wild-type plants are functionally important.

Experimental procedures

Plant lines

Landsberg erecta, fca-1, fca-4 (Ler alleles) were obtained from Prof. M. Koornneef. ify-1, ap1-1 (Ler alleles) were from the Nottingham stock centre. ify-5 and ify-6 (Ler alleles) and the 35S-LFY transgenic line DW191.2.5 (in Ler) were obtained from Dr D. Weigel. tfl1–1, ify-2, ify-10, ap1–3 (Col alleles) were from the Ohio stock centre. fca-9, an fca allele in the Columbia background, was isolated by C.-H.Y. whilst in Dr R. Sung’s laboratory (University of California, Berkeley, USA). Double mutant lines were isolated from F2 populations generated by cross-pollinating parental lines homozygous for individual mutations. In the case of the emf1 and ify-6 mutants, heterozygous lines were used as mutant parents. Double mutants were easily identified segregating in the expected 1/16th ratio in the F2 progeny. A line showing a typical double mutant phenotype (as compared to the other double mutants segregating in the F2) was selfed and F3 seed collected. Flowering time, leaf number, coflorescence and floral node number were scored on F3 individuals grown alongside relevant controls. For fca ify-6 and fca emf1, where the double mutant plant was sterile, F2 individuals homozygous for fca and heterozygous for either ify 6 or emf1 were selfed and the phenotype was scored on the 1/4 of the progeny that were homozygous for both mutations. The plants were regularly moved around in the growth room to minimize effects of microenvironment in the chamber.

Growth conditions

Plants were grown in soil (mixture of Levingtons M3 compost with grit) either in a greenhouse (for fca-9 analyses) or a Sanyo Gallenkamp controlled environment room under short day or extended short day conditions (for the fca-1, fca-4 analyses). Light conditions for the short-day room were 10 h illumination by 400 W Woton metal halide power star lamps, PAR 113.7 µmol m–2 sec–1 and an R/FR ratio of 2.41. Light for extended short-days was as for short-days (10 h) followed by 8 h illumination with tungsten halide lamps only, PAR 14.27 µmol m–2 sec–1, and an R/FR ratio of 0.66. Plants were transferred to partitioned trays at about the four-leaf stage.

RNase protection assays

RNA isolation and RNase protection assays for FCA transcripts β and γ were performed as described in Macknight et al. (1997).

Acknowledgements

This work was funded through the BBSRC CSG grant to the JIC, BBSRC Plant Molecular Biology II programme and EC Bridge grant (BIOT CT 90–0207). C.-H.Y. was supported by a grant from the National Science Council, Taiwan, Republic of China, grant number NSC 85–2321-B-005–076. The authors thank Dr Detlef Weigel for transgenic lines and extremely useful discussion. They also thank Drs Jose Martinez-Zapater, George Coupland and Gordon Simpson for comments on the manuscript.

References


