

Photoinduction of Flower Identity in Vegetatively Biased Primordia

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Far-red light and long photoperiods promote flowering in Arabidopsis. We report here that when 30-day-old vegetative plants were induced with a continuous light treatment enriched in far-red light, flowers developed directly from previously initiated primordia. Specifically, plants induced with our continuous incandescent-enriched (CI) treatment produced an average of two primary-axis nodes with a leaf/flower phenotype, indicating that approximately two leaf/paraclade primordia per plant produced an individual flower from tissue that typically would differentiate into a paraclade (secondary inflorescence). Assays for APETALA1:: β -glucuronidase activity during the CI photoinduction treatment indicated that the floral meristem identity gene *APETALA1* was transcriptionally activated in primordia with a leaf/paraclade bias and in primordia committed to leaf/paraclade development. APETALA1:: β -glucuronidase activity levels were initially highest in young primordia but were not correlated strictly with primordium fate. These results indicate that primordium fate can be modified after primordium initiation and that developing primordia respond quantitatively to floral induction signals.

INTRODUCTION

After the transition to flowering in Arabidopsis, flowers are initiated from the apical meristem instead of leaves or paraclades (secondary shoots). In early-flowering ecotypes such as Landsberg *erecta* (*Ler*) and Nossen (*No*), strong photoperiodic treatments can induce mature vegetative plants to initiate flowers from the primary meristem within one plastochron (Hempel and Feldman, 1994, 1995). These flowers typically are not associated with leaves, although Arabidopsis flower primordia evidently have a minor leaf component that is expressed when flowers are genetically ablated (Nilsson et al., 1998). The last leaves initiated on a photoinduced shoot apex develop as cauline leaves, largely due to their position and their developmental state at the time that the flower-inducing signals reach the shoot apex. In Arabidopsis, both cauline and rosette leaves develop in conjunction with secondary flowering shoots (paraclades), that is, leaves and paraclades develop from a common primordium—the leaf/paraclade primordium. The flowering of the paraclades associated with the cauline leaves typically occurs shortly after flowering begins on the primary shoot and after the initiation of one or more leaves from the paraclade meristem (Hempel and Feldman, 1994; Grbić and Bleecker, 1996).

Although the switch from leaf/paraclade to flower initiation on the primary shoot represents a relatively abrupt morphological change, a number of more gradual changes also occur during plant maturation. For example, the surface characteristics and shapes of the leaves change gradually in response

to gibberellins and other developmental signals (Chien and Sussex, 1996; Telfer et al., 1997). The flowering-response potential within the shoot apex generally increases with maturity, because older plants are more responsive to photoinduction treatments (Corbesier et al., 1996). These changes likely are due to a progressive increase in the sensitivity of primordia to photoinductive signals (Blázquez et al., 1997) and/or to changes in the balance of flower-promoting versus flower-repressing signals in the plant (Bernier et al., 1993; Schultz and Haughn, 1993).

Within the shoot apex, floral meristem identity genes regulate flowering. One of these genes, *LEAFY* (*LFY*), is a critical regulator of the flowering response within the shoot apex, because above-threshold levels of *LFY* are required for establishing floral meristem identity (Weigel and Nilsson, 1995; Blázquez et al., 1997) and for suppressing indeterminate meristem growth (Okamoto et al., 1996). A second floral meristem identity gene, *APETALA1* (*AP1*), is also important for establishing floral meristems (Irish and Sussex, 1990; Bowman et al., 1993). It is expressed predominately in young flower meristems (Mandel et al., 1992) and triggers flowering when ectopically expressed in transgenic plants (Mandel and Yanofsky, 1995). *AP1* promoter activity is useful as a marker for floral determination in Arabidopsis, because the *AP1* promoter is active only in florally determined plants (Hempel et al., 1997).

The expression of above-threshold levels of floral meristem identity genes within primordia at the shoot apex requires above-threshold levels of flower-promoting signals from outside of the shoot apex (Bernier et al., 1981). It is the leaves that perceive the light signals that induce flowering

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and promote the transmission of flower-promoting signals to the shoot apex (Knott, 1934; Corbesier et al., 1996). Genes likely to be involved in the production, transmission, and perception of flower-promoting signals have been identified in genetic screens for late-flowering mutants (Koorneef et al., 1991; Martínez-Zapater et al., 1994).

One of the mutants identified—*constans* (*co*)—has a semidominant phenotype, which might be expected of a gene involved in quantitative (threshold-based) signaling. *CO* is a putative transcription factor, and its expression is regulated by photoperiod (Putterill et al., 1995). Furthermore, constitutive expression of *CO* induces early flowering and the expression of floral meristem identity genes (Simon et al., 1996); recent work indicates that *CO* is positively regulated by blue light acting through a cryptochrome (CRY2) and negatively regulated by red light acting through a phytochrome (PHYB) (Guo et al., 1998).

The *phyB* mutant, which flowers early under both long-day and short-day conditions (Halliday et al., 1994; Reed et al., 1994), belongs to another broad class of mutants, the early-flowering mutants, that identify genes acting directly or indirectly to suppress flowering (Weigel, 1995). Because phytochromes directly perceive light signals in the leaves, *PHYB* is likely involved in leaf-to-apex signaling during the induction of flowering. Interestingly, *CO* is expressed both in young leaves and in the shoot apex (Putterill et al., 1995); thus, *CO* also may be involved in leaf-to-apex signaling, although the precise location of *CO* activity still is not known.

It also is not known whether the flower-inducing signals from outside the shoot apex instruct meristems to make flower primordia or whether they directly instruct developing primordia to become flowers. In this report, we present results suggesting that primordia can respond directly to flower-inducing signals after they are initiated. Specifically, we describe the formation of flowers from young vegetatively biased leaf/paraclyde primordia during a continuous incandescent-enriched (CI) photoinduction treatment. Analyses of AP1:: β -glucuronidase (GUS) activity within the shoot apices of photoinduced plants indicate that preexisting leaf/paraclyde primordia express the floral meristem identity gene *AP1* in response to the CI photoinduction treatment. The expression of *AP1* in primordia initiated before the start

of the photoinduction treatment and the formation of individual flowers from the youngest of the previously initiated primordia indicate that vegetatively biased primordia are not committed strictly to a specific fate at the time they are initiated.

RESULTS

Photoinduction of a Leaf/Flower Phenotype

Previously, it was hypothesized that when vegetative Arabidopsis plants are induced to flower by strong photoinduction treatments, the chimeric flower/paraclyde shoots that commonly appear on the primary shoot (below the lowest complete flower) represent vegetative primordia that were converted partially into flowers (Hempel and Feldman, 1995). To test this “conversion” hypothesis, we induced a population of vegetative *Ler* plants with a CI photoinduction treatment to determine whether this very strong photoinduction treatment would induce the complete conversion of paraclyde primordia into flower primordia. Table 1 provides a comparison of the CI treatment used in this study with the strong photoinductive treatment of Hempel and Feldman (1994) that was used to induce flowering in *Ler* plants. An average of six additional flower primordia was evident after 72 hr for the plants photoinduced with the CI treatment (Table 1).

Figure 1 shows a “leaf/flower” nodal phenotype that was common on the primary inflorescences of the plants subjected to the CI treatment. Leaf/flower nodes were formed directly above the uppermost leaf/paraclyde on the primary shoot (Figures 1A and 1B). Arabidopsis plants do not usually have leaves (bracts) subtending individual flowers, and in the previous study mentioned above, none of the flowers on the primary shoot was subtended by leaves (Hempel and Feldman, 1994). Figure 2, however, indicates that most of the plants in the CI-induced population (83%) had at least one node with a macroscopic leaf subtending a flower (Figures 2C to 2E) and that ~14% had two nodes with macro-

Table 1. Comparison of Strong and CI Photoinduction Treatments^a

Treatment	Photoperiod	Total Irradiance ($\mu\text{E m}^{-2} \text{sec}^{-1}$)	Light Quality	No. Flowers Initiated after 72 Hr of Induction
Strong ^b	16 hr light/ 8 hr dark	100 to 130	90% cool white 10% incandescent	7.9
CI ^c	Continuous light	120 to 140	70% cool white 30% incandescent	14.2

^aIn both studies, photoinduction followed 30 days of vegetative growth under short-day conditions (8 hr of light/16 hr of dark; see Methods).

^bHempel and Feldman (1994).

^cThis study.

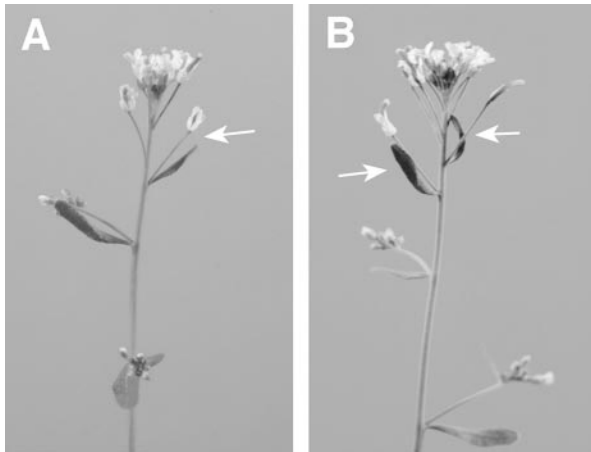


Figure 1. Leaf/Flower Nodes Produced on Plants Exposed to CI Photoinduction Conditions after 30 Short Days.

- (A) Primary inflorescence with one leaf/flower node (arrow).
 (B) Primary inflorescence with two leaf/flower nodes (arrows). The sepals and petals of the flower to the right have fallen away.

scopic leaves subtending flowers (Figure 2E). Chimeric flower/paraclyde shoots also were present on a subset of the plants. When present, they were located directly below the lowest complete flower on the primary shoot (Figures 2B and 2D).

We also found that fingerlike protrusions of tissue often subtended flowers low on the primary inflorescence (Figures 2A to 2D) and that all of the plants with no leaf/flower nodes had multicellular fingerlike protrusions subtending the lowest flower on the primary shoot. These structures were found below a flower on approximately three-fourths of the plants with one leaf/flower node but basically were absent from the plants with two leaf/flower nodes. In a previous study, similar structures were commonly evident under the uppermost paraclyde on the primary shoot and were considered to be vestigial cauline leaves (Hempel and Feldman, 1994). In that study, however, vestigial leaves were not present below individual flowers. In both cases, the fingerlike protrusions are indicative of the highly suppressed leaf component of a node; in this study, a leaf/flower node. Thus, the modal inflorescence phenotype within the CI-induced population was one with two leaf/flower nodes per plant (one with a macroscopic cauline leaf and one with a vestigial leaf).

Shoot Apex Morphogenesis and Flower Primordium Initiation

Figure 3 indicates that flower primordia were initiated rapidly after the start of the CI photoinduction treatment. After 72 hr

of induction, ~ 14 flower primordia were present. The solid line drawn through the data points in Figure 3 indicates the number of flower primordia present between 72 and 120 hr after the start of the CI treatment. Before the 72-hr time point, the definitive morphological markers for leaf versus flower primordia (see Figure 4) were not evident. However, a curve roughly estimating the number of flower primordia present before the 72-hr time point is indicated by a dashed line (Figure 3). An acceleration of the primordium initiation rate is indicated. This acceleration is consistent with results showing that the rate of primordium initiation increases dramatically after the start of photoinduction (Hempel and Feldman, 1995) and with data indicating that a photoinduced increase in mitotic activity in the closely related *Sinapis alba* (white mustard) occurs after an initial 26- to 30-hr lag period (Gonthier et al., 1987). The curve intercepts the y-axis at a value of 2. This value is equal to the total number of leaf/flower nodes found on plants with the modal phenotype (Figure 2C; see Discussion for details and rationale).

Figure 4 illustrates the patterns of primordium initiation and primordium differentiation evident at the shoot apices of the induced plants. The oldest flower primordia were clearly distinguishable from leaf and paraclyde primordia at the 72-hr time point. At this time, leaf/flower nodes were evident, and their morphology indicated a common developmental origin for the leaf and flower (Figure 4A). The total number of leaf/flower nodes, however, was not always evident after 72 hr. For example, a second leaf/flower node may be present in Figure 4A, at the node below the evident leaf/flower node, but the identity of the shoot at this node (denoted by a question mark in Figure 4A) is not yet evident. In addition, the second flower (F2 in Figures 4A and 4B) often is subtended by vestigial leaf tissue (Figures 2, 4D, and 4E).

After 96 hr of photoinduction, floral organ primordia were evident on the oldest flowers (Figures 4B and 4C), and chimeric shoots were evident on a subset of the plants (Figure 4C). A consistent pattern evident at this time point was that the plants with fewer leaf/flower nodes typically were those that were less responsive to the induction treatment (as measured by total flower number). For example, Figure 4B shows the shoot apex of a plant that has 20 initiated flower primordia and a clearly evident leaf/flower node (with its flower component labeled F1), whereas Figure 4C shows the shoot apex of a plant with 16 initiated flower primordia and no clearly evident leaf/flower node. The shoot apex in Figure 4C, however, does have a chimeric flower/paraclyde shoot subtended by a leaf. This shoot evidently represents the partial conversion of a paraclyde primordium into a flower primordium.

Flower primordia that developed in conjunction with a macroscopic leaf primordium consistently developed and matured after the flower primordia immediately above them on the primary shoot (Figures 4A and 4B). These differences typically persisted throughout the development of the inflorescence, because anthesis and fruit maturation also were delayed in the flowers subtended by leaves (Figure 1B).

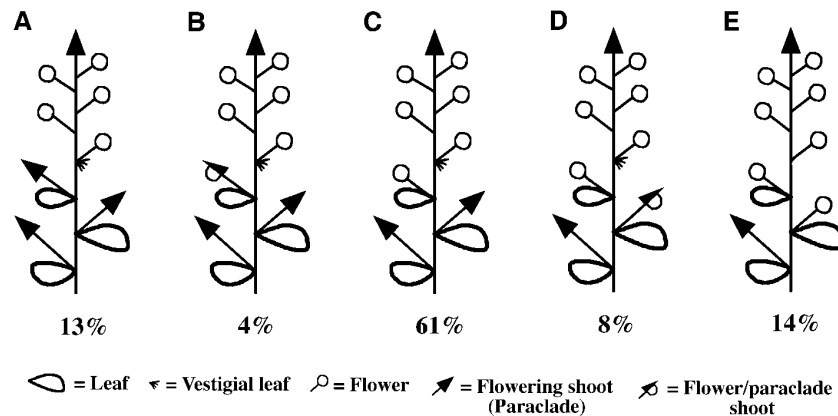


Figure 2. Primary Inflorescence Phenotypes Found within a Population of *Ler* Plants Induced with CI Photoinduction Conditions.

(A) "Normal" inflorescence. The lowest flower, however, was always subtended by vestigial leaf tissue.

(B) Inflorescence with a leaf/flower/paraclade node below a vestigial leaf/flower node.

(C) Inflorescence with a single macroscopic leaf/flower node. On ~75% of these plants, the second lowest flower on the primary inflorescence was subtended by vestigial leaf tissue.

(D) Inflorescence with a leaf/flower/paraclade node and a macroscopic leaf/flower node. An additional vestigial leaf/flower node also was evident on ~50% of these plants.

(E) Inflorescence with two macroscopic leaf/flower nodes. Vestigial leaf/flower nodes were rare in this class of plants.

In control populations of plants grown in long days, none of the plants had flowers on the primary inflorescence that were subtended by macroscopic or vestigial leaves.

Photoinduced Expression of *AP1::GUS* and Floral Meristem Identity

To characterize further the flowering response within the shoot apices of photoinduced plants, we measured the transcriptional regulation of the floral meristem identity gene *AP1* by assaying for the activity of an *AP1::GUS* reporter. Previous work suggested that *AP1::GUS* activity is limited to plants that are committed to flowering, although activity was not limited strictly to flower primordia (Hempel et al., 1997). The purpose of analyzing *AP1::GUS* activity was to correlate the induction of flower meristem identity gene expression with the induction of flowers from previously vegetative primordia. The activation of the *AP1::GUS* transgene was characterized in the *Ler* and *No* ecotypes, both of which carried the transgene at the same locus (see Methods). Populations of *Ler* and *No* plants harboring the *AP1::GUS* gene were induced after 30 short days under CI photoinduction conditions in a single experiment. Table 2 shows that leaf/flower nodes commonly occurred on the plants within both the *Ler* and *No* populations, although at a slightly lower frequency than in the first experiment (Figure 2).

Figure 5 indicates that the two ecotypes responded similarly to the photoinduction treatment. No *AP1::GUS* activity was visible before the start of the photoinduction treatment (Figures 5A and 5E). After 24 hr, however, *AP1::GUS* activity was evident in both the Landsberg *erecta* and Nossen apices, and expression was strongest in the youngest leaf/paraclade primordia. After 48 hr, *AP1::GUS* activity was evident

in primordia throughout the shoot apex in both ecotypes, and flower primordia were evident on the flanks of the apical meristem (Figures 5C and 5G). To identify the regions within the shoot apex with the highest levels of *AP1::GUS* activity, we used a diluted 5-bromo-4-chloro-3-indolyl β -D-glucuronic acid (X-gluc) solution (one-quarter strength). Assays using the dilute solution indicated that the highest levels of activity occurred in the flower primordia (Figures 5D and 5H) and that the patterns of expression were similar to reported patterns of mRNA expression (Mandel et al., 1992).

AP1::GUS activity after 72 hr of induction is shown in Figures 5I and 5J. Activity was relatively low in the paraclade primordia (Figure 5I) but high in the flower primordia, including the flower primordia, which developed in conjunction with a leaf primordium (Figure 5J). Relatively low levels of *AP1::GUS* activity were detected also in the developing leaf primordia at this time point (Figures 5I and 5J).

AP1::GUS activity also was examined during the transition to flowering in plants grown only in long days. These results are shown in Figure 6. We expected that *AP1::GUS* activity would be lower in the young leaf primordia of these plants, because leaf/flower nodes were not found in long-day-grown populations (Table 2). Initially, no *AP1::GUS* activity was present in the long-day-grown plants (Figures 6A, 6E, and 6F). However, *AP1::GUS* activity was evident in the leaf primordia of the plants undergoing the transition to flowering in long days; contrary to initial expectations, levels of activity (Figures 6B and 6G) were similar to those found in plants that were photoinduced with the CI photoinduction treat-

ment (Figures 5B and 5F). AP1::GUS activity was evident also throughout the shoot apices of the long-day-grown plants, and there was significant activity in nonmeristematic tissues, particularly after flower primordia had been initiated (Figures 6C, 6D, and 6H). AP1::GUS activity was first evident ~2 days earlier in the *Ler* ecotype. The overall patterns of AP1::GUS activity, however, were similar in the two lines (Figure 6).

DISCUSSION

Photoinduced Conversion of Vegetative Primordia into Flowers

Primordia initiated within vegetative *Arabidopsis* shoot apices typically produce a node comprised of both a leaf and a paraclade and thus are referred to as leaf/paraclade primordia (Hempel and Feldman, 1995). The data reported here, however, indicate that very strong photoinduction treatments can induce the paraclade (shoot) region of a young leaf/paraclade primordium to become a flower, even when the leaf region of the primordium is committed to developing as a leaf. The result is an atypical "leaf/flower" phenotype. This phenotype appeared on approximately two nodes per plant in populations exposed to CI photoinduction conditions after 30 short days. One of the two nodes typically had a macroscopic cauline leaf associated with a flower, whereas the second node typically had only a vestigial leaf at the base of the flower (Figures 2 and 4). We have concluded that these two leaf/flower nodes are derived from leaf/paraclade primordia initiated before the start of the CI photoinduction treatment and that the flower of a leaf/flower node develops from the converted paraclade region of a leaf/paraclade primordium. This conclusion is denoted graphically in Figure 3 by a curve that intercepts the Y-axis at a value of 2.

Two lines of reasoning are consistent with this interpretation. First, in a previous study, no leaves (either macroscopic or vestigial) were found under individual flowers, and the production of leaves ceased within the first day of a less potent photoinduction treatment (Hempel and Feldman, 1994). The presence of two leaf/flower nodes coupled with the fact that six additional flowers were initiated during the first 72 hr of the CI photoinduction treatment (Table 1) suggest that the flowers with subtending leaves developed from primordia initiated before the start of the CI treatment; that is, it is improbable that a stronger photoinduction treatment (the CI treatment) would induce the *de novo* formation of leaves under flowers when a weaker treatment did not (Table 1; Hempel and Feldman, 1994).

Second, photoinduction experiments with white mustard indicate that increases in mitotic activity within the shoot apex do not occur until after 1 day of induction (Gonthier et al., 1987). The relevance of the white mustard study to *Arabidopsis* flowering is indicated by experiments showing that

the responses of the two mustards to photoinduction treatments are qualitatively similar (Corbesier et al., 1996). Thus, the curve indicating the time of initiation of the first flower primordia (Figure 3, dashed line) is consistent with the assumption that the rapid acceleration of mitotic activity and the primordium initiation rate is preceded by an initial lag period during which the activity of the shoot apex is similar to that of the vegetative shoot apex (Hempel and Feldman, 1995). The hypothesized slope of the curve during the first 24 hr of induction is estimated at one primordium per day, which is slightly more than the rate of primordium initiation calculated for vegetative plants (0.8 primordia per day; Hempel and Feldman, 1994).

Alternatively, if it is assumed that the initiation of flowers did not begin until after the start of the induction treatment (i.e., after hr 0), it is implied that the rate of primordium initiation at the shoot apex would have to increase almost instantaneously from less than one primordium per day to >4.5 primordia per day. Such a rapid increase in shoot apex activity is not consistent with what is known about the activation of the shoot apex during floral induction in mustards (Gonthier et al., 1987; Corbesier et al., 1996).

Not all of the leaf/paraclade-to-leaf/flower conversions induced by the CI treatment were complete, because chimeric flower/paraclade shoots (Hempel and Feldman, 1995) were common within the populations of plants induced with CI light. The chimeric flower/paraclade shoots formed on the

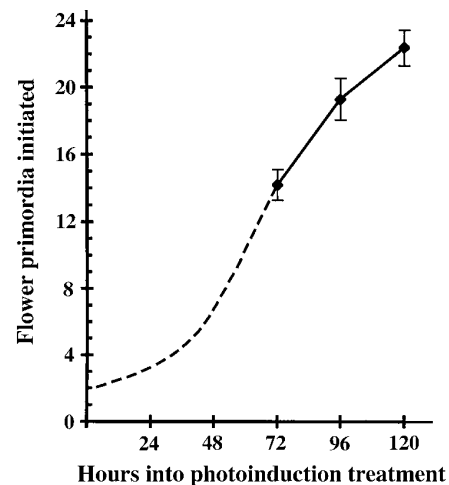


Figure 3. Initiation of Flower Primordia during a CI Photoinduction Treatment.

A population of *Ler* plants was photoinduced under CI photoinduction conditions (Table 1) after 30 short days. Initiated flower primordia were evident clearly at 72, 96, and 120 hr after the start of the photoinduction treatment. A solid line is drawn through the data points; the dashed line represents an estimation of the number of flower primordia present at earlier time points in the photoinduction treatment (see Discussion). Error bars indicate standard deviation.

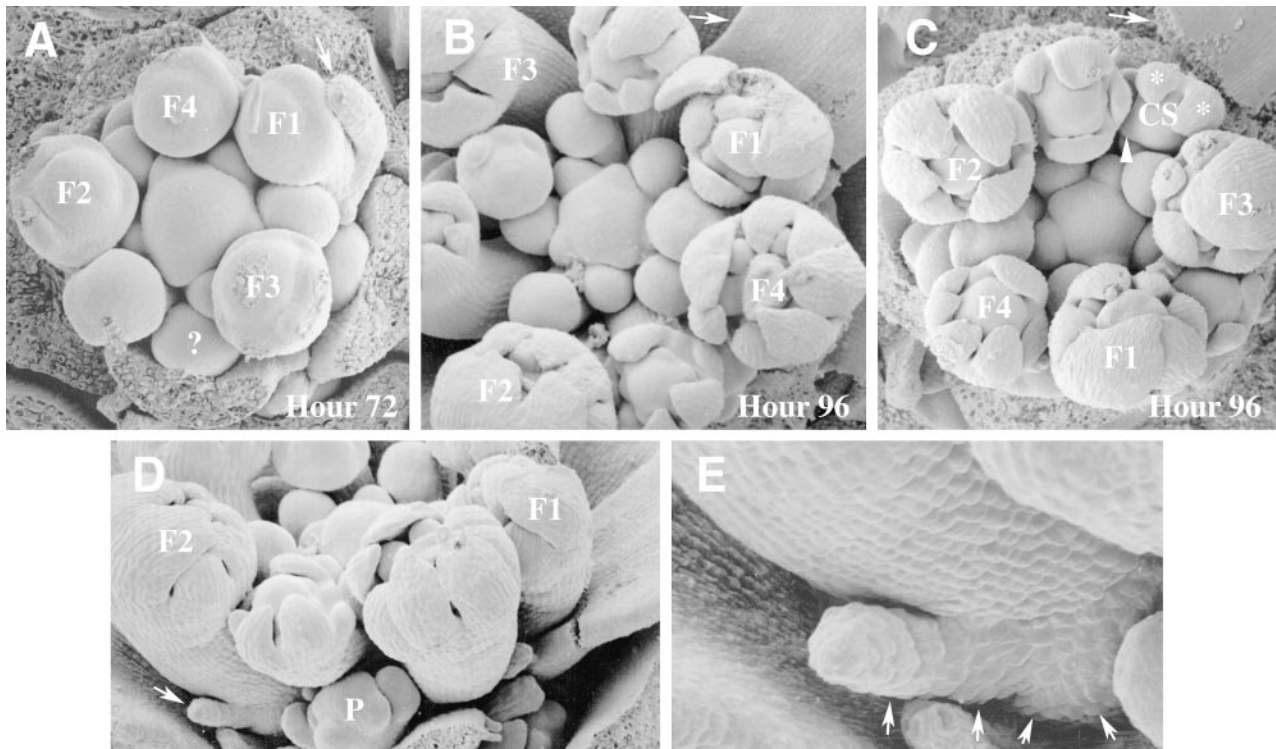


Figure 4. Shoot Apex Morphology of Plants Treated with the CI Photoinduction Treatment.

(A) Shoot apex after 72 hr of CI photoinduction. The first four flower primordia are numbered in the order in which they were initiated (F1 to F4). The identity of these primordia is indicated by the sepal primordia that encircle their periphery. The first flower (F1) and the leaf primordium subtending it (arrow) developed from the same original leaf/paraclade primordium. The identity of a shoot primordium (question mark) initiated at the node below F1 is not yet evident. Although it is most likely a paraclade, it may be a flower or a flower/paraclade (see Figure 2).

(B) Shoot apex after 96 hr of CI photoinduction. The first four flower primordia are labeled (F1 to F4). F1 is subtended by a leaf primordium (arrow) that will develop into a macroscopic leaf.

(C) A second shoot apex after 96 hr of CI photoinduction. The first four flowers are labeled (F1 to F4). A chimeric flower/paraclade shoot (CS) is evident at the node below F1. A leaf primordium subtends this shoot (arrow), and the identity of the chimeric shoot is indicated by the abaxial sepal primordia (asterisks) and the initiation of a secondary flower primordium (arrowhead) on the abaxial side of the shoot.

(D) Side view of the shoot apex shown in **(B)**. The first two flowers (F1 and F2) and a paraclade (P) are labeled. F2 is subtended by a multicellular "finger" of leaf tissue (arrow).

(E) Close-up of the leaf tissue subtending F2 in **(D)**. Arrows indicate the leaf tissue and its close association with the developing flower primordium above it.

primary inflorescence always were located below the lowest complete flower (Figures 2 and 4), which is consistent with our conclusion that they represent partially converted primordia that also were initiated before the start of the CI photoinduction treatment.

Primordium Plasticity and the Progressive Specification of Primordium Identity

For simplicity, we have described the formation of flowers from leaf/paraclade primordia initiated on vegetative plants as "conversions," implying that the primordia initially had leaf/paraclade identity. Although it is known that the converted primordia would have differentiated into leaves and

paraclades if the plants were not induced after 30 short days (Hempel and Feldman, 1994), they clearly were not strictly fated to become leaves and paraclades early in their development. The converted primordia, however, presumably had partial leaf/paraclade identity early in their development, although that identity was overridden by the influx of above-threshold levels of flower-promoting signals into the shoot apex. This partial leaf/paraclade identity, which increases as a primordium develops on a vegetative plant, may be referred to as leaf/paraclade bias.

The term bias denotes a developmental tendency but not commitment; that is, it denotes a presumptive developmental fate, based on the inductive signaling that has already occurred (Grainger, 1992). Primordia with a developmental bias, however, can take on alternative fates in response to

signaling that overrides their bias. Although the concept of developmental bias has been used primarily to characterize animal primordia (Grainger, 1992), it seems particularly useful for describing plant development, because the fates of plant cells and primordia often can be altered during their development by experimentally controlled changes in signaling (Battey and Lyndon, 1990; Irish and Nelson, 1991; van den Berg et al., 1995; Zachgo et al., 1995).

Figure 7 illustrates how the fates of existing primordia with leaf/paraclade bias are affected by photoinduction treatments and indicates the potential fates of the various vegetative primordia at the shoot apex of a 30-day-old plant (Figure 7A). If no induction treatment is given to a 30-day-old vegetative plant, all of the primordia present at the shoot apex develop into leaves and paraclades (Figure 7B; Hempel and Feldman, 1994). However, the application of a strong photoinduction treatment induces anlagen to develop as flowers and initiated leaf/paraclade primordia to develop as cauline leaves and paraclades (Figure 7C; Hempel and Feldman, 1994, 1995). The application of a CI photoinduction treatment (this study), however, induced both the anlagen and approximately two initiated primordia to produce individual flowers. It is also possible that an additional flower(s) may have been formed from an initiated primordium that had its leaf/paraclade bias overridden to the degree that no leaf identity was expressed morphologically (see Figure 7D).

The development of flowers from pre-existing vegetative primordia also occurs at the apex of photoinduced *Lolium temulentum* (Evans and Blundell, 1996); in other cases, natural changes in temperature, hydration, and photoperiod affect the fates of primordia after they have developed bias and/or partial commitment toward a certain fate (Wycherley, 1954; Deschamp and Cooke, 1985; Battey and Lyndon, 1990; Goliber and Feldman, 1990). The fact that bias can be partially or fully nullified indicates that the specification of plant primordia is progressive and not instantaneous (Christianson and Warnick, 1985; Kerstetter and Poethig, 1998). It also is consistent with previous conclusions that primordia are not specified in accordance with strict cell division patterns or cell lineage (Poethig, 1987; Kaplan and Hagemann, 1991; van den Berg et al., 1995; Smith et al., 1996). One reason that plants can easily tolerate flexible specification processes and the teratologies that are an occasional by-product (Battey

and Lyndon, 1990; Hempel and Feldman, 1995) is that they are iterative organisms. Because most plants produce numerous primordia, the precise specification of each individual primordium presumably is not of great importance.

Leaf/Flower Node Formation in Arabidopsis

The leaf/flower phenotypes discussed here differentiated in response to a potent CI photoinduction treatment, but the same general phenotype can occur on *terminal flower1 (tfl1)* mutants under standard growth conditions. *tfl1* mutants produce leaf/flower nodes under long days (Shannon and Meeks-Wagner, 1991; Alvarez et al., 1992), and their primary shoot meristems also terminate early, usually in a cluster of partial flowers (Alvarez et al., 1992). Furthermore, the severity of the *tfl1* phenotype is moderated by photoperiod (Shannon and Meeks-Wagner, 1991; Schultz and Haughn, 1993). Thus, one general interpretation of the *tfl1* mutant phenotype is that its meristems are highly sensitive to floral induction signals and therefore that *TFL1* acts to prevent the activity of floral signals within indeterminate meristems (Alvarez et al., 1992; Bowman et al., 1993). The extension of prefloral development in plants that ectopically express *TFL1* (Ratcliffe et al., 1998) is consistent with this interpretation. This suggests the possibility that *TFL1*, which is expressed at the base of the inflorescence meristem (Bradley et al., 1997), has a role in shielding indeterminate meristems from the signals that promote flower development (Liljegren and Yanofsky, 1996; Ratcliffe et al., 1998).

Transgenic plants ectopically expressing the flower meristem identity genes *LFY* and *AP1* also produce leaf/flower phenotypes throughout the primary shoot (Mandel and Yanofsky, 1995; Weigel and Nilsson, 1995), indicating that *LFY* and *AP1* are critical integrators of the flower-promoting signals produced in response to photoinduction treatments. The *LFY* and *AP1* overexpression phenotypes, however, do not indicate whether *LFY* or *AP1* responds directly to signals produced outside the shoot apex. However, the rapid induction of these genes in the developing primordia of photoinduced plants (Blázquez et al., 1997; Hempel et al., 1997) indicates that this is a possibility (see also Huijser et al., 1992). The rapid response of *LFY* to *CO* expression (Simon et al., 1996) and to gibberellins (Blázquez et al., 1998) also suggests a

Table 2. Inflorescence Phenotypes within Populations of *AP1::GUS* Plants Photoinduced under CI Conditions

Genotype (Background)	Inflorescence Phenotype ^a				
	Normal (A) (%)	Leaf/Flower/Paraclade (B) (%)	Leaf/Flower (C) (%)	1 Leaf/Flower + 1 Leaf/Paraclade (D) (%)	2 Leaf/Flowers (E) (%)
<i>AP1::GUS</i> (Ler)	13.8	12.5	52.5	7.5	13.8
<i>AP1::GUS</i> (No)	19.7	12.1	50.0	10.6	7.6

^a See Figure 2 for descriptions of each phenotype (A to E). *n* = 80 for each of the two populations. Under long-day (16 hr of light/8 hr of dark) conditions, no leaf/flower nodes were formed, and ~5% of the plants had flower/paraclade shoots. Vestigial leaf/flower nodes were not recorded.

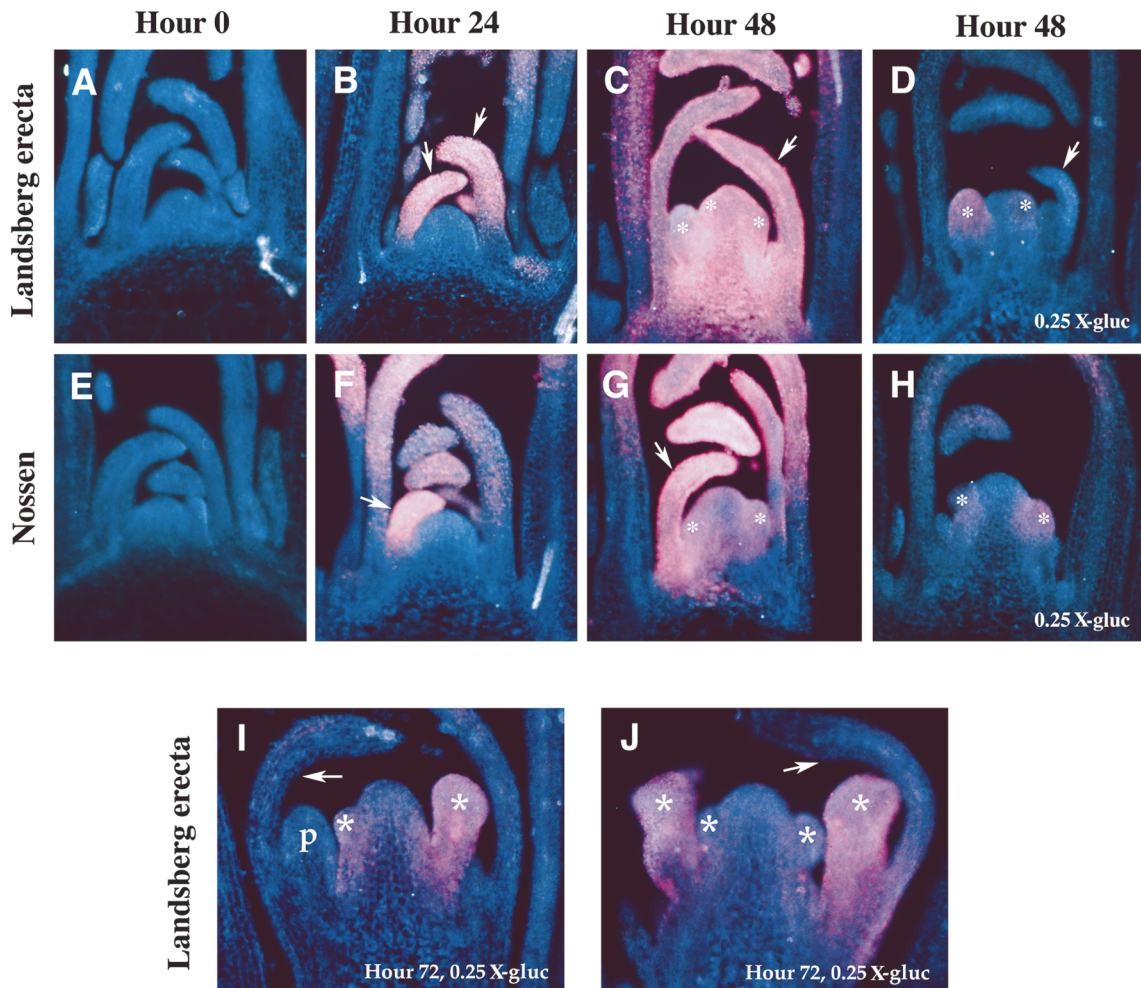


Figure 5. AP1::GUS Activity in CI-Photoinduced Ler and No Plants.

In (A) to (C) and (E) to (G), incubation was with full-strength (2 mM) X-gluc solution. In (D) and (H) to (J), incubation was with quarter-strength (0.5 mM) X-gluc solution. GUS activity is indicated by the pink color.

(A) and (E) No AP1::GUS activity is apparent at hour 0 of the CI photoinduction treatment.

(B) and (F) At hour 24, the highest levels of AP1::GUS activity are evident in the youngest leaf primordia (arrows), which were initiated before the start of the CI treatment.

(C) and (G) At hour 48, AP1::GUS activity is evident throughout the shoot apex. Arrows indicate cauline leaf primordia, and asterisks indicate flower primordia.

(D) and (H) AP1::GUS activity is strongest within flower primordia (asterisks) at hour 48, whereas young leaf primordia (arrow) have lower levels of activity. Incubation with a dilute X-gluc solution was required to detect relative differences of activity at this time point.

(I) Young paraclade primordia (p) had lower levels of AP1::GUS activity than did flower primordia (asterisks) at hour 72. The arrow indicates the leaf primordium subtending the paraclade primordium.

(J) Flower primordia subtended by leaves (arrow) had similar levels of AP1::GUS activity when compared with other flower primordia at approximately the same stage of development. Asterisks indicate four flowers in the plane of the section.

relatively direct interaction between flower-inducing signals and the *LFY* promoter.

Although we have concluded that the leaf/flower nodes that were produced in response to the CI treatment represent conversions of primordia with a leaf/paraclade bias, the

same phenotype may also arise by other mechanisms in wild-type plants. For example, in populations of No and Ler plants grown only in short days, leaves commonly subtend the lowest flower on the primary inflorescence (F.D. Hempel, unpublished data). One likely explanation for the appearance

of leaf/flower nodes under short-day conditions is that mixed signals simultaneously promote both vegetative (leaf) and reproductive (flower) development in young primordia during the transition to flowering in short days (Hempel, 1996). The appearance of a leaf under a flower in short days also may indicate the incomplete suppression of a normally cryptic leaf component that subtends the flower (see Nilsson et al., 1998).

Responses to Floral Induction Signals within the Shoot Apex

AP1::GUS activity was induced in vegetative primordia throughout the shoot apex after the start of the CI photoinduction treatment. The response of numerous primordia to signals that promote flowering is consistent with the hypothesis that primordia respond directly to developmental signals (Hempel, 1996; Blázquez et al., 1997; Kerstetter and Poethig, 1998). The last primordia initiated before the start of the photoinduction treatment were generally the most sensitive; they had the highest levels of AP1::GUS activity 24 hr after the induction treatment started (Figure 5F). This relatively strong response in these particular primordia is

consistent with our conclusion that individual flowers developed from the last primordia initiated before the start of the CI photoinduction treatment (Figures 3 and 7).

Interestingly, no clear differences in the levels of AP1::GUS activity were detected between CI-photoinduced plants and long-day-grown plants at approximately similar stages of development (cf. Figures 5 and 6); under both growth conditions, AP1::GUS activity also was present in nodes that were committed to a leaf/paraclyde fate. These results indicate that AP1 expression is not correlated strictly with a primordium's competence to develop into a flower and that the young primordia on older plants are more sensitive to the expression of floral meristem identity genes such as *LFY* and *AP1* (Mandel and Yanofsky, 1995; Weigel and Nilsson, 1995).

Although the expression of floral meristem identity genes occurs throughout the apex during the transition to flowering (Figures 5 and 6; Blázquez et al., 1997; Hempel et al., 1997), the developmental effects on leaf primordia are evidently minimal under normal circumstances. This, however, does not rule out the possibility that floral meristem identity genes can function within leaf primordia under certain circumstances. Interestingly, the ectopic expression of *AP3* and *PISTILLATA (P)* can induce petal tissue at the margins

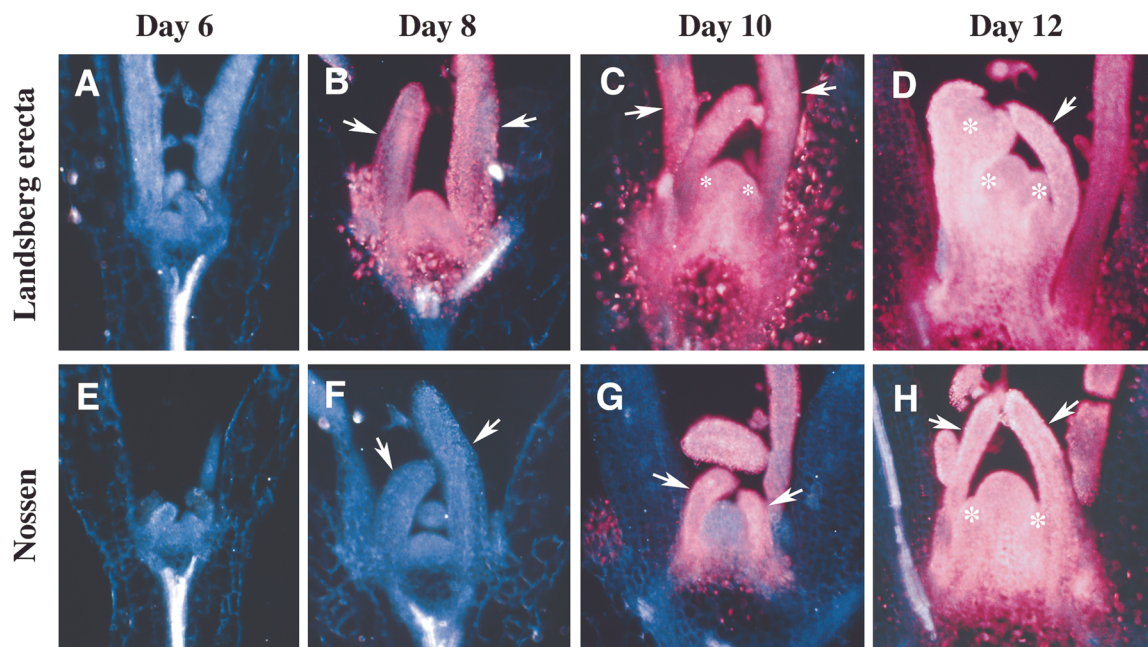


Figure 6. AP1::GUS Activity in Long-Day-Grown *Ler* and *No* Plants.

Plants were grown under long-day conditions (16 hr of light/8 hr of dark). Arrows indicate leaf primordia, and asterisks indicate flower primordia. All apices were incubated with full-strength (2 mM) X-gluc.

(A) to (D) AP1::GUS activity in *Ler*. Activity was first evident on day 8, and flower primordia were first clearly evident on day 10.

(E) to (H) AP1::GUS activity in *No*. Activity was first evident on day 10, and flower primordia were first clearly evident on day 12.

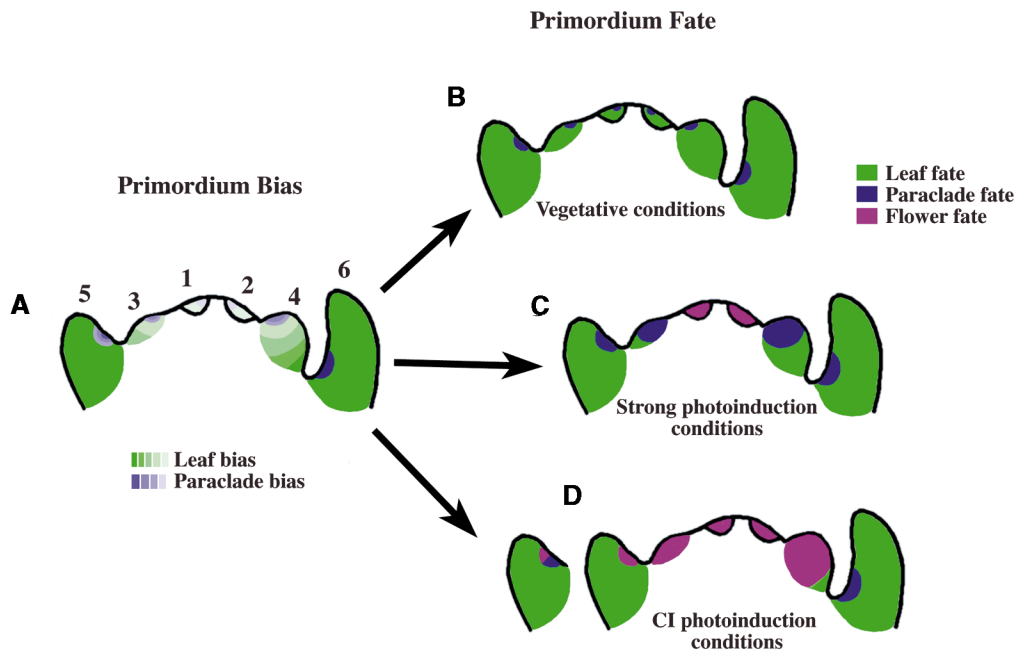


Figure 7. Primordium Bias and Primordium Fates in 30-Day-Old Arabidopsis Shoot Apices.

For simplicity, the shoot apices indicate all primordia present on a shoot apex (as though they were visible in a single plane). Primordia are numbered from youngest to oldest (1 to 6). Anlagen number is estimated at 2 (primordia 1 and 2) based on the domains of *KNAT1* and *LFY* expression in the apical dome (Weigel et al., 1992; Lincoln et al., 1994).

(A) Before photoinduction, all primordia at a 30-day-old apex are vegetative leaf/paraclade primordia. Hypothetical increases in primordium bias toward leaf and paraclade fate are indicated by increasing color density.

(B) If no photoinduction treatment is given, all of the primordia present in **(A)** will develop as leaves and paraclades.

(C) If a strong photoinduction treatment is given (Hempel and Feldman, 1994), the anlagen (primordia 1 and 2) develop as flowers, whereas all initiated primordia (3 to 6) develop as leaves and paraclades.

(D) If a CI photoinduction treatment is given (this study), the anlagen (primordia 1 and 2) develop as flowers. Initiated leaf/paraclade primordia committed to the expression of leaf fate (primordia 4 and 5) also produce individual flowers. Additional initiated primordia (e.g., primordium 3) also may develop as flowers without subtending leaves, if there is no commitment to leaf or paraclade fate within the primordium at the start of the inductive treatment.

of cauline leaves (Krizek and Meyerowitz, 1996), and it is at the margins of the cauline leaves that *AP1::GUS* activity is highest (F.D. Hempel, unpublished data). This suggests that the level of *AP1* expression at the cauline leaf margin is sufficient for the specification of petal identity, that is, if the other organ identity genes required for petal identity, namely, *AP3* and *PI* (Bowman et al., 1991), are also expressed.

It is clear from other studies that above-threshold levels of *LFY* and *AP1* expression are critical for the assignment of floral identity to meristems that are competent to respond and that the amount of *LFY* and/or *AP1* expression required for flower specification decreases as a plant matures (Mandel and Yanofsky, 1995; Weigel and Nilsson, 1995; Blázquez et al., 1997). Similar conclusions have also been reached with regard to flowering in Antirrhinum, in which above-threshold levels of the *LFY* ortholog *FLORICAULA* and the *AP1* ortholog *SQUAMOSA* are required for flower specification

(Coen et al., 1990; Huijser et al., 1992; Meijer et al., 1995; Bradley et al., 1996).

A more dramatic example of threshold-based flower induction, however, is that of *Impatiens balsamina*. In *I. balsamina*, continuous flower-promoting signals are required for complete flower specification (Krishnamoorthy and Nanda, 1968; Debraux and Simon, 1969; Battey and Lyndon, 1988; Pouteau et al., 1997), and the dampening of leaf-to-apex induction signals results in the reversion of flowers to vegetative growth. This indicates that flower specification in *I. balsamina* is controlled by the leaves, even during floral organ differentiation (Pouteau et al., 1997). Whether there is a similar need for continuous leaf-to-apex signaling in Arabidopsis is not known. Although this may seem unlikely, because Arabidopsis inflorescence shoots do not undergo a reversion to vegetative growth when moved from florally inductive to noninductive conditions (Hempel et al., 1997), we

cannot rule out the possibility that the seemingly irreversible commitment to flowering in *Arabidopsis* inflorescences is due to irreversible changes that occur outside the shoot apex.

METHODS

Plant Materials and Growth Conditions

Wild-type *Arabidopsis thaliana* Landsberg *erecta* (*Ler*) plants were used in the first photoinduction experiment (Table 1 and Figures 1 to 4). The generation of a standard *APETALA1* and β -glucuronidase (*AP1::GUS*) line (AM154.5c/Nossen [No]) was described previously (Hempel et al., 1997). A second line (AM154.5c/*Ler*), with a reporter gene at the same genetic locus, was constructed by crossing AM154.5c/No into the *Ler* background four times. These lines were used in the second pair of experiments (Table 2 and Figures 5 and 6).

See Hempel and Feldman (1995) for general growth conditions. Short-day (8-hr light/16-hr dark) preinduction light conditions and long-day (16-hr light/8-hr dark) light conditions were 100 $\mu\text{E m}^{-2} \text{sec}^{-1}$, with 90% from cool-white bulbs and 10% from incandescent bulbs. Continuous incandescent-enriched (CI) photoinduction conditions are described in Table 1. The addition of incandescent light bulbs, which are high in far-red light (and decrease the red/far-red ratio), enhances the flowering response of *Arabidopsis* (Martinez-Zapater and Somerville, 1990; Lee and Amasino, 1995). Although we chose to promote flowering by adding far-red light and extending the light period, flowering also can be promoted by the addition of blue light (Guo et al., 1998).

The CI photoinduction treatment started after 30 days of vegetative growth in short days. Hr 0 of the photoinduction treatment occurred 8 hr into the continuous light period, when the plants were first exposed to a light extension. After \sim 1 week of CI photoinduction, the plants showed signs of stress, including anthocyanin production and slight bleaching. This may indicate that stress is partially responsible for the very strong flowering response in the induced plants.

Scanning Electron Microscopy, Morphological Analyses, and GUS Assays

Scanning electron microscopy and morphological analyses were as described previously (Hempel and Feldman, 1994, 1995). All GUS assays, tissue sectioning, and microscopy were as described previously (Hempel et al., 1997). In some assays, the 2 mM 5-bromo-4-chloro-3-indolyl β -D-glucuronic acid (X-gluc) solution was diluted with PBS buffer (three parts PBS to one part X-gluc solution) to indicate regions of relatively high *AP1::GUS* activity within shoot apices (Figures 5D and 5H to 5J). Under dark-field optics, the X-gluc product appears pink.

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