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Photo and Hormonal Control of Meristem Identity in the Arabidopsis Flower Mutants *apetala2* and *apetala1*

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We have analyzed the contributions of phytochrome and gibberellin signal transduction to the control of flower meristem identity in the Arabidopsis mutants *apetala1* (*ap1*) and *apetala2* (*ap2*). *ap1* flowers are partially defective for the establishment of flower meristem identity and are characterized by the production of ectopic secondary or axillary flowers and by branching. Axillary flower production is also induced in *ap2-1* flowers by short-day photoperiod and is suppressed by *hy1*, a mutation blocking phytochrome activity. The production of axillary flowers by *ap2-1* is also suppressed by exogenous gibberellins and by *spindly* (*spy*), a mutation that activates basal gibberellin signal transduction in a hormone-independent manner. Ectopic axillary flower production and floral branching by *ap1* flowers are also suppressed by *spy*. We conclude that gibberellins promote flower meristem identity and that the inflorescence-like traits of *ap2-1* and *ap1-1* flowers are due in part to *SPY* gene activity.

INTRODUCTION

In Arabidopsis, the inflorescence shoot apical meristem is indeterminate and capable of producing numerous rosette and cauline leaves, lateral shoots, and floral buds that arise in a spiral pattern of phyllotaxis (Hempel and Feldman, 1994; Medford et al., 1994). Although the floral meristem is closely related spatially and by cell lineage to the inflorescence meristem, it proceeds along a determinate developmental pathway and is characterized by the production of four sets of organs—sepals, petals, stamens, and carpels—that arise sequentially in a whorled pattern of phyllotaxis (Smyth et al., 1990). Genetic and molecular studies in Arabidopsis and other systems have defined a network of genes that control the establishment and maintenance of flower meristem identity and determinacy, including *APETALA1* (*AP1*), *APETALA2* (*AP2*), *LEAFY* (*LFY*), *CAULIFLOWER* (*CAL*), *TERMINAL FLOWER* (*TFL*), and *AGAMOUS* (*AG*) (reviewed in Schultz and Haughn, 1993; Weigel, 1995). However, little is known about the signals that control the activities of these genes. Previously, we showed that the floral meristem mutants *ag* and *lfy* can be used to characterize the signals that control the maintenance of flower meristem identity in Arabidopsis (Okamuro et al., 1993, 1996). In this study, we have begun to analyze the signals that control the establishment of flower meristem identity and meristem determinacy by using the floral mutants *ap2* and *ap1*.

AP2 is best known for its role in the specification of flower organ identity and the negative regulation of *AG* homeotic gene expression (Komaki et al., 1988; Kunst et al., 1989; Bowman et al., 1991a, 1991b; Coen and Meyerowitz, 1991; Drews et al., 1991; Meyerowitz et al., 1991). In wild-type flowers, *AG* gene expression is temporally and spatially restricted to stamens and carpels. In strong *ap2* mutants, *AG* is prematurely activated and ectopically expressed in flower development, resulting in the homeotic transformation of sepals into ovule-bearing carpels and the repression of petal development (Bowman et al., 1991a, 1991b; Drews et al., 1991).

In addition to its functions in the control of floral organ identity, *AP2* has also been shown to control the establishment of flower meristem identity, in part through its interaction with the floral meristem identity gene *AP1* (Irish and Sussex, 1990; Bowman et al., 1993; Shannon and Meeks-Wagner, 1993). Strong *ap1* mutants produce highly branched, inflorescence-like flowers that are characterized by the lack of petals, by the replacement of sepals with bractlike leaves, and by the production of ectopic secondary flowers in the axils of the first-whorl leaves (Irish and Sussex, 1990; Bowman et al., 1993; Schultz and Haughn, 1993). These ectopic secondary or axillary flowers may in turn produce tertiary axillary flowers. By contrast, *ap1-1 ap2-1* double mutants produce flowers that are more indeterminate and inflorescence-like than either single mutant alone (Irish and Sussex, 1990; Bowman et al., 1993; Schultz and Haughn, 1993; Shannon and Meeks-Wagner, 1993).

The role of *AP2* and *AP1* in the establishment of flower meristem identity is further revealed under short-day (SD) photoperiod. SD-grown *ap2-1* flowers reportedly show enhanced inflorescence-like characteristics (Komaki et al., 1988; Okamuro et al., 1993; Schultz and Haughn, 1993). Similarly, the inflorescence-like nature of *ap1* flower development

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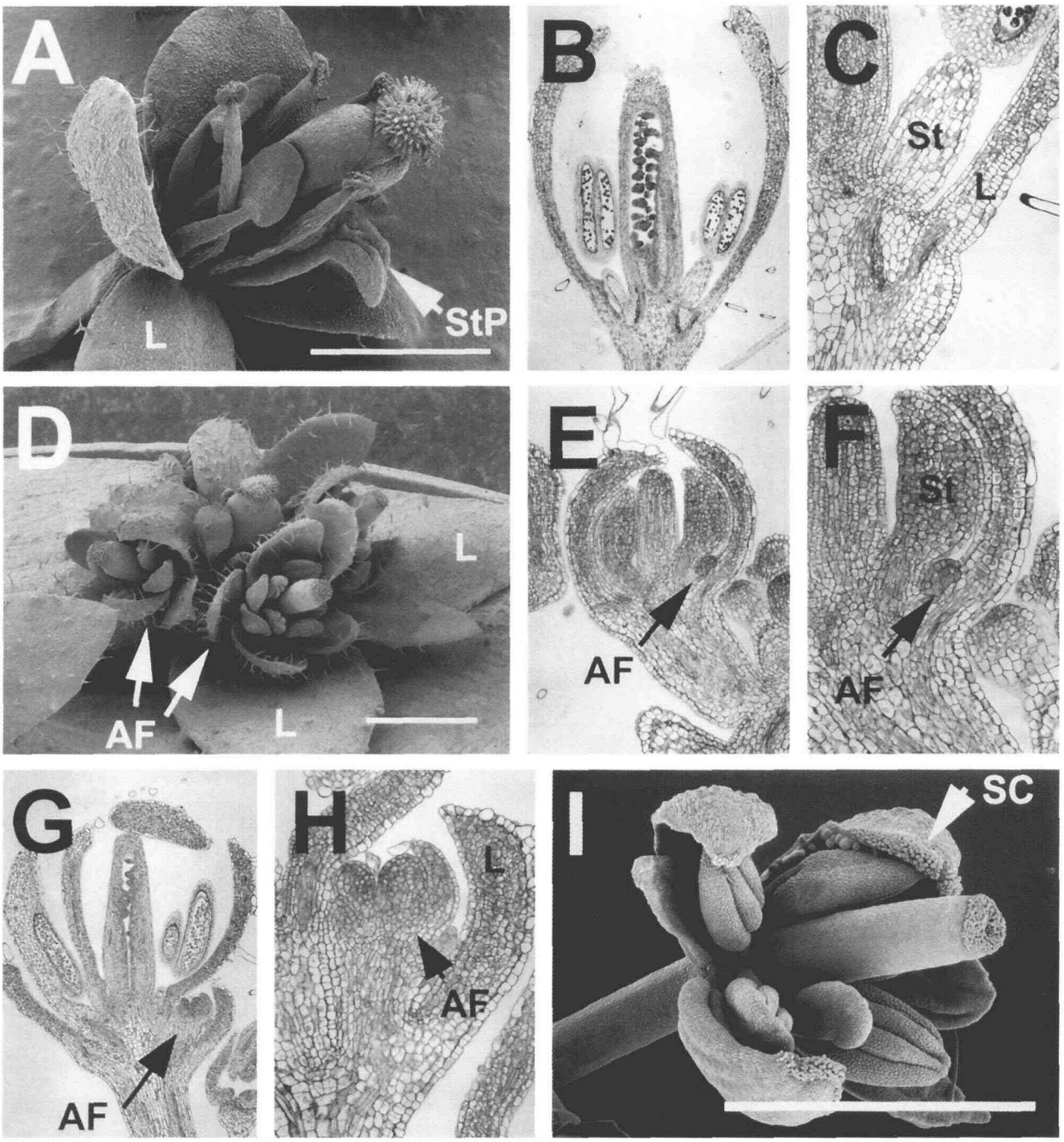

Figure 1. Comparison of LD- and SD-Grown *ap2-1* Flower Structure.

(A) LD *ap2-1* flower taken from a basipetal position on the primary inflorescence. This LD *ap2-1* flower has produced four leaves (L), four staminoid petals (StP), six stamens, and two fused carpels. Bar = 1 mm.

(B) and **(C)** Longitudinal section through an LD *ap2-1* flower. The flower shows the whorled phyllotaxis and the production of leaves, stamens, and carpels typical of *ap2-1* flower development under LD conditions **(B)**. A higher magnification **(C)** shows that there is no detectable flower primordia in the axil of the floral leaf (L). St, stamen.

(D) SD *ap2-1* flower taken from a basipetal position on the primary inflorescence. This SD *ap2-1* flower has produced six leaves (L), four secondary axillary flowers (AF), six stamens, and two fused carpels. Bar = 1 mm.

is enhanced under SD photoperiod, with mutant flowers replaced by highly branched indeterminate shoots (Schultz and Haughn, 1993). Together, these studies suggest that *ap2* and *ap1* are hypersensitive to the intrinsic and environmental signals that control the establishment of flower meristem identity and suppress meristem indeterminacy. We have used the *ap2* and *ap1* mutants to begin to analyze the signals and signal transduction pathways involved in the control of flower meristem identity.

RESULTS

Transforming *ap2-1* Flowers into Shoots

To understand how *ap2* flower development is affected by photoperiod, we compared the structure of long-day (LD)–grown and SD-grown *ap2-1* flowers. We chose *ap2-1* for this analysis because it has been well characterized both phenotypically and at the molecular level (Bowman et al., 1989, 1991b; Jofuku et al., 1994). As shown in Figure 1A, under LD conditions, *ap2-1* mutant flowers are characterized by the homeotic transformation of first-whorl sepals into leaves that are distinguished by the presence of branched or stellate trichomes. Second-whorl organs are transformed from petals into staminoid petals. By contrast, third- and fourth-whorl organs develop into normal pollen-producing stamens and ovule-bearing carpels, respectively. *ap2-1* flowers also display a compact and whorled pattern of floral organogenesis, as illustrated in Figures 1B and 1C.

The transformation of sepals into leaves in *ap2-1* flowers is thought to reflect the absence of *AP2* gene activity in the specification of floral organ identity (Coen and Meyerowitz, 1991; Meyerowitz et al., 1991). Alternatively, the production of leaves might reflect a partial transformation in meristem identity from flower to shoot due to the role of *AP2* in the establishment of floral meristem identity. Consistent with this hypothesis, previous studies have reported that the inflorescence-like traits of weak *ap2* flowers are dramatically enhanced by SD photoperiod (Komaki et al., 1988; Okamuro et al., 1993; Schultz and Haughn, 1993). As shown in Figure 1D, the most striking alteration in *ap2-1* flower development is the production of ectopic secondary or axillary flowers. Table 1 shows that this SD-induced change in meristem activity is photoperiod dependent and not due to the decrease in total light per day because a compensatory increase in SD light intensity did not suppress axillary flower formation.

Table 1. Photo and Hormonal Control of Secondary Flower Production in *ap2-1* Flowers

Genotype	Light and Hormone Treatments[a]	Flowers per Plant with Visible Secondary Flowers[b]	No. of Plants Examined
ap2-1	LD	0 (0)	10
ap2-1	SD	7 (3.0)	13
ap2-1	SD (D)	15 (4.4)	15
ap2-1 hy1-1	SD (D)	3.3 (3.3)	18
ap2-1	SD + 10^{-5} M GA_3	0 (1.1)	15
ap2-1	SD (D) + 10^{-5} M GA_3	0 (0.6)	15
spy-2 ap2-1	SD (D)	0 (0)	7
WT *Ler*[c]	LD	0 (0)	15
WT *Ler*	SD (D)	0 (0)	15

[a] Plant growth conditions and light regimes are described in Methods. LD, long day; SD, short day; SD (D), short-day double incident light. Exogenous application of gibberellin A_3 (GA_3) was performed as described in Methods.

[b] Indicated are the median number of flowers per primary inflorescence that produce secondary flowers which range in number from one to five. Standard deviation values are given in parentheses. No secondary flower production was observed in *hy1*, *spy*-2, or wild-type Landsberg *erecta* + 10^{-5} M GA_3 control plants grown under SD conditions.

[c] Wild-type Landsberg *erecta*.

Longitudinal sections through SD-grown *ap2-1* flowers show that secondary flowers emerge from densely staining meristematic cells located in the axils of the first-whorl leaves (Figures 1E to 1H). These densely staining cells appear after stamen and carpel primordia have been initiated (Figures 1E and 1F) and are not detectable in LD-grown *ap2-1* flowers (Figure 1C). As the axillary flowers develop, the regions between the first-whorl leaves elongate to form a floral internode (data not shown). The transformation of sepals into leaves and the production of axillary flowers under SD photoperiod are not specific to the *ap2-1* allele but have also been observed in *ap2-3*, *ap2-4*, *ap2-5*, and *ap2-7* flowers (Komaki et al., 1988; C. Lotys-Prass and J.K. Okamuro, data not shown). Taken together, the production of leaves and axillary flowers as well as floral internode elongation in *ap2* flowers support the hypothesis that the *ap2-1* floral meristem is partially inflorescence-like in identity.

Figure 1. (continued).

(E) to **(H)** Longitudinal section through developing SD *ap2-1* flowers. The secondary flower primordium (AF) is visible as a densely staining group of cells located at the base of the first-whorl leaf [**(E)** and **(F)**] that develops into a young axillary flower bud, as shown in **(G)** and **(H)**. The separation of leaves by floral internode elongation is also visible. L, floral leaf; St, stamen.

(I) LD *ap2-1 spy-2* flower taken from a basipetal position on the primary inflorescence. This flower is characterized by the absence of petals, the homeotic transformation of leaves into carpelloid sepals (SC), and normal stamens and carpels. Bar = 1 mm.

Apical–Basal Gradient of *ap2-1* Axillary Flower Production

Previous studies have reported that *ap2* flower development can vary dramatically according to the apical–basal position of a flower on the inflorescence stem (Bowman et al., 1989, 1991b; Kunst et al., 1989; Irish and Sussex, 1990). The strongest defects in *ap2-1* flower development are displayed by the distal or acropetal flowers on the primary inflorescence. By contrast, we observed that axillary flower production is highest in early-arising basipetal flowers and that axillary flower production declines acropetally or toward the apex of the inflorescence. Figure 2A shows that between 75 and 97% of the first 10 basipetal flowers produced by the primary inflorescence will produce axillary flowers under SD conditions. The frequency of axillary flower production declines gradually to zero by position 22. Figure 2B shows that the number of axillary flowers produced by each flower also declines acropetally from an average of 2.4 secondary flowers at position 1 to less than one by position 13.

There are several hypotheses to explain why the frequency of axillary flower production forms a gradient along the primary inflorescence. First, axillary flower production may reflect an intrinsic and progressive acropetal decrease in the requirement for *AP2* gene activity for the establishment of flower meristem identity. Second, *ap2* flowers may be defective for the ability to perceive or to respond to a signal that either represses inflorescence shoot meristem identity or that promotes flower meristem identity.

Control of *ap2-1* Axillary Flower Production by Phytochrome

The observation that *ap2-1* axillary flower production and flower meristem identity are photoperiod dependent suggested to us that meristem identity may be governed in part by the phytochrome system of photoreceptors. To explore this hypothesis, we used a genetic approach to test whether phytochrome activity is required for SD axillary flower formation. There are at least five phytochrome genes in Arabidopsis (*PHYA* to *PHYE*; Sharrock and Quail, 1989; Clack et al., 1994). The activities of all five genes are strongly suppressed by the *hy1* mutation that blocks phytochrome chromophore biosynthesis (Parks and Quail, 1991). We used the *hy1-1* allele (Koornneef et al., 1980) to generate *ap2-1 hy1-1* double mutants and grew these plants under SD conditions. Table 1 shows that *hy1-1* was able to suppress *ap2-1* axillary flower production by >75%. On average, only 3.3 flowers per primary inflorescence produced a single axillary flower in *ap2-1 hy1-1* plants compared with 15 flowers per plant in *ap2-1* single mutants. A similar result was obtained by using *ap2-1 hy2-1* mutant plants (data not shown). Together, these results support the proposal that the shootlike activity of *ap2-1* flowers is due in part to phytochrome.

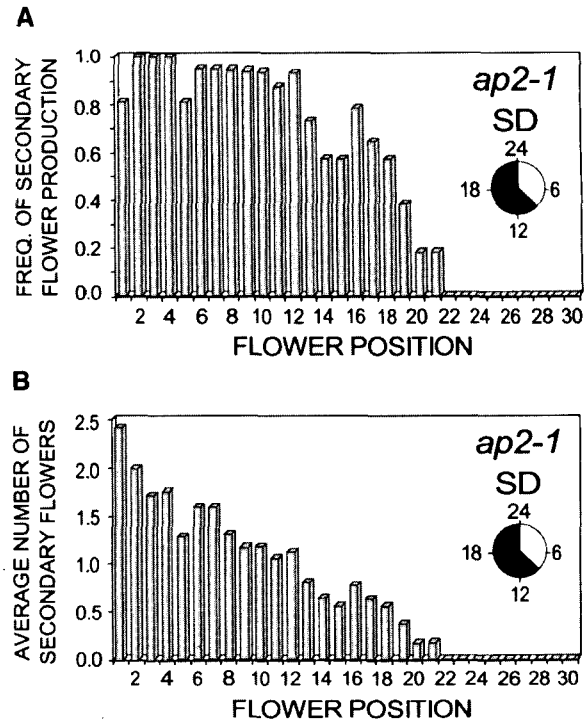

Figure 2. Secondary Axillary Flower Production by SD *ap2-1* Flowers Reveals a Basipetal–Acropetal Gradient on the Primary Inflorescence.

(A) Shown is the frequency of SD *ap2-1* secondary flower production, according to position on the primary inflorescence. Flowers were numbered sequentially, beginning with the first flower produced by the inflorescence.

(B) Shown is the average number of secondary flowers per *ap2-1* floral meristem by position on the primary inflorescence.

Gibberellins Suppress *ap2-1* Flower Meristem Indeterminacy

How does the perception of photoperiod by phytochrome control *ap2-1* flower development? One hypothesis is that photoperiod affects gibberellin (GA) synthesis or activity. GA activity is governed by photoperiod in many plant species, including Arabidopsis (Gianfagna et al., 1983; Pharis et al., 1987; Talón and Zeevaart, 1992; Zeevaart and Gage, 1993; Jordan et al., 1995; Xu et al., 1995). GAs are also one of at least two signals that have been shown to promote flowering in Arabidopsis (Langridge, 1957; Napp-Zinn, 1969; Wilson et al., 1992). Therefore, we reasoned that *ap2-1* axillary flower production may be a programmed response to a decrease in GA levels, in GA activity, or in the ability to perceive GAs under SD photoperiod.

To test these hypotheses, we treated SD *ap2-1* plants with 10^{-5} M gibberellin A_3 (GA_3) and with 10^{-5} M gibberellin A_{4+7} (GA_{4+7}) (see Methods), both of which have been shown to be biologically active in Arabidopsis (Langridge, 1957;

Kobayashi et al., 1993). Table 1 shows that GA_3 completely suppressed axillary flower production in *ap2-1*. Similarly, GA_{4+7} was equally effective in suppressing axillary flower production (K.D. Jofuku, C. Lotys-Prass, and J.K. Okamuro, data not shown). Thus, the *ap2-1* flower meristem is capable of responding developmentally to exogenous GAs.

To confirm genetically that GA signaling can suppress *ap2-1* axillary flower production, we used the *SPINDLY* (*SPY*) gene mutation *spy-2* (Jacobsen and Olszewski, 1993). Homozygous mutations in *SPY* activate a basal level of GA signal transduction in a hormone-independent manner. In general, *spy* mutants exhibit no dramatic effect on flower meristem identity. However, *spy-2* mutants do occasionally produce three and sometimes four carpels (Jacobsen and Olszewski, 1993). Table 1 shows that *spy-2* was able to completely suppress secondary flower production by SD-grown *ap2-1* flowers. In addition, Figure 1I shows that the inflorescence-like character of the *ap2-1* flower is suppressed by *spy*. Under LD conditions, *ap2-1 spy-2* double mutant flowers display an enhanced transformation of first-whorl organs from leaves into carpelloid sepals, including the production of stigmatic papillae and ovule primordia. In addition, the production of second-whorl staminoid petals is strongly suppressed. We conclude from these results that *SPY* promotes the inflorescence-like character of *ap2-1* flowers and that the SD-induced changes in *ap2-1* flower development can be attributed to changes in GA synthesis, activity, or perception.

Photocontrol of Axillary Flower Production in *ap1* Flowers

ap1 flowers, unlike *ap2*, produce axillary flowers under LD conditions. *ap1-1* is among the best studied of the *ap1* mutants and is presumed to be a null allele (Irish and Sussex, 1990; Mandel et al., 1992; Bowman et al., 1993). As shown in Figure 3A, LD-grown *ap1-1* flowers are characterized by the absence of petals and the production of axillary flowers. Stamen and carpel development are normal. Figures 3B and 3C show that LD *ap1-1* flower development is structurally similar to SD *ap2-1* flower development (Figure 1). First-whorl organs are punctuated by the production of secondary flowers that develop in the axils of the first-whorl organs after the initiation of stamen and carpel primordia. Moreover, floral internode elongation occurs between first-whorl organs as the flower matures. Thus, like SD *ap2-1* flowers, LD *ap1-1* flowers are inflorescence-like in meristem activity yet also produce normal stamens and carpels.

The phenotype of *ap1-1* flowers is also dictated by their position on the inflorescence (Irish and Sussex, 1990; Schultz and Haughn, 1993). Figure 4A shows that LD *ap1-1* plants display a gradient of decreasing axillary flower production similar to that shown by SD *ap2-1* plants (Figure 2). The frequency of axillary flower production is highest in basipetal flowers, ranging from 77 to 100% through flower position 11 on the primary inflorescence, and then gradually declines to 10% at position 28. Figure 4B shows that the average number of secondary flowers produced by each primary flower also declines acropetally in *ap1-1*.

Like *ap2* flowers, the phenotype of *ap1* flowers is also sensitive to photoperiod (Irish and Sussex, 1990; Bowman et al., 1993). Figures 3D to 3F show that SD growth conditions result in the enhanced transformation of the *ap1-1* flower into a highly branched inflorescence shoot. Thus, SD conditions strongly influence the establishment of floral meristem identity and determinate flower development in *ap1-1*. Based on the similarity between *ap1-1* and *ap2-1* flower development, the photocontrol of mutant flower development, and the gradient of mutant flower phenotypes, we hypothesized that the pattern of *ap1-1* flower development may also be controlled by GAs.

GA Signal Transduction Promotes *ap1* Flower Meristem Identity and Organogenesis

Can GAs influence *ap1* flower meristem identity and axillary flower production as they do in SD *ap2-1* flowers? To test this hypothesis, we generated *ap1-1 spy-3* double mutant plants and grew them under LD conditions. Table 2 shows that *spy-3* strongly suppressed *ap1-1* axillary flower production. *ap1-1 spy-3* double mutant flowers produced less than two secondary flowers per primary inflorescence. *spy-3* also strongly suppressed the inflorescence-like character of *ap1-1* flowers. Figure 3G shows that under LD photoperiod, basipetal *ap1-1 spy-3* flowers are characterized by the production of leaflike organs, the absence of petals, and the occurrence of normal stamens and carpels. Others failed to make leaves and produced only stamens and carpels due to the abortion of organ primordia early in flower development (data not shown). Moreover, Figure 3H shows that when compared with SD *ap1-1* flowers (Figure 3D), SD *ap1-1 spy-3* flowers are less inflorescence-like and display a reduction in branching and in floral bud formation. In addition, the floral leaves can display partial staminoid and carpelloid transformations that include the production of pollen sac and stigmatic papillae, respectively. From these results, we conclude that *SPY* promotes inflorescence-like development in LD *ap1* flowers as it does in SD *ap2-1* flowers.

To confirm the GA hypothesis for *ap1-1* flower development, we sprayed *ap1-1* flowers with GA_3 (see Methods). As shown in Figure 5, we observed that axillary flower production in *ap1-1* was strongly but not completely suppressed by exogenous GAs. We infer from the incomplete suppression of axillary flower production by exogenous GAs that this floral phenotype is intermediate between that of *ap1-1* and *ap1-1 spy-3*. These results support the hypothesis that a GA signal can promote *ap1* flower meristem identity and suppress inflorescence meristem identity and axillary flower

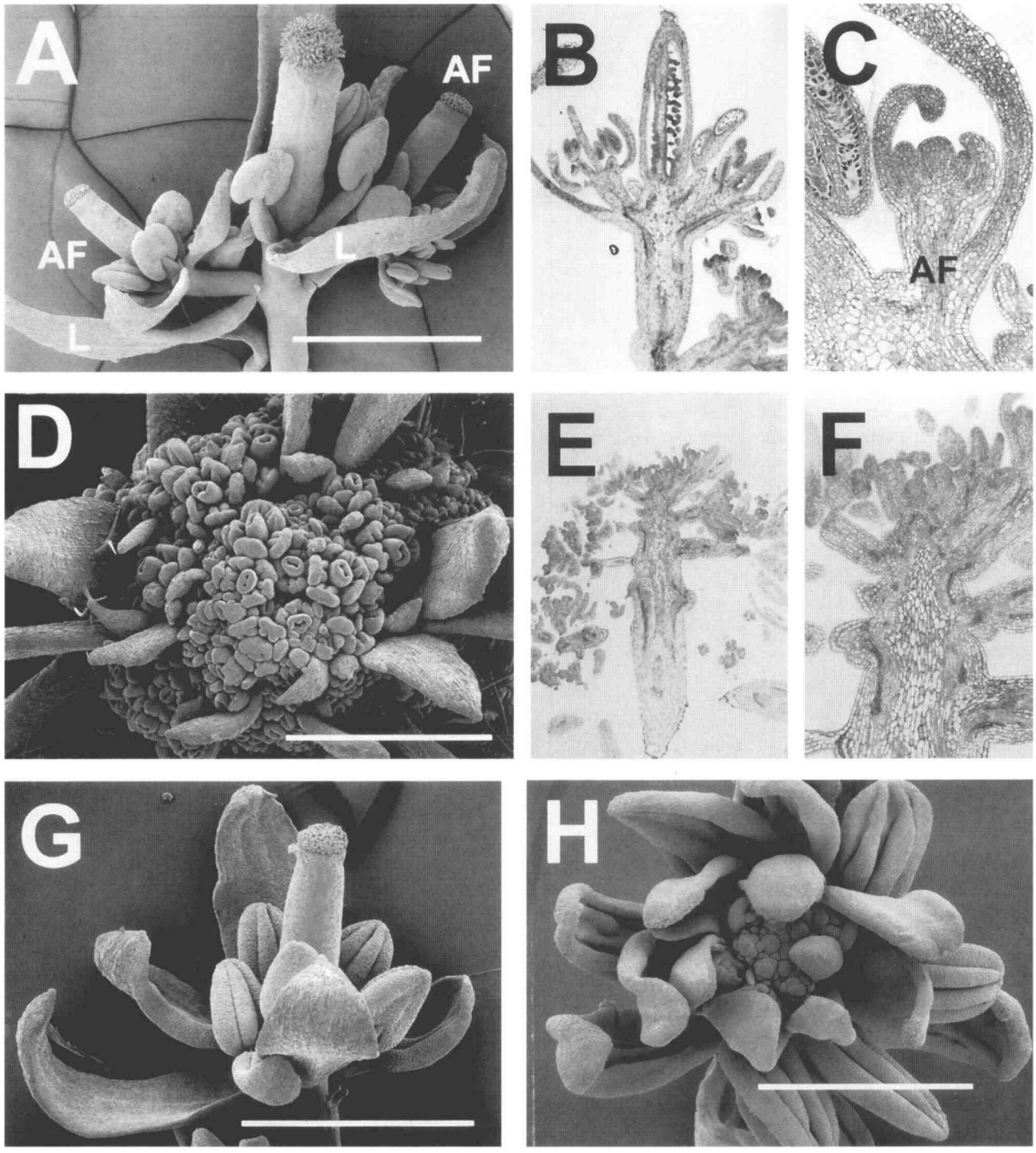

Figure 3. Axillary Flower Production by *ap1-1* Flowers.

(A) LD *ap1-1* flower taken from a basipetal position on the primary inflorescence. This LD *ap1-1* flower lacks petals and has produced four leaves (L), three axillary flowers (AF), six stamens, and two fused carpels. Bar = 1 mm.

(B) and **(C)** Longitudinal section through an LD *ap1-1* flower. This LD *ap1-1* flower is distinguished by the production of secondary flowers from the axils of two floral leaves and is similar in structure to the SD *ap2-1* flower shown in Figures 1G and 1H. A higher magnification of **(B)** is shown in **(C)**.

(D) SD-grown *ap1-1* flower. This structure has produced numerous leaves and flower buds and shows the enhanced highly branched inflorescence-like character typical of SD-grown *ap1-1* flowers. Bar = 1 mm.

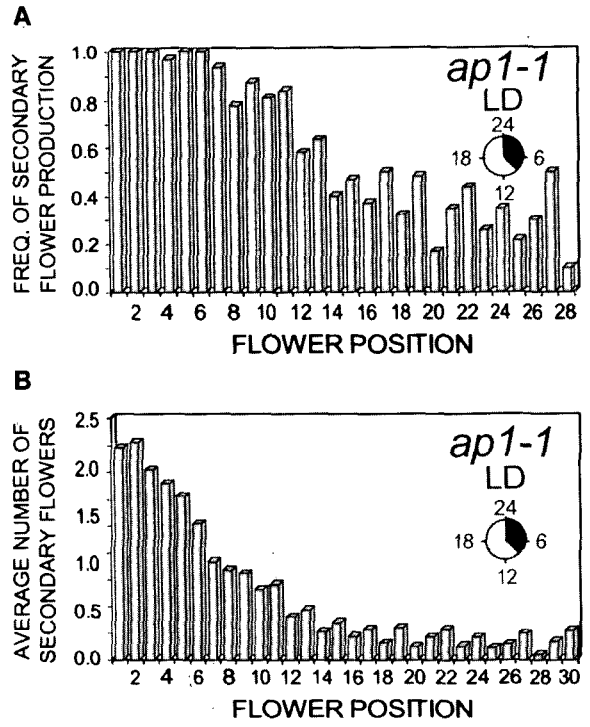

Figure 4. Secondary Axillary Flower Production by LD *ap1-1* Flowers Reveals a Basipetal–Acropetal Gradient on the Primary Inflorescence.

(A) Shown is the frequency of secondary flower production by LD *ap1-1* flowers, according to their position on the primary inflorescence.

(B) Shown is the average number of secondary flowers produced by LD *ap1-1* flowers, according to their position on the primary inflorescence.

production. One striking additional effect of exogenous GAs on *ap1-1* flower development was the enhanced production of petals (Figure 5B). Normally, petals are rarely produced by LD-grown *ap1-1* flowers (Irish and Sussex, 1990; Bowman et al., 1993). We observed that exogenous GAs also promote petal development in SD-grown *ap2-1* flowers (Figure 5C). Together, these data suggest that the effects of *ap1-1* and *ap2-1* on petal development may be due in part to defects in GA signaling.

DISCUSSION

The inflorescence-like traits of *ap2-1* and *ap1-1* flower development, such as the production of leaves, axillary flowers, and branching, are regulated by photoperiod. We have used these traits to analyze the signals that control the establishment of flower meristem identity in Arabidopsis. Our results show that *ap2-1* flower development is governed in part by phytochrome, *SPY*, and GAs. Similarly, we conclude that *ap1-1* flower development is governed in part by *SPY* and by GAs.

Phytochrome and *SPY* Promote Flower Meristem Indeterminacy

An important conclusion from this study is that phytochrome promotes inflorescence meristem identity in *ap2-1* flowers.

Table 2. Photo and Hormonal Control of Secondary Flower Production in *ap1-1* Flowers

Genotype	Light Treatment[a]	Flowers per Plant with Visible Secondary Flowers[b]	No. of Plants Examined
ap1-1	LD	13 (3)	26
ap1-1 spy-3	LD	1.5 (2.1)	13
WT *Ler*[c]	LD	0 (0)	15
WT *Ler*	SD (D)	0 (0)	15

[a] Plant growth conditions and light regimes are as described in Methods and in Table 1.

[b] *ap1-1* plants were scored for the number of flowers on the primary inflorescence that made at least one visible secondary flower. The median number of flowers per primary inflorescence that produce secondary flowers is given. Standard deviation values are given in parentheses. No secondary flower production was observed in *spy-3* control plants grown under LD conditions.

[c] Wild-type Landsberg *erecta*.

Figure 3. (continued).

(E) and **(F)** Longitudinal section through a single SD *ap1-1* flower similiar in developmental stage to that shown in **(A)**. This section illustrates the enhanced indeterminate nature of *ap1-1* flower development induced by an SD photoperiod **(E)**. A higher magnification of **(E)** is shown in **(F)**.

(G) *spy-3* suppresses axillary flower production in LD *ap1-1* plants. This flower was taken from position six on the primary inflorescence and is representative of *ap1-1 spy-3* basipetal flowers. It has no axillary flowers and has produced four leaves, one staminoid leaf, six stamens, and two fused carpels. Bar = 1 mm.

(H) *spy-3* suppresses the inflorescence-like character of SD *ap1-1* flower development. This *ap1-1 spy-3* flower displays a reduction in SD-induced branching and the production of floral buds. In addition, floral leaves display partial staminoid and carpelloid characteristics, including the production of pollen sacs and stigmatic papillae, respectively. Bar = 1 mm.

Figure 5. Exogenous GAs Suppress Inflorescence-like Traits in *ap1-1* and *ap2-1* Flowers.

(A) LD *ap1-1* control flower.

(B) GA-treated LD *ap1-1* plants produce flowers showing partial suppression of axillary flower production and the induction of petals. GA treatments were performed as described in Methods.

(C) SD *ap2-1* primary inflorescence from a GA-treated plant (left), showing production of petals compared with an untreated SD *ap2-1* control inflorescence (right). GA treatments were performed as described in Methods.

Axillary flower production in *ap2-1* is induced under SD photoperiod and suppressed under LD photoperiod (Table 1). SD-induced secondary flower formation has also been observed in other *ap2* mutants, including *ap2-3*, *ap2-4*, *ap2-5*, and *ap2-7* (Komaki et al., 1988; Bowman et al., 1993; C. Lotys-Prass and J.K. Okamuro, unpublished results). This form of floral meristem indeterminacy is strongly suppressed by *hy1*, which blocks phytochrome activity (Table 1).

SD photoperiod also promotes the transformation of *ap1-1* flowers into inflorescence-like shoots (Figure 3D). Both SD *ap2-1* and SD *ap1-1* flower phenotypes are due in part to *SPY* gene activity. *SPY* has been shown to function genetically as a negative regulator of GA signal transduction (Jacobsen and Olszewski, 1993; Jacobsen et al., 1996). We observed that *spy-2* suppresses leaf development and axillary flower production in *ap2-1* flowers under SD photoperiod (Table 1). Similarly, *spy-3* suppresses axillary flower development, floral branching, and meristem indeterminacy in *ap1-1* flowers under both LD and SD conditions (Table 2, and Figures 3G and 3H). Thus, we conclude that *SPY* is required to promote meristem indeterminacy in both *ap2-1* and *ap1-1* flowers.

ap2-1 hy1-1 double mutant flowers produce a low but significant number of residual axillary flowers under SD photoperiod (Table 1). One hypothesis to explain this result is that there remains a low level of physiologically active and biochemically detectable phytochrome in *hy1-1* (Cone and Kendrick, 1985; Parks et al., 1989; Lifschitz et al., 1990; Whitelam and Smith, 1991). Alternatively, the residual axillary flower production by SD *ap2-1 hy1-1* flowers may indicate the activity of a second phytochrome-independent signal that promotes axillary flower production. Consistent with this hypothesis, we observed that axillary flower production in LD *ap1-1* flowers, unlike SD *ap2-1* flowers, is not suppressed by *hy1-1* (J.K. Okamuro, data not shown). Moreover, the effect of SD photoperiod on *ap1-1* meristem indeterminacy (Figure 3D) is only partially suppressed by *hy1-1* (J.K. Okamuro, data not shown). The photoreceptor system responsible for this control is not yet known.

Control of Flower Meristem Identity by GAs

A second important conclusion from this study is that GAs act antagonistically to phytochrome and to *SPY* to promote the establishment of flower meristem identity in *ap2* and *ap1* flower development. Our data showed that the inflorescence-like traits of both *ap2-1* and *ap1-1* flowers were strongly suppressed by *spy* and by exogenously applied GAs. Previously, we showed that phytochrome and GAs also control the maintenance of flower meristem identity both in *ag* and in heterozygous *lfy* mutant flowers. In these mutants, the SD photoperiod induces a heterochronic switch from flower to shoot development, a dramatic transformation known as floral meristem reversion (Okamuro et al., 1993, 1996). Floral meristem reversion in *ag* and in heterozygous *lfy* flowers is phytochrome dependent and is genetically suppressed by *spy* and by exogenous GAs (Okamuro et al., 1996). Thus, by using the floral meristem mutants *ap2*, *ap1*, *ag*, and *lfy*, we have linked GA and phytochrome signaling to the establishment and maintenance of flower meristem identity in Arabidopsis.

Previously, we proposed that the effects of SD photoperiod on flower meristem identity in *ag* and in heterozygous *lfy* flowers are due in part to the regulation of floral meristem identity gene activity by GA signaling (Okamuro et al., 1996). This may also be the case for the photocontrol of *ap1* and *ap2* flower development. One hypothesis is that the activity of one or more genes responsible for the establishment of floral meristem identity, such as *AP1*, *AP2*, *CAL*, or *LFY*, may be positively regulated by GAs. Alternatively, GAs may control the activity of inflorescence meristem–promoting genes, such as *TFL* (Shannon and Meeks-Wagner, 1991), *STM* (Barton and Poethig, 1993), or *KNAT1* (Lincoln et al.,

1994), resulting in photoperiod-dependent changes in *ap1* and *ap2* flower development. Experiments to test these hypotheses are possible because many of the genes that control flower and shoot meristem identity have now been cloned.

Our observations that *spy* enhances the transformation of *ap2-1* floral leaves into carpelloid sepals under LDs (Figure 1) and that exogenous GAs promote petal development in *ap1-1* and *ap2-1* flowers (Figure 5) suggest that defects in *ap2* and *ap1* flower organ development may result in part from a reduction in GA signaling in the floral meristem. To date, however, there has been little evidence to implicate GAs in the establishment of floral meristem identity or the specification of floral organ identity in Arabidopsis. Mutations that reduce GA biosynthesis (*ga*) or interrupt GA signal transduction (*gai*) inhibit petal and stamen development but do not affect flower meristem or organ identity (Koornneef and van der Veen, 1980; Koornneef et al., 1985). One hypothesis to explain this paradox is that the effects of GA signaling on flower development in *ga* mutants may be compensated for by the activity of the floral regulatory gene network such that flower meristem and floral organ identity are not dramatically perturbed. By contrast, a loss-of-function mutation in one key link in this genetic network, either *AP2*, *AP1*, *AG*, or *LFY*, renders the floral meristem hypersensitive to signals that either promote or repress flower development. Together, these mutants provide an exciting new opportunity to study the signals regulating flower meristem identity and organogenesis.

METHODS

Plant Material

Arabidopsis thaliana Landsberg *erecta* was used as the wild-type flower control. *ap2-1*, *ap1-1*, and *hy1-1* are in the Landsberg *erecta* background and were provided by M. Koornneef (Wageninen Agricultural University, Wageninen, The Netherlands). *spy-2* and *spy-3* are in the Columbia background and were provided by N. Olszewski (University of Minnesota, St. Paul).

Plant Growth Conditions

Plants were grown under a mixture of cool-white fluorescent (Sylvania CW/VHO; Osram Sylvania, Versailles, KY) and incandescent lights (Phillips, Somerset, NJ) in a Conviron E15 chamber (Controlled Environments, Asheville, NC) in a 1:1:1 mixture containing vermiculite, perlite, and peat moss. Long-day (LD) growth conditions consisted of 16 hr of light at 150 to 180 μmol m^{-2} sec^{-1} and 8 hr of dark. Short-day conditions (SD) consisted of 9 hr of light at 150 to 180 μmol m^{-2} sec^{-1} and 15 hr of dark. Short-day double incident light [SD (D)] conditions consisted of 9 hr of light at 300 μmol m^{-2} sec^{-1}. Plants were watered with one-quarter–strength Peter's solution (Grace-Sierra Co., Milpitas, CA). For gibberellin (GA) spray experiments, the exogenous application of gibberellin A_3 (GA_3) (Sigma) or gibberellin A_{4+7} (GA_{4+7}) was performed once a week for SD conditions and twice a week for LD conditions, as described by Wilson et al. (1992). GA_{4+7} was kindly provided by P. Grau (Abbott Laboratories, Long Grove, IL).

Analysis of Axillary Flower Production

To generate *ap2-1 spy* and *ap1-1 spy* double mutants, we crossed homozygous flower mutants with *spy-2* and *spy-3*. F_2 seedlings homozygous for *spy* were selected by germination on 1.2×10^{-4} M paclobutrazol (Jacobsen and Olszewski, 1993), washed extensively with H_2O, transplanted to soil, and grown under SD (D) conditions. *ap2-1 spy* is kept as a heterozygote for *ap2-1* because the double mutant is female sterile.

Structural Analysis Using Light Microscopy

Flowers were fixed in FAA (3.7% formaldehyde, 5% acetic acid, 50% ethanol), dehydrated in a graded ethanol series, infiltrated, and embedded in London Resin White (Polysciences Inc., Warrington, PA). Serial 2-μm longitudinal sections were obtained by using a glass knife and ultramicrotome and stained with toluidine blue–basic fuchsin. Images were obtained using bright-field optics.

Scanning Electron Microscopy

Flowers were fixed in FAA and dehydrated in a graded ethanol series. Fixed tissues were critical point dried, mounted onto stubs, and coated with gold palladium. Specimens were examined in a scanning electron microscope (Topcon Technologies, Paramus, NJ) with an accelerating voltage of 10 kV.

Image Processing

All images were scanned and digitized by using a Polaroid Sprintscan 35 (Polaroid, Inc., Cambridge, MA) or an AGFA Arcus II flatbed scanner (AGFA Division, Miles Inc., Ridgefield, NJ). Contrast and brightness were adjusted by using Adobe Photoshop 3.0.1 (Mountain View, CA). Printed images were generated using a Codonics NP1600 printer (Codonics, Inc., Middleburg Heights, OH).

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