**SHORT INTEGUMENT (SIN1), a gene required for ovule development in Arabidopsis, also controls flowering time**

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**SUMMARY**

The short integument (sin1) mutation causes a female-specific infertility, and a defect in the control of time to flowering in Arabidopsis. Female sterility of Sin-1 plants is due to abnormal ovule integument development and aberrant differentiation of the megagametophyte in a subset of ovules. An additional defect of sin1 mutants is the production of an increased number of vegetative leaf and inflorescence primordia leading to delayed flowering. The delayed flowering phenotype of sin1-1 is not due to a defect in the perception of day length periodicity or in gibberellic acid metabolism. Phenotypes of double mutant combinations of sin1 with terminal flower (tfl1) indicate that SIN1 activity is required for precocious floral induction typical in a tfl1 mutant. Unexpectedly, sin1-1 tfl1-1 plants do not make pollen, thus revealing a novel role for TFL1 in the anther. Early flowers of sin1-1 ap1-1 double mutants are transformed to long inflorescence-like shoots. A genetic model for the role of SIN1 in flowering time control is proposed.

Key words: Arabidopsis, meristem, flowering time, ovule, short integument

**INTRODUCTION**

The form of higher plants is governed by temporal expression of a morphogenetic program that interacts with the environment. In the apical region of the shoot, a set of undifferentiated and rapidly dividing cells, meristem, constitutes a developmental machine that by division and differentiation generates a series of anlagen destined to make specialized organs such as leaves, inflorescence and, finally, flowers. These organs appear in a precise temporal and spatial order in the flanks of dividing meristem cells (Poethig, 1990). The genetic regulation of meristem phase change leading ultimately to flowering is a central problem in plant biology. In Arabidopsis thaliana, meristem development progresses through at least three distinct phases: from vegetative (V) through inflorescence (I) to the floral (F) mode (V —› I —› F switch). In Arabidopsis this switch is under multiple genetic controls and responds to day length and temperature (Coupland, 1995; Lee et al., 1994; Martinez-Zapater et al., 1994; Sung et al., 1992; Weigel, 1995; Wilson et al., 1992; Yang et al., 1995; Zagotta et al., 1992). A number of genes that participate in the above processes have been identified by their effects on flowering time (Coupland, 1995; Koornneef et al., 1991). Two genes, EMBRYONIC FLOWER1 and 2 (EMF1, and EMF2) have been proposed to be central negative regulators of flowering, which integrate the effects of environmental factors and thereby control the expression of floral meristem identity genes (Coupland, 1995; Yang et al., 1995).

Three genes, LEAFY (LFY), APETALA1 (AP1), and CAULIFLOWER (CAL), are important in the I —› F switch (Bowman, 1992; Gustafson-Brown, 1994; Huala and Sussex, 1992; Irish and Sussex, 1990; Kempin et al., 1995; Mandel and Yanofsky, 1995; Weigel et al., 1992; Weigel and Nilsson, 1995). AP1 and LFY genes are required to induce the transcription of floral organ identity genes APETALA2 (AP2), APETALA3 (AP3), PISTILLATA (PI) and AGAMOUS (AG) in the developing floral anlagen (Huala and Sussex, 1992; Ma, 1994; Mandel et al., 1992; Weigel and Meyerowitz, 1993, 1994; Weigel et al., 1992). These genes and their homologues in Antirrhinum appear to code for transcription factors (Kempin et al., 1995; Mandel et al., 1992; Weigel et al., 1992; Weigel and Meyerowitz, 1994). In accordance with their inferred role in the floral meristem, LFY and AP1 messages accumulate in the floral but not in the inflorescence meristem (Bowman, 1992; Huala and Sussex, 1992; Weigel et al., 1992). TFL1 (TERMINAL FLOWER) is a proposed negative regulatory gene of LFY and AP1 in the inflorescence meristem (Gustafson-Brown, 1994; Schultz and Haughn, 1993; Shannon and Meeks-Wagner, 1993). This is because tfl1 mutations cause lateral and apical inflorescence meristems to be converted to floral meristem (Alvarez et al., 1992; Shannon and Meeks-Wagner, 1991). Genetic and molecular studies indicate that TFL1 prevents LEAFY and AP1 activity in the apical and lateral inflorescence meristems (Gustafson-Brown et al., 1994; Shannon and Meeks-Wagner, 1993). Therefore, the current model of the I —› F switch postulates a down regulation of TFL1 activity that leads to increased expression of LFY, AP1 and CAL genes in the flowering meristem.

The evolution of flowering plants may have entailed a modification of primitive leaf or leaf-like structures that contained naked ovules on their surfaces, to specify floral organs that ultimately evolved to surround the ovules (Herr, 1995; Stebbins,
This view of angiosperm evolution predicts that the genetic regulatory network that controls ovule development should be interfaced with that which triggers flowering. Several genes important for ovule development have been identified in Arabidopsis (Reiser and Fischer, 1993). BELL1, a so-called cadastral gene that encodes a homeodomain protein (Reiser et al., 1995), controls the expression of the floral organ identity gene AG within the ovule and thereby controls morphogenesis of ovule integuments (Modrusan et al., 1994; Ray et al., 1994). SUPERMAN, another cadastral gene that restricts the spatial expression pattern of the floral organ identity gene AP3 (Sakai et al., 1995), is important in ovule integument development (Gaiser et al., 1995). The organ identity gene AP2 is also known to control ovule morphogenesis (Modrusan et al., 1994). By contrast, no known meristem identity or flowering control gene has a demonstrated role in ovule development.

The gene SHORT INTEGUMENT (SIN1) is required for normal ovule development (Lang et al., 1994; Reiser and Fischer, 1993; Robinson-Beers et al., 1992). We have determined that the sin1 mutation additionally causes a defect in the V → I → F switch. SIN1 is needed for the expression of the early flowering phenotype imparted by a tfl1 mutation and tfl1 sin1 double mutants do not produce pollen. Furthermore, we demonstrate that the sin1-1 allele enhances the effect of an ap1 mutation. Thus, SIN1 represents a genetic connection between ovule development and control of flowering.

MATERIALS AND METHODS

Strains

Strain WC1 was gl1 Columbia (Co), sin1 ER strain was Sin1-C described before (Lang et al., 1994). The source of tfl1-1 was CS6167 (Co) (Shannon and Meeks-Wagner, 1993), tfl1-2 was CS3091 in the Landsberg erecta background (La-er) (Alvarez et al., 1992), ap1-1 was NW4 (La-er) (Lang et al., 1994). The strain containing the sin1-2 (La-er) mutation was generously provided by Dr Charles S. Gasser (University of California, Davis).

Plant growth and physiological conditions

Conditions for germination and growth of Arabidopsis have been described before (Lang et al., 1994). Long day photoperiod (LD) was 18 hours light followed by 6 hours of dark. Short day (SD) was 14 hours light and 10 hours dark. To determine the effect of gibberellic acid (GA), a 50 μM solution of GA3 (Sigma) in 10% acetone (Oak) was sprayed, with a nebulizer, as a fine mist on the plants on every third day. Control plants were sprayed with 10% acetone. The spraying was done in an open area during the light period. The applied solution was allowed to evaporate for 1 hour at the room temperature before the plants were replaced in the growth chamber.

Genetic double mutants

All double mutant combinations except sin1-1/sin1-2 were constructed by applying pollen from Sin1-C plants to the stigma of the recipient mutants. Wherever possible morphological markers, such as glabra1 (gl1: absence of trichomes on leaf), erecta (er; compact rosette leaves, short pedicel, blunt fruits) and chlorina (ch1, light green color of leaves and stems), were monitored for segregation in subsequent progeny to verify the crosses. Double or triple (involving gl1) mutants were identified in F2 by their respective phenotypes. Unless stated explicitly, only those progenies that were phenotypically Er+ were analyzed. Allelism test between sin1-1 and sin1-2 was done by crossing pollen from Sin1-C plants into phenotypically Sin+ plants in a La-er population segregating the sin1-2 allele. F1 seeds derived from individual crosses were separately germinated and tested for the appearance of Sin+ progeny with an Er+ phenotype. One out of the five independent crosses (tested produced 6 Sin-:7 Sin+ F1 progeny (expect 1:1 for non-complementation), all of which were Er+, establishing allelism. The Sin+ plants produced in this batch of F1 were sin1-1/sin1-2.

Microscopic techniques

Techniques for tissue preparation and scanning electron microscopy were as described before (Lang et al., 1994).

RESULTS

sin1 mutation causes a defect in programmed meristem phase change

When grown under the long day condition, wild-type Arabidopsis initially produces a succession of five to eight (seven, on average) vegetative leaves in a helical arrangement from meristematic cells set aside in the flanks of the primary apical meristem (Fig. 1; Table 1). Next, two to three inflorescence meristem clusters, each subtended by a cauline leaf (or bract), initiate from the flanks of the growing apical meristem. These inflorescence primordia are termed coflorescences as they are borne on the flanks of the main inflorescence axis (Schultz and Haughn, 1993). Cells of the vegetative internode, the column of cells between two successive leaves, do not expand appreciably while internodal cells of the inflorescence (those between two successive coflorescence axes, and those between two flowers) usually do expand. Finally, a series of flowers without subtending bracts initiates from the flanking lateral meristem. The first leaf primordium appears on day 5 and the inflorescence axis starts to elongate on day 18. By day 18, at least five floral buds have formed. Thus, organ primordia appear at an average rate of approximately one per day until fertilization begins. Secondary inflorescence branches appear at a later stage from axils of rosette leaves. Since morphogenesis of these secondary inflorescences can be variable, we will not consider these any further.

Plants homozygous for the sin1-1 mutation (Fig. 1C) produce approximately 16 rosette leaves, 13 inflorescence primordia with subtending bracts, and five to six floral buds before starting to expand shoot internodes on the 40th day (Table 1). Thus, the meristem initials in the sin1 mutants appear at the same rate of approximately one per day as for a Sin+ plant, but the duration spent in each phase of development is at least doubled compared to the wild type. The resulting plants are taller and are more luxuriant. The total number of flowers borne on a wild-type or on a sin1-1 plant is indefinite: the wild type usually bears fewer flowers than sin1-1 on the...
primary inflorescence (the main shoot) because successful fertilization in the wild type causes a cessation of flowering, while the mutant, being female sterile, flowers indefinitely. The apical meristem in both the wild type and the mutant remains indeterminate (Fig. 2A,B).

A second allele of sin1 (designated sin1-2) has the same associated phenotypes of female sterility and meristem transition defects. Allelism test (see Methods) confirmed that sin1-2 does not complement sin1-1 in any of the phenotypes. First, sin1-2 mutant plants are late flowering and produce many more rosette leaves and coflorescences compared to the wild type. Second, sin1-2 mutants are female sterile because of ovule defects. The ovule defect in sin1-2 was less severe in morphological terms than those associated with sin1-1 allele. The ovule defect of sin1-1/sin1-2 heterozygotes was of intermediate severity. The phenotypic series for ovule defect is sin1-1 > sin1-1/sin1-2 > sin1-2. Therefore, both sin alleles are loss-of-function alleles. There was, however, no significant difference in flowering time between homozygous sin1-1 and sin1-2 mutants: 36±1 days \((n=16)\) for 1 cm bolt with sin1-2 compared to 39±0.5 days \((n=46)\) for sin1-1. sin1-1/sin1-2 heterozygous plants were approximately equally affected for flowering time \((33±1\text{ days, } n=7)\) and the number of organs produced \((\text{number of rosette leaves plus coflorescence on the primary axis}) = 30±1, n=7)\). Thus the meristem transition phenotype does not show the same phenotypic series as shown by the ovule phenotype. One interpretation of these results is that meristem transition is more sensitive to the level of SIN1 product than is ovule morphogenesis.

**Environmental effects on sin1-1 phenotype**

Several classes of late flowering mutants of *Arabidopsis* show an altered response to day length and/or to the plant hormone, gibberellic acid (GA) (Coupland 1995; Koornneef et al., 1994).

**Fig. 1.** Effects of mutations on vegetative development. A-D are photographs of plants grown in long days until the inflorescence shoots reached approximately 1-2 cm. (A) gl1 tfl1-1; (B) strain WC1, mutant for gl1 but otherwise wild type; (C) gl1 sin1-1; (D) gl1 sin1-1 tfl1-1. E-H are photographs of leaves of a WC1 plant (grown in SD to make more than 13 rosette leaves). E,F and H show the second, seventh and the 13th rosette leaf, respectively, with no trichomes on leaf margin. G shows a cauline leaf with trichomes (arrow) which subtends a coflorescence shoot. Scale bars: (E,F,H) 0.4 cm; (G) 0.2 cm.

**Fig. 2.** Scanning electron micrographs of inflorescence apices. Mature inflorescence tips are shown in (A) WC1; (B) sin1-1; (D) tfl1-1; and (E) sin1-1 tfl1-1. C shows an immature apex of a tfl1-1 mutant. Arrowheads in A and B point to the undifferentiated apical meristem dome. In C, the arrowhead points to the developing carpel primordium of the terminal flower. Abbreviations: b, a flower bud; ca, carpel. Scale bars are 0.25 mm in all panels.
1991). In fact, phenotypic analysis of the sin1-1 mutant had earlier led us to propose that it may be defective in some aspect of GA metabolism. The following observations make this possibility unlikely. Application of GA3 did not reverse the late flowering phenotype but accelerated flowering of sin1-1 growing in long days (LD) to approximately the same extent as it did to the wild type (Table 1). Growth of sin1-1 in short days (SD) prolonged the flowering time to 280±15 days (number of plants, n=8) compared to 7±5 days (n=29) for the wild type in SD. The ratio of flowering times of the mutant to the wild type was not significantly different in short and long days. Repeated applications of GA3 accelerated the flowering time of Sin+ plants in SD as it did to Sin+ plants (data not shown). Both Sin+ and Sin− plants responded to GA3 by growing longer and more slender leaves and shoots. Both Sin+ and Sin− plants treated with GA3 were partially male sterile. In summary, floral induction in sin1-1 plants was responsive to both day length periodicity and gibberellic acid treatment. Vernalization, cold treatment of hydrated seeds before germination, had no effect on flowering time. These results suggest that the sin1-1 mutation does not directly cause a defect in photoperiod response, and in metabolism of or signal transduction by GA.

**Origin of extra organs in sin1-1**

The delayed meristem transition phenotype of sin mutants may be due to a defect in the internal developmental program. The extra rosette leaves in a sin1 mutant may represent organs normally found associated with the inflorescence but which are now converted to vegetative leaves. If true, it may be possible to obtain a morphological marker that is normally specific for an inflorescence organ but which are ectopically in the super-numerary rosette leaves of sin1-1 mutant plants. The number and density of trichomes on the abaxial surface of rosette leaves change with developmental time: late-appearing leaves have more abaxial trichomes than early leaves (Schultz and Haughn, 1993). This criterion has previously been used to mark developmental time (Schultz and Haughn, 1993; Weigel and Nilsson, 1995). By contrast, we wished to use an organ-specific marker that never appears on rosette leaves, but does so on cauline leaves (bracts) that subtend a coflorescence. We noted that bract-specific trichomes appear on rosette leaf edge in a gl1 mutant background, the extended vegetative phase in the sin1-1 mutant is correlated with at least a partial operation of an inflorescence-like program in which bract-specific trichomes appear on rosette leaf margins.

Leaf shape was used in other related studies to monitor developmental transition in Arabidopsis (Schultz and Haughn, 1993). We could not detect a sharp transition in our metric for leaf shape (Fig. 3) between the seventh and the eighth leaf; hence a change in leaf shape was not a reliable marker of developmental transition in these strains.

**Tfl− phenotype is modified by sin1 mutation**

The mutation tfl1 causes early flowering, a reduction in the number of lateral inflorescence primordia (coflorescence), and produces a terminal differentiation of the apical meristem to a floral meristem (Alvarez et al., 1992; Shannon and Meeks-Wagner, 1991, 1993). In the sense that the tfl1 mutation pre-

### Table 2. Interaction of sin1 with tfl1

<table>
<thead>
<tr>
<th>Strain</th>
<th>Days to 1 cm bolt (± s.e.)</th>
<th>Without trichome (± s.e.)</th>
<th>With trichome (± s.e.)</th>
<th>Total (± s.e.)</th>
<th>Number of coflorescence (± s.e.)</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>WC1</td>
<td>23.6 (±0.4)</td>
<td>6.5 (±0.1)</td>
<td>0</td>
<td>6.5</td>
<td>2.2 (±0.1)</td>
<td>48</td>
</tr>
<tr>
<td>sin1 gl1</td>
<td>41.7 (±1.7)</td>
<td>8.5 (±1.0)</td>
<td>16.8 (±1.6)</td>
<td>25.3</td>
<td>8.3 (±0.7)</td>
<td>13</td>
</tr>
<tr>
<td>tfl1-1 gl1</td>
<td>21.6 (±0.1)</td>
<td>5.9 (±0.2)</td>
<td>0</td>
<td>5.9</td>
<td>1.0 (±0.1)</td>
<td>20</td>
</tr>
<tr>
<td>tfl1-2</td>
<td>18.5 (±0.3)</td>
<td>0</td>
<td>4.3 (±0.1)</td>
<td>4.3</td>
<td>1.0 (±0.1)</td>
<td>16</td>
</tr>
<tr>
<td>tfl1-1 sin1 gl1</td>
<td>39.3 (±0.5)</td>
<td>5.4 (±0.2)</td>
<td>13.3 (±1.6)</td>
<td>18.7</td>
<td>7.2 (±0.4)</td>
<td>21</td>
</tr>
<tr>
<td>tfl1-2 sin1 gl1</td>
<td>32.3 (±0.7)</td>
<td>10.7 (±0.6)</td>
<td>10.2 (±1.2)</td>
<td>20.9</td>
<td>4.2 (±0.4)</td>
<td>6</td>
</tr>
</tbody>
</table>

The sin1 allele tested was sin1-1. n is the number of plants tested. All plants were grown in LD.

Fig. 3. Variation in the number of leaf-edge trichomes, and leaf shape throughout development. The developmental time is represented by rosette leaf number in the order of appearance: leaf 1 is the earliest leaf. All data are from plants grown simultaneously in LD, except those denoted by filled circles in A which were grown in SD. Error bars represent standard errors of mean. Curves were drawn by fitting a polynomial through the means.
maturely commits the vegetative meristem to reproductive development, sin1 causes an opposite effect. sin1 is pleiotropic on meristem transition during both vegetative and inflorescence phases and on ovule morphology. It has been argued elsewhere that ovules are independent floral organs (Colombo et al., 1995; Herr, 1995; Meyerowitz, 1994; Ray et al., 1994; Taylor and Taylor, 1993). The TFL1 gene negatively regulates the activities of other genes, such as APETALA1 and LFY, that are important for both floral meristem identity and floral organ identity (Gustafson-Brown et al., 1994; Schultz and Haughn, 1993; Shannon and Meeks-Wagner, 1993; Weigel et al., 1992). Therefore it is interesting to determine if TFL1 also controls SIN1.

In the LD growth regime, the Sin+ strain WC1 produced on average seven rosette leaves and two coflorescence branches (identified by subtending bracts) that in turn bore secondary and tertiary coflorescence primordia (Table 2). Homozygous sin1-1 mutant plants under an identical condition produced on average 25 rosette leaves and eight coflorescence branches (Table 2). The apical inflorescence meristem of WC1 and sin1-1 plants remained indeterminate (Fig. 2A,B); compared to WC1, many more organ primordia were arranged in a spiral motif around the central dome of the apical meristem in the sin1 mutant. We tested two mutant alleles of tfl1. Each tfl1 mutant plant in LD generated on average four to six rosette leaves and one bract that subtended a flower, not a coflower (Fig. 1). The inflorescence apices differentiated to one or more terminal flowers (Fig. 2CD).

In LD condition, double mutants of tfl1 sin1-1 and tfl1-2 sin1-1 generated on average 19 to 21 rosette leaves, and four to seven coflorescence branches, many of which bore several secondary coflorescence primordia, branches, or both (Table 2, Fig. 1D). However, the apex of each inflorescence (and coflower) of the double mutant plants differentiated into terminal flowers (Fig. 2E).

Consistent with our idea that supernumerary rosette leaves in strains involving the sin1 mutation have partial inflorescence characteristics, the extra leaves of tfl1 sin1 gl1 triple mutant plants displayed many trichomes on their margins (Table 2; Fig. 3D).

Besides reversing the early flowering phenotype of tfl1 mutation, the sin1 tfl1 double mutations produced female sterility due to ovule defects typical of a sin1-1 single mutant. Ovule morphology of tfl1 sin1-1 double mutants was identical to those of sin1-1 single mutants (data not shown). Unexpectedly, the double mutants were completely male sterile: the androecium development was morphologically normal (Fig. 2E) but the anthers remained white and failed to develop pollen grains. This synergistic interaction between sin1 and tfl1 revealed a cryptic role for TFL1 in pollen development. A cryptic role for SIN1 in pollen development was reported earlier (Lang et al., 1994; Robinson-Beers et al., 1992).

**sin1 interacts with ap1 mutation**

Since TFL1 is postulated to regulate meristem transition at least partially through its action on AP1 (Gustafson-Brown et al., 1994; Shannon and Meeks-Wagner, 1993), we investigated whether SIN1 also does so. If SIN1 is a positive regulator of AP1, the effect of an ap1 mutation should be enhanced in a sin1 ap1 double mutant.

The strong mutant allele ap1-1 (in a background wild type for ER) produced a normal number of rosette organs and coflorescences but its flowers had partial inflorescence characteristics (Schultz and Haughn, 1993; Table 3; Fig. 4A). The floral stalk, as usual, was not subtended by a bract; in place of a sepal often there was a bract and a flower. Each floral stalk in the ap1-1 mutant had at most five flowers, four originating from the site of four sepal and one that represented the main floral axis. The number of supernumerary flowers borne on a floral stalk decreased acropetally. These supernumerary flowers were arranged in a radial symmetry, a characteristic of floral organs, as opposed to the helical symmetry of flower series along the main inflorescence axis. On the basis of these characteristics, it is thought that the supernumerary flowers of ap1 are transformed floral organs (Ma, 1994).

### Table 3. Interaction between sin1-1 and ap1-1 mutations

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Strain</th>
<th>Flowers that are</th>
<th>First normal flower</th>
</tr>
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<tbody>
<tr>
<td>25°C</td>
<td>ap1-1</td>
<td>1.5 to 14th</td>
<td>13th</td>
</tr>
<tr>
<td></td>
<td>sin1-1</td>
<td>2.5 to 21st</td>
<td>&gt;30th</td>
</tr>
<tr>
<td>17°C</td>
<td>ap1-1</td>
<td>1.5 to 11th</td>
<td>12th</td>
</tr>
<tr>
<td></td>
<td>sin1-1</td>
<td>1.5 to 9th</td>
<td>10th</td>
</tr>
</tbody>
</table>

*The structure that occupied the position of the first flower (the first organ primordium without a subtended bract) was a determinate inflorescence with 29 nodes and two expanded secondary coflorescences with 3 and 6 nodes, respectively.

†Not recorded. All plants were grown in LD.

For each temperature and strain, the flower positions are listed from younger to older. Positions 13th through 29 were occupied by structures strongly resembling inflorescences.
ing that floral characteristics eventually dominate in these modified organs. The severity of the sin1-1 effect on ap1-1 decreases acropetally, such that late-arising flowers are less inflorescence-like, and later flowers resemble the canonical ap1-1 mutant flowers (Table 3). Since the ap1-1 mutant is known to be cold sensitive, we grew and observed mutant plants at 17°C. The first flowers of the double mutants were more severely affected at 17°C than those in the ap1-1 single mutant (Table 3). For example, in one ap1-1 sin1-1 double mutant plant, an elaborate inflorescence with over a hundred organs developed in place of a flower. Thus, the sin1-1 mutation enhanced the effect of ap1-1.

DISCUSSION

The basis of late flowering in sin1

The late flowering phenotype of sin1 may be interpreted in one of several ways. First, as is the case with several late flowering mutations, the sin1 mutation may cause a defect in the transduction of environmental signals. For example, the co, gi, and the fha mutations cause a delay in flowering when grown in long days but not in short days (Kooymen et al., 1991; Martinez-Zapater et al., 1994; Putterill et al., 1995). The sin1 mutation does not belong in this category of mutations because growth in short days causes a further delay when compared to long day growth: while Sin+ plants flowered after approximately 80 days, sin1 mutants flowered after approximately 270 days. Short day grown sin1 mutant plants develop hundreds of vegetative leaves, more than 50 coflorescences, and grow to gigantic proportions. Late flowering phenotypes imparted by fy, fpa, fve, fca, ld, fe, and fri mutations can be reversed by vernalization (Clarke and Dean, 1994; Kooymen et al., 1991). Vernalization had no effect on the late flowering phenotype of sin1, so it cannot be included in this class. Repeated applications of gibberellic acid on vegetative leaves failed to rescue the late flowering phenotype of sin1 mutants grown in long day conditions. In short days, repeated gibberellic acid application accelerated the time to flowering of both the wild type and sin plants. Thus, sin1 mutation endows the plants with neither a gibberellin requirement nor a gibberellin insensitivity. Superficially, sin1 resembles a class of poorly understood mutations, including fwa, fd and fi, that are late flowering in both long and short days and are insensitive to vernalization (Kooymen et al., 1991). Note, however, that fwa is a dominant mutation while sin1 is recessive. Furthermore, none of these other three mutations has any effect on fertility. Therefore, the sin1 mutation defines a novel class by its phenotypic effects: late flowering in both long and short days, cannot be rescued by gibberellin acid or vernalization, and the associated female sterility. The map location of sin1 (at 1.0 cM from an on chromosome 1; Lang et al., 1994) rules out the possibility that it is a mutation in a meristem identity gene in the same sense as is the leafy mutation. While the fy mutation affects only the transition from the inflorescence to the floral meristem, sin1 affects both V — à I and I — à F switches. Thus, on average in LD, the fates of the eighth to the 16th organ primordia in a sin1 mutant are changed from that of inflorescences or of flowers to that of leaves, and the fates of
the 17th to the 20th primordia are changed from that of flowers to inflorescences. Consistent with this interpretation, supernumerary rosette leaves of sin1 gl1 plants contain trichomes on their margins, as do the cauline leaves (bracts) but not the seven vegetative leaves of SIN1 gl1 plants.

**Interaction of SIN1 with AP1 and TFL1**

In a sin mutant, the meristem appears to spend a longer time at each developmental phase. It is formally possible that the wild-type SIN product encodes a general positive regulator of meristem phase change. The absence of epistasis of tfl1 on sin1 (Fig. 4) allows us to reject the simple possibility that SIN1 is a negative regulator of TFL1. SIN1 may act through a genetic pathway independent of TFL1. In that case, if tfl has an effect opposite to that of sin1 on the delayed flowering phenotype, tfl sin1 double mutants should be intermediate between the two single mutants. Thus, we expect on average nine supernumerary leaves (with trichomes on the leaf margin) and five co-florescence primordia in the double mutant growing in LD. The observed results are not significantly different (Table 2). Furthermore, each apical meristem eventually developed into a terminal flower. Therefore, I → F transition at the inflorescence apex does not require SIN1 activity. While SIN1 may act independently of TFL1, it could still be affecting through AP1 and LFY. A further possibility is that TFL1 indirectly controls LFY and AP1 expressions through its effect on SIN1. The predicted outcome is that sin1 should be epistatic over tfl1. This is partially true. In LD, a sin1 tfl1 double mutant plant produces on average 25-26 non floral organs as opposed to only 5-7 in tfl1 single mutants and approximately 33 in sin1 single mutants. The values for sin1 and tfl1 sin1 mutants overlap at the 99% confidence level (Table 2), and are not significantly different. Each coflorescence of the double mutant in turn develops several secondary coflorescence primordia and many female sterile flowers (Fig. 4), unlike what is observed for a tfl1 mutant. In summary, sin1 is a partial suppressor of tfl1, and TFL1 may regulate meristem fate through SIN1.

The phenotype of a sin1-1 ap1-1 double mutant (Fig. 4) is significantly different from that of an ap1 cal double mutant where the inflorescence meristem fails to extend and the apex develops into a tightly packed, helical, cluster of floral primordia resembling a cauliflower (Bowman, 1992; Kempin et al., 1995). The effect of sin1 appears to be additive on ap1. Therefore, sin1 is unlikely to directly regulate AP1 activity. The effect of the sin1-1 allele on the ap1-1 mutant phenotype of the first flower is enhanced at 17°C, a temperature at which ap1-1, the most severe allele of ap1 known, is more expressive (Bowman et al., 1993). These results indicate that both SIN1 and AP1 products are simultaneously required for early floral meristem identity (Weigel and Nilsson, 1995). Alternatively, SIN1 may indirectly regulate flowering by controlling the general competence of the meristematic cells to respond to the activity of meristem identity genes (Weigel and Nilsson, 1975). Synergistic interaction has been reported between the weak lfy-5 allele and constans, which results in late flowering (Putterill et al., 1995), suggesting that flowering time genes may directly or indirectly regulate activities of meristem identity genes such as LFY.

Tests of genetic models by epistasis analysis require the mutant alleles to be null. The allele sin1-2 is weaker than sin1-1 in its effect on ovule morphology because sin1-1/sin1-2 heterozygotes show intermediate levels of ovule defect compared to either homozygous mutant (Golden, Ray and Ray, unpublished). Expressivity of the delayed flowering phenotype of sin1-2, however, is approximately equal to that of sin1-1 and that of the sin1-1/sin1-2 heterozygote. If ovule and meristem defects are pleiotropic effects of the same molecular lesion, then the meristem defect is more sensitive to an alteration of the SIN1 gene product than is the ovule defect, and the effect of the sin1-1 allele on meristem transition is complete. Therefore, we suggest that the sin1-1 allele can be adequately used for epistasis analysis. It is not known whether tfl1-1 or tfl1-2 are null alleles, and ap1-1, the strongest known allele, is not null. Therefore, all our conclusions based on epistasis analysis, as are most conclusions based on epistasis in Arabidopsis, are subject to future revision. The higher sensitivity of the meristem defect to a sin1 mutational allele suggests that a sharper threshold of SIN1 activity is required for normal meristem transition than for normal ovule development. Alternatively, SIN1 may encode a bifunctional product having organ-specific functional domains.

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