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ORIGINAL ARTICLE

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UFO in the *Arabidopsis* inflorescence apex is required for floral-meristem identity and bract suppression

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Abstract The *UNUSUAL FLORAL ORGANS* (*UFO*) gene of *Arabidopsis* encodes an F-box protein required for the determination of floral-organ and floral-meristem identity. Mutation of *UFO* leads to dramatic changes in floral-organ type which are well-characterized whereas inflorescence defects are more subtle and less understood. These defects include an increase in the number of secondary inflorescences, nodes that alternate between forming flowers and secondary inflorescences, and nodes in which a single flower is subtended by a bract. Here, we show how inflorescence defects correlate with the abnormal development of floral primordia and establish a temporal requirement for UFO in this process. At the inflorescence apex of *ufo* mutants, newly formed primordia are initially bract-like. Expression of the floral-meristem identity genes *LFY* and *AP1* are confined to a relatively small adaxial region of these primordia with expression of the bract-identity marker FIL observed in cells that comprise the balance of the primordia. Proliferation of cells in the adaxial region of these early primordia is delayed by several nodes such that primordia appear ''chimeric'' at several nodes, having visible floral and bract components. However, by late stage 2 of floral development, growth of the bract generally ceases and is overtaken by development of the floral primordium. This abnormal pattern of floral meristem development is not rescued by expression of *UFO* from the *AP1* promoter, indicating that UFO is required prior to *AP1* activation for normal development of floral primordia. We propose that UFO and LFY are jointly required in the inflorescence meristem to both promote floral meristem development and inhibit, in a non-cell autonomous manner, growth of the bract.

Keywords *Arabidopsis thaliana* · Cryptic bract · Floral-meristem identity · *LFY* · *UFO*

Abbreviations Col: Columbia · IM: Inflorescence meristem · L*er*: Landsberg erecta · SCF: Skp1-Cullin-F-box · SEM: Scanning electron microscopy · SAM: Shoot apical meristem

Introduction

The shoot apical meristem (SAM) comprises a small group of dividing cells that give rise to the aerial parts of the *Arabidopsis* plant. During vegetative development, the SAM produces a compact rosette consisting of a short stem and a variable number of leaves. The transition to reproductive growth is marked by formation of the inflorescence meristem (IM), which produces an elongated stem punctuated by narrow cauline leaves, secondary inflorescences, and flowers. Floral meristems are formed in a spiral pattern on the periphery of the IM and generate four types of floral organs in a characteristic whorled pattern: sepals, petals, stamens, and carpels (Bowman et al. 1989; Hill and Lord 1989). Genes that are required for the proper development of these floral organs have been isolated and the genetic interactions between many have been determined. The combinatorial specification of floral-organ identity by three classes of homeotic genes (A, B, and C) has been summarized in the ABC model (Haughn and Somerville 1988; Bowman et al. 1991; Coen and Meyerowitz 1991; Weigel and Meyerowitz 1994).

Molecular and genetic studies have identified several genes whose activities are induced by the transition to flowering and that play a primary role in the determination of both floral-meristem and floral-organ identity. Two key genes in this process are *LEAFY* (*LFY*), and *APETALA1* (*AP1*). Loss-of-function mutations in either

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gene result in floral to vegetative meristem transformation phenotypes and floral-organ defects consistent with a loss of Class A, B, and C or Class A floral-organ identity gene activity, respectively (Irish and Sussex 1990; Schultz and Haughn 1991, 1993; Huala and Sussex 1992; Weigel et al. 1992; Shannon and Meeks-Wagner 1993; Bowman et al. 1993). In addition, ectopic expression of either gene during the vegetative phase results in precocious flower formation, indicating that they can activate the floral program (Weigel and Nilsson 1995; Mandel and Yanofsky 1995). Both genes are strongly expressed in floral primordia, *LFY* slightly earlier than *AP1*, and encode DNA-binding transcription factors (Weigel et al. 1992; Mandel et al. 1992). LFY has been shown to bind directly to the promoter of *AP1* and the Class C floral-organ identity gene *AG* to activate transcription (Parcy et al. 1998; Busch et al. 1999; Wagner et al. 1999).

UNUSUAL FLORAL ORGANS (*UFO*) is a third gene implicated in the determination of both floral-organ and floral-meristem identity. *UFO* encodes an F-box protein, many of which provide substrate specificity to a class of E3 ubiquitin ligases known as SCFs (reviewed in Patton et al. 1998; Samach et al. 1999; Wang et al. 2003). UFO interacts both in vitro and in vivo with ASK1, another SCF subunit, supporting the notion that UFO acts by controlling the ubiquitination of target proteins (Samach et al. 1999; Zhao et al. 1999, 2001; Ni et al. 2004). The expression of *UFO* is observed in all shoot apical meristems in a dynamic pattern that is particularly complex in the developing flower (Ingram et al. 1995; Lee et al. 1997; Samach et al. 1999). Loss-of-function mutations in *UFO* produce flowers with abnormal petals and stamens whereas *35S::UFO* transgenic plants produce flowers with extra petals and stamens (Levin and Meyerowitz 1995; Wilkinson and Haughn 1995; Lee et al. 1997) indicating the UFO is both necessary and sufficient for Class B floral organ identity. Indeed, it has been shown that UFO, together with LFY, positively regulates class B function at least in part, by activating *APETALA3* (*AP3*) and *PISTILLATA* (*PI*) transcription (Parcy et al. 1998; Samach et al. 1999; Honma and Goto 2000; Zhao et al. 2001). Recently, it was shown that LFY interacts directly with regulatory sites in the promoter of *AP3* (Lamb et al. 2002). Since UFO does not appear to regulate LFY transcript abundance (Levin and Meyerowitz 1995), it has been proposed that UFO targets for proteolysis a repressor of *AP3* expression that must be removed prior to LFY action (reviewed in Callis and Vierstra 2000; Lohmann and Weigel 2002). Genetic analyses indicate that while UFO requires LFY for all known activities, LFY has functions that do not require UFO (Lee et al. 1997; Parcy et al. 1998; Wagner et al. 1999).

The requirement for UFO in promoting floral-meristem identity is less apparent than its role in determining floral-organ type, but is based on several observations. First, loss-of-function mutations in *ufo* lead to subtle changes in inflorescence structure including an increased number of secondary inflorescences relative to wild-type and a variable number of floral nodes with no defined structure, a bract with no associated shoot, or a bract leaf or filament underlying a single flower. These defects are more severe in non-inductive flowering conditions (short-day photoperiods) than in inductive flowering conditions (long-day or continuous-light photoperiods) indicating that they are enhanced by diminished commitment to a floral fate. However the majority of inflorescence nodes in *ufo* mutants have a single flower with no subtending organ as observed in wild-type inflorescences (Levin and Meyerowitz 1995; Wilkinson and Haughn 1995). The best evidence for the role of UFO in floral meristem identity comes from double mutant studies where mutant alleles of *ufo* were shown to strongly enhance the floral-meristem identity defects of *ap1* and weak *lfy* alleles (Levin and Meyerowitz 1995; Wilkinson and Haughn 1995). As yet there is little information indicating when, where, and how UFO promotes floral-meristem identity.

To further understand the role of UFO in this capacity development of the *ufo* inflorescence apex was examined in more detail. Defects in the inflorescence architecture were correlated with altered development of floral primordia in the IM. Molecular genetic analyses was also used to determine when, during floral development UFO promotes meristem identity and to clarify its relationship to AP1 and LFY function. It is demonstrated that UFO has a role in promoting floral meristem and suppressing bract development at all nodes prior to the emergence of the floral primordium from the inflorescence meristem. As is the case for promotion of floral-organ identity, the role of UFO in floral-meristem identity is required in addition to and distinct from the activities of AP1 and LFY.

Materials and methods

Plant materials and growth conditions

Wild-type was the Col-2 ecotype of *Arabidopsis thaliana* unless stated otherwise. *ufo-1* (Col-2 ecotype), *ufo-2* (L*er* ecotype), and *fil-1* mutant plants were previously described (Wilkinson and Haughn 1995; Levin and Meyerowitz 1995, Sawa et al. 1999a). *35S::UFO* plants were provided by D. Weigel (Weigel and Nilsson 1995; Lee et al. 1997). The *35S::UFO* and *AP1::UFO* transgenes (see below) were introduced into *ufo-1* and *ufo-2* plants by crossing. All plants were grown in continuous light conditions as described (Modrusan et al. 1994). Floral stages were determined according to Smyth et al. (1990).

Construction of the *AP1::UFO* transgenic line

The *AP1::UFO* transgenic line was a gift of Ilha Lee, Takuji Wada, and Detlef Weigel (Salk Institute, La Jolla and Max Planck Institute for Developmental Biology,

Tübingen) and is previously unpublished. Briefly, the *AP1::UFO* transgene comprises a 1.9-kb fragment of *AP1* promoter (including the untranslated leader region) fused to the coding sequence of *UFO* (Hempel et al. 1997; Lee et al. 1997). To generate pIL2, this transgene was cloned between the *Xba*1 and *Eco*RI sites of the binary vector pCGN1547 (McBride and Summerfelt 1990). Each construct was introduced into wild-type Col-2 plants by vacuum infiltration (Bechtold et al. 1993). The *Agrobacterium* strain used was C58C1 pGV3101 pMP90 (Koncz and Schell 1986). Kanamycin-resistant transformants were selected on GM agar plates that contained 50 mg/l kanamycin sulfate (Sigma, St. Louis, MO).

Scanning electron microscopy

SEM samples were prepared as in Modrusan et al. (1994). Inflorescences were mounted on stubs and the organs surrounding the IM were dissected away. Stubs were coated with gold–palladium in a SEMPrep2 sputter coater (Nanotech, Manchester, UK) and observed using a Cambridge 250T scanning electron microscope (Leica, Wetzlar, Germany) with an accelerating voltage of 20 kV.

In situ analysis

Tissue was fixed and sectioned for RNA in situ hybridization as described (Samach et al. 1999). Sections were viewed using an Axioskop 2 microscope (Carl Zeiss, Germany) using brightfield or differential interference contrast optics where the presence of transcript is visible as dark-brown stained regions. All micrographs presented were digitized and manipulated using PHOTOSHOP 5.0 (Adobe Systems, Inc. San Jose, CA, USA). Digoxigenin-labeled anti-sense probes *AP1* and *LFY* were prepared and used as described (Wilkinson and Haughn 1995). The digoxigenin-labeled anti-sense probe for *FIL* comprised the full-length coding region of the gene and was generated from a cDNA template by the method described (Hooker et al. 2002).

Results

Abnormal pattern of development for floral primordia in *ufo* mutants

To examine the basis of *ufo* defects in inflorescence architecture more fully, the inflorescence apices of wild-

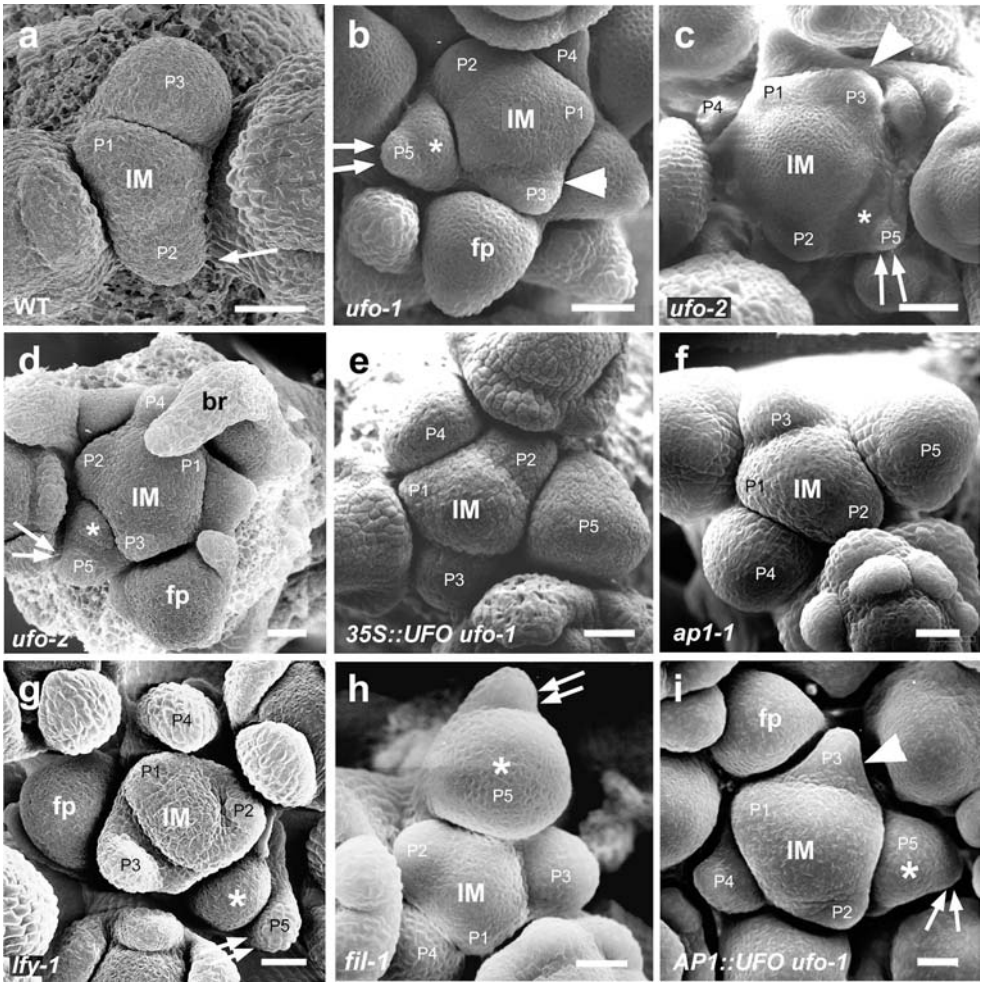

Fig. 1 a–i Scanning electron micrographs comparing wild-type and mutant inflorescence apices. For all applicable panels, an *arrowhead* indicates a bract-like stage 1 primordium, *double arrows* indicate the bract-like distal portion of stage 2 primordia, and an *asterisk* indicates the bulge on the adaxial side of this primordium that is a floral meristem. (**a**) wild-type control plant; *single arrow* indicates a normal rounded stage 1 floral primordia. (**b**) *ufo-1* mutant. (**c**) *ufo-2* mutant. (**d**) *ufo-2* mutant; *br* denotes a bract that has developed at the expense of the floral meristem. (**e**) *35S::UFO ufo-1* plant. (**f**) *ap1-1* mutant. (**g**) *lfy-1* mutant. (**h**) *fil-1* mutant; stage 1 primordium is wild-type in appearance; bract is visible in stage 2 primordium. (**i**) *AP1::UFO ufo-1* plant. *IM*, inflorescence meristem; *fp*, floral primordium. *Scale bars*, 25 μm. Floral and/or bract primordia (*P*) are numbered sequentially in all panels

type control plants and *ufo* mutants were examined using SEM (Fig. 1). As expected, early floral primordia produced by the IM of wild-type control plants had a domed appearance (Fig. 1a, see arrow) and were of a well-defined developmental age depending on their position in the inflorescence. In contrast, the initial primordia (stage 1) produced in *ufo-1* and *ufo-2* IMs were flatter and triangular-shaped, similar to that of bract primordia (Fig. 1b, c, see arrowheads). This structure will therefore be referred to as a bract-like primordium regardless of its eventual fate. About 1 day after the appearance of the bract-like primordium and coinciding with formation of a cleft between the bract primordium and the IM (stage 2), a bulge developed on the adaxial

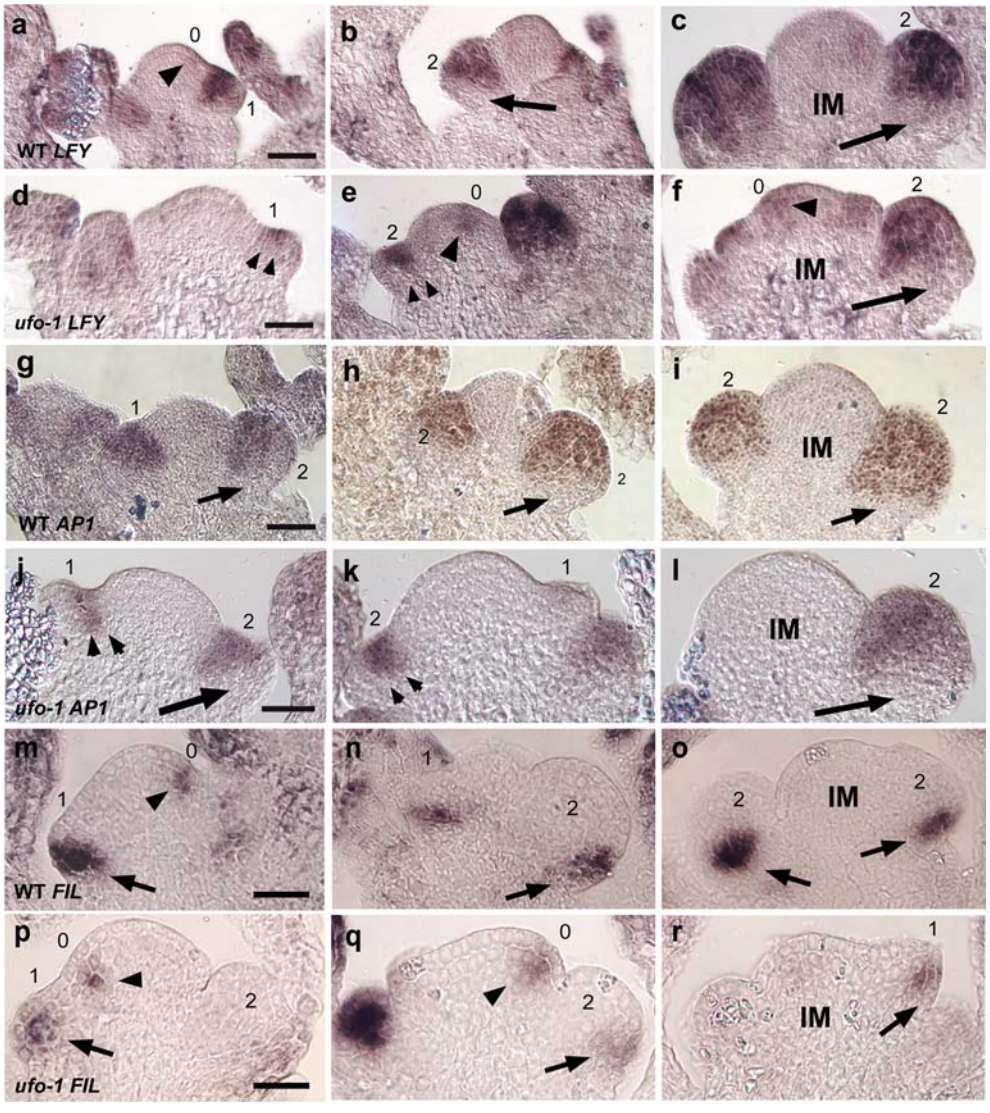

Fig. 2 **a**–**l** *LFY*, *AP1*, and *FIL* expression in wild-type and *ufo-1* mutant plants as monitored by in situ hybridization. *LFY* expression in wild-type (**a**–**c**) and *ufo-1* mutant plants (**d**–**f**). In wild-type plants *LFY* is first detected in the floral anlagen (indicated by *arrowhead* in **a**). *LFY* expression continues in stages 1 and 2 primordia but is absent from a sector of cells at the base of these structures (marked by *arrows* in **a**–**c**). In *ufo-1* mutant plants, *LFY* is first detected in floral anlagen (indicated by *single arrowheads* in **e, f**). *LFY* expression continues in primordia that are the morphological equivalent of stage 1 and early stage 2, but is confined to a small adaxial region of these primordia (marked by *double arrowheads* in **d, e**) and is always absent from the bract-like portion of these structures (**d**–**f**). *AP1* expression in wild-type (**g**–**i**) and *ufo-1* mutant (**j**–**l**) plants. In wild-type control plants, *AP1* expression is first detected in stage 1 primordia and continues to be expressed in stage 2 primordia, but is absent from cells at the base of these structures, which correspond to the cryptic bract (marked by *arrows* for stage 2 primordia in **g**–**i**). In *ufo-1* mutant plants, *AP1* is first detected in the adaxial region of primordia that are morphologically equivalent to stage 1 (marked by *double arrowheads* in **j, k**) and stage 2, but is absent from the bract-like region of these structures (marked by *single arrows* in **j, l**). *FIL* expression in wild-type (**m**–**o**) and *ufo-1* mutant (**p**–**r**) plants. For both genotypes *FIL* is first detected in floral anlagen (see *arrowheads* in **m**, **p**, **q**) but becomes confined to the cryptic bract region (base) of stages 1 and 2 primordia (marked by *arrows* in **m**–**r**). *Scale bars*, 25 μm. *IM*, inflorescence meristem. Floral stages are labeled based on morphology (Smyth et al. 1990) and are indicated as follows: floral anlagen (*0*); stage 1 primordium or equivalent (*1*); stage 2 primordium or equivalent (*2*)

side of the bract primordium (Fig. 1b–d, see asterisks). Typically, this bulge grew out to attain the domed appearance of a floral primordium (Fig. 1b, d, denoted fp) whereas growth of the bract-like primordia was retarded or arrested. This axillary *ufo* floral primordium appeared two to three nodes later than the floral primordium in wild-type and lagged in development behind that of a wild-type flower at the same position relative to the inflorescence shoot apex. Thus, *ufo-1* inflorescences had a greater number of primordia that were morphologically equivalent to stage 1 and early stage 2 than in comparison with the wild-type. For example, the stage 2 floral primordium of wild-type is in the P3 position (Fig. 1a, P3), whereas the similar stage of floral primordium is in the P5 position of the *ufo* inflorescence (Fig. 1b, d, P5*). This caused the *ufo* IM to appear larger due to an increased number of under-developed primordia on its flanks (Fig. 1, compare a with b–d). By stages 3–4 of floral development, the initial bract-like structure was generally no longer visible. Occasionally, the bract continued development either concomitant with or in place of the floral shoot (Fig. 1d, denoted br). This same pattern of floral meristem development was observed in young inflorescences (15–20 nodes), in old inflorescences (30–40 nodes), and was present in all light conditions tested (data not shown). It is concluded that the normal pattern of floral primordium development at every node of the inflorescence meristem requires UFO activity to promote the floral primordium and to suppress bract development.

Floral meristems in *Arabidopsis* and other members of the *Brassicaceae* develop in the absence of a visible subtending leaf. However, the expression pattern of meristem markers, such as *SHOOTMERISTEMLESS* (*STM*) and the lateral organ marker *AINTEGUMENTA* (*ANT*), suggest the presence of a highly reduced "cryptic" bract that subtends the flower and that becomes repressed in its growth very early in development (Long and Barton 2000). To test the correlation between enhanced bract development and the abnormal development of floral primordia, the inflorescence apices of *35S::UFO ufo-1* transgenic plants, and *ap1-1* mutants, which do not produce single flowers subtended by bracts were first examined. Previously, it has been shown that floral and inflorescence morphological defects in *ufo* mutants can be rescued by expression of *UFO* from the strong constitutive CaMV *35S* promoter (Lee et al. 1997). In accord, *35S::UFO ufo-1* plants had normal stage 1 floral primordia similar to those of wild-type control plants (Fig. 1, compare a and e). The inflorescences of *ap1-1* plants which are defective in very early floral development were also examined (Irish and Sussex 1990; Bowman et al. 1993). These plants also produced normal rounded early floral primordia, similar to those of wild-type control plants (Fig. 1, compare a and f).

The inflorescence apices of *lfy-1* and *filamentous flower-1 (fil-1)* mutants were examined next. Mutations in the *LFY* gene cause strong floral-meristem identity defects and like *ufo*, form single flowers subtended by bracts (Schulz and Haughn 1991; Huala and Sussex 1992; Weigel et al. 1992). The pattern of floral meristem development in *lfy-1* mutants was nearly identical to that of *ufo* mutants: the initial primordia were bract-like, and a bulge that appeared on the adaxial side of these primordia gradually developed into a dome-shaped floral primordium at the expense of bract development (Fig. 1g). Mutations in *FIL* which encodes a YABBY protein also produce flowers that are subtended by bracts or filamentous structures (Sawa et al. 1999ab). However, in *fil-1* mutants stage 1 primordia were wild-type in appearance and bract formation was not apparent until stage 2 (Fig. 1h). It is therefore concluded that the floral meristem defect for *ufo* and *lfy* was very similar and that formation of flowers with visible subtending bracts and filaments correlates with a delay in the formation of visible floral primordia and enhanced development of the cryptic bract, which subtends floral primordia.

Analysis of *LFY* expression in wild-type and *ufo* inflorescence meristems

The first known marker in the process of specifying floral meristem identity is *LFY*. Expression of *LFY* is required for determination of floral meristem identity, but precedes commitment to a floral fate (Hempel et al. 1997). Therefore the pattern of *LFY* mRNA expression was examined in wild-type and *ufo-1* inflorescence meristems using in situ hybridization (Fig. 2a–f). In wild-type inflorescences, *LFY* expression was first detected in floral anlagen (P0) within the IM (Fig. 2a, see arrowhead). *LFY* expression persisted in stages 1 and 2 primordia, but was absent from a sector of cells on the abaxial side of these primordia (Fig. 2b, c, see arrows). This basal region of the floral primordium corresponds to the "cryptic bract" and has been shown to express the leaf markers *ASYMMETRIC LEAVES1* (*AS1*) (Long and Barton 2000; Byrne et al. 2000) and *FIL* (Sawa et al. 1999b; Dinneny et al. 2004; see also Fig. 2m–o, see arrows).

In *ufo-1* mutants, *LFY* expression was also detected prior to stage 1 in floral anlagen within the IM (Fig. 2e, f, see arrowheads). *LFY* expression was maintained in early floral primordia (morphologically equivalent to stage 1 and early stage 2 in wild-type), but was restricted to a smaller adaxial portion of the primordia than was observed in wild-type (Fig. 2d, e, see double arrowheads) until the morphological equivalent of late stage 2 (Fig. 2f). The lack of *LFY* expression in abaxial cells of early primordia along with the expression of the bract-identity marker *FIL* in these locations (Fig. 2p–r, see arrows) suggests that these regions of the primordium do not have floral identity and thus correspond to the cryptic bract. These results indicate that the relative proportion of adaxial floral to abaxial bract tissue in the young primordia (morphologically equivalent to stage 1

and early stage 2) of the *ufo-1* mutant is consistently smaller than that of wild-type.

Analysis of *AP1* expression in wild-type and *ufo* inflorescence meristems

Expression of *AP1* is the earliest marker of commitment to floral meristem fate and is first detected in stage 1 floral primordia of wild-type IMs (Hempel et al. 1997). Therefore the pattern of *AP1* expression was examined in wild-type and *ufo-1* IMs by in situ hybridization (Fig. 2g–l). As expected, expression of *AP1* was first detected in the stage 1 floral primordia of wild-type control plants. A small group of abaxial cells of these early floral primordia, which did not express *AP1* (Fig. 2g), but expressed *FIL*, were detected (Fig. 2m, see arrow) and thus correspond to the cryptic bract region of the floral meristem. *AP1* expression persisted in stage 2 floral primordia and was again absent from the cryptic bract (Fig. 2g–i, see arrows). In *ufo-1* mutants, *AP1* expression like that of *LFY* was first detected in a much smaller proportion of adaxial cells of young (morphologically equivalent to stage 1 or early stage 2) primordia (Fig. 2j, k, see double arrowheads). The remaining cells of these primordia expressed *FIL*, indicating that they had bract identity (Fig. 2p–r, see arrows). By the morphological equivalent of late stage 2, the group of adaxial cells that expressed *AP1* appeared to have proliferated whereas cells in the cryptic bract region of the primordia did not (Fig. 2l, see arrow).

These results are consistent with those from the analysis of *LFY* expression (previous para) in supporting the hypothesis that the relative proportion of adaxial floral to abaxial bract tissue in the young primordia of the *ufo-1* mutant is consistently smaller than that of wild type.

UFO is required sequentially for determination of floral-meristem and floral-organ identity

UFO is expressed in a complex temporal and spatial pattern in the inflorescence. *UFO* transcript in the IM is excluded from the center and periphery of the IM and appears to predict where the floral anlagen will be situated on the flanks of the IM. Expression is absent in stage 1 floral primordia but resumes at stage 2 in the central region. By stage 3, expression is lost in the central meristem, but it expands laterally in a cone-shaped pattern. Expression becomes restricted to the petal primordia by stage 5 and is maintained there through most of the floral organ development (Ingram et al. 1995; Lee et al. 1997; Samach et al. 1999). To examine the temporal requirement for UFO in promoting floral-meristem identity, testing was done to determine whether expression of *UFO* under the control of a promoter for the floral-meristem identity gene *AP1* could complement defects in floral-meristem and floral-organ identity in *ufo* mutants. *AP1* is an early marker of floral determination and is first detected in stage 1 floral primordia (Fig. 2, see also Mandel et al. 1992; Hempel et al. 1997).

An *AP1::UFO* transgene was crossed into the *ufo-1* and *ufo-2* mutant backgrounds. The resulting plant lines were first scored for the restoration of petal and stamen formation, which are largely absent in *ufo* mutant flowers. As expected, examination of *AP1::UFO ufo-1* flowers revealed the presence of petals and stamens (Fig. 3). These data indicate that expression of *UFO* in stage 1 and later flowers is sufficient to restore petal and stamen development. This was anticipated since *AP1* is activated prior to the formation of visible petal and stamen primordia. However, many of the flowers had extra organs, or organs that were fused or were of mixed identity (Table 1). Therefore the number of floral organs produced in wild-type control plants was compared with that produced by plants expressing the *AP1::UFO* transgene. This analysis confirmed that expression of *UFO* from the *AP1* promoter caused an increase in organ number similar to that described for *35S::UFO* (Lee et al. 1997). These data are consistent with the idea that UFO has a role in promoting growth within the floral primordium.

Next, testing was done to determine whether expression of *UFO* from the *AP1* promoter was sufficient to complement defects in inflorescence architecture and restore a normal pattern of development to floral primordia in the IM. First the structure of the IM in wild-type, *ufo-1*, *AP1::UFO ufo-1* plants were compared using

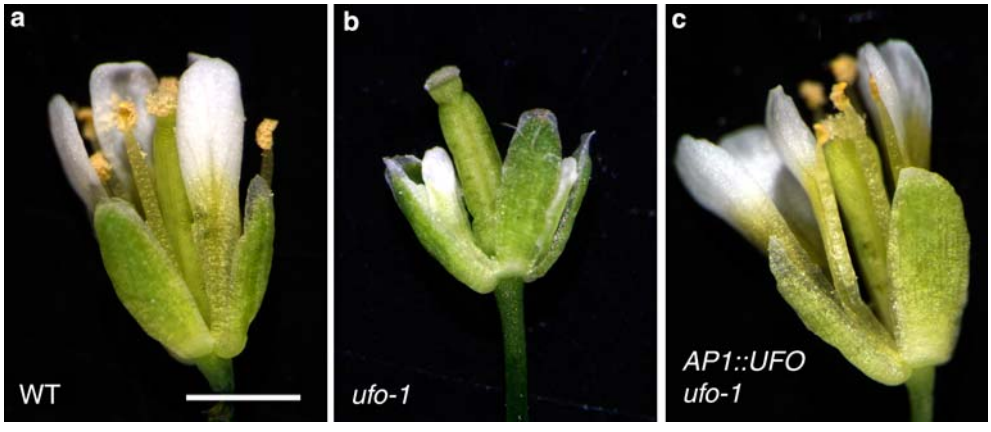

Fig. 3 **a–c** *AP1::UFO* and *AP3::UFO* transgenes restore petal and stamen development in *ufo-1* mutants. A representative flower is shown for: (**a**) wild-type control plant. (**b**) *ufo-1* mutant plant. (**c**) *AP1::UFO ufo-1* plant. *Scale bar*, 1 mm

Table 1 Effect of *AP1::UFO* expression on wild-type and *ufo* plant development

Genotype	Sepal/sepal-like[a]	Petal/petal-like[a]	Stamen/stamen-like[a]	Carpel[a]	Number of 2° inflorescences[b]
*C*ol-2	4.0 ± 0.0	4.0 ± 0.0	5.9 ± 0.4	2.0 ± 0.0	2.0 ± 0.1
L*er*	4.0 ± 0.0	4.0 ± 0.0	5.9 ± 0.3	2.0 ± 0.0	1.8 ± 0.1
ufo-1 (Col-2)	4.4 ± 0.6	2.5 ± 0.8	0.1 ± 0.2	2.5 ± 0.6	2.7 ± 0.1
ufo-2 (L*er*)	4.0 ± 0.0	3.3 ± 1.0	0.1 ± 0.5	2.6 ± 0.9	2.7 ± 0.2
Col *AP1::UFO*	4.2 ± 0.6	5.1 ± 1.1	7.1 ± 1.4	2.6 ± 0.8	2.1 ± 0.1
ufo-1 AP1::UFO	4.5 ± 1.2	4.8 ± 0.9	6.7 ± 0.8	3.2 ± 0.6	2.7 ± 0.2
ufo-2 AP1::UFO	4.1 ± 1.2	4.5 ± 0.8	6.6 ± 0.9	3.4 ± 0.8	3.0 ± 0.2

[a]Values are given as mean ± standard deviation
[b]Values are given as mean ± standard error

SEM (Fig. 1). This analysis revealed that inflorescence apices of *AP1::UFO ufo-1* transgenic plants resembled those of *ufo* plants and produced stage 1 primordia that were bract-like in appearance (Fig. 1, compare c and d with h and i). The number of secondary inflorescences in the above genotypes were also scored confirming that *AP1::UFO ufo-1* plants had a small but significant increase in the number of secondary inflorescences, similar to that observed for *ufo-1* and *ufo-2* mutants (Table 1; see also Levin and Meyerowitz 1995; Wilkinson and Haughn 1995). It was therefore concluded that expression of *UFO* from the *AP1* promoter was not sufficient to complement *ufo*-related defects in inflorescence architecture, nor restore a normal pattern of development to floral primordia in the IM. This analysis revealed that *UFO* expression is required prior to the emergence of stage 1 primordia, likely in the IM itself, to complement defects in the development of floral primordia.

Discussion

UFO is required for both promotion of floral-meristem development and bract suppression

The establishment of floral-meristem identity in *Arabidopsis* results in lateral inflorescences subtended by bracts being replaced by floral shoots without bracts at inflorescence nodes. Two genes that play key roles in this process are *LFY* and *AP1* although several other genes including *UFO* have also been implicated. A number of structural abnormalities within the *ufo* mutant inflorescence suggest weak defects in floral-meristem identity. These include a slight increase in the number of lateral inflorescences produced, flowers subtended by bracts or filamentous structures, and nodes with bracts but no associated shoot. However, mature floral morphology at many nodes appears normal and it is only under short photoperiods or in certain genetic backgrounds that meristem identity defects are more obvious (Levin and Meyerowitz 1995; Wilkinson and Haughn 1995). It has been shown here that alterations in the inflorescence architecture of *ufo* mutants are preceded by consistent defects in the pattern of floral primordium formation in the IM.

Floral meristems in *Arabidopsis* and most other members of the *Brassicaceae* develop in the absence of a visible subtending leaf. However, the expression patterns of the meristem marker *STM*, and the lateral organ marker *ANT* during flower development suggest the presence of a highly reduced "cryptic" bract, which subtends the flower (Long and Barton 2000). It has therefore been proposed that this *STM*-negative region that develops on the flanks of the inflorescence meristem is a bract primordium and that the floral meristem proper develops on the adaxial side of this bract primordium. The bract primordium, although initially specified, becomes repressed in its growth very early in development (Long and Barton 2000). Consistent with this model, Hempel and Feldman have argued that cauline leaves are the leaves that are already initiated at the meristem when the plant is subject to a floral stimulus and that the rapid development of the axillary meristem and the suppression of leaf size is a consequence of this induction. Indeed, in wild-type plants a floral inductive signal can alter the fate of leaf primordia already initiated at the vegetative meristem such that some flowers are subtended by reduced bracts form, similar to those observed in *ufo* mutants (Hempel and Feldman 1995; Hempel et al. 1998). However in *ufo* mutants all floral primordia appear to arise from the adaxial side of an initial bract-like primordium. Growth of the floral primordium does not overtake that of the bract primordium until at least stage 2. This delay in the formation of the floral primordium is correlated with the development of primordia that are chimeric in nature possessing both floral and bract components (see also Hempel and Feldman 1995) and provides an explanation for the variable inflorescence defects observed in *ufo* mutants. Thus, the *ufo* mutant phenotype suggests that UFO is required both to promote floral meristem development and to suppress growth of the bract.

UFO required sequentially for determination of floral-meristem and floral-organ identity

The results presented here indicate that the earliest detectable defects in the *ufo* mutant inflorescence occur before the emergence of the stage 1 floral primordium

and prior to the earliest expression of *UFO* in the floral meristem (stage 2). It has also been shown that expression of *UFO* from the *AP1* promoter is sufficient to restore petal and stamen formation, but cannot complement defects in inflorescence architecture or restore a wild-type pattern of floral-primordia development. Thus, promotion of floral-meristem identity and bract suppression must rely on expression of UFO in the IM. It is interesting that this expression is excluded from the center and periphery of the IM and appears to predict where the floral anlagen will be situated (Ingram et al. 1995; Lee et al. 1997; Samach et al. 1999). These data also reinforce that UFO is required sequentially throughout floral development, first in the IM and later in the floral meristem. In the floral meristem, UFO is first required to promote petal and stamen formation by influencing both patterning and identity of primordia in whorls 2 and 3. UFO acts in part, with LFY, to activate *AP3* expression (Levin and Meyerowitz 1995; Wilkinson and Haughn 1995; Krizek and Meyerowitz 1996; Lee et al. 1997; Parcy et al. 1998; Samach et al. 1999). In addition, the changes in organ number associated with loss and gain of UFO function (Levin and Meyerowitz 1995; Wilkinson and Haughn 1995; Lee et al. 1997; this study, Table 1) suggests that UFO promotes growth within the floral meristem. Both of these roles presumably depend on the *UFO* expression detected in middle whorls between stages 2 and 4, which coincides with the time when *AP3*/*PI* expression patterns are established (Jack et al. 1992; Goto and Meyerowitz 1994). After patterning and identity have been properly established, UFO is next required for initiating petal primordia, correlating with the restriction of *UFO* expression to this region beginning during stages 4–5, at or just before organ initiation (Smyth et al. 1990). This late function for UFO was uncovered through analysis of weak *ufo* alleles that affect only petal development (Durfee et al. 2003) and through the use of an inducible form of UFO (Laufs et al. 2003).

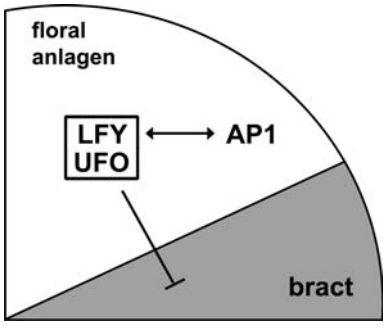

Fig. 4 Model for promotion of floral meristem identity and suppression of cryptic bract development by *UFO* and *LFY*. It is proposed that *UFO* and *LFY* are jointly required in the inflorescence meristem to both promote floral meristem development and inhibit, in a non-cell autonomous manner, bract formation. It is proposed that this shared function occurs in a manner analogous to their co-regulation of floral-organ type (reviewed in Lohmann and Weigel 2002) and is distinct from the *UFO*-independent role of *LFY* in promoting floral-meristem identity by direct activation of *AP1* expression (Parcy et al. 1998; Busch et al. 1999; Wagner et al. 1999). *Arrow* indicates promotion of gene expression and the *T-Bar* indicates non-cell autonomous repression of cryptic bract development. Bract domain of developing floral primordium is shown in *light grey*

Co-regulation of inflorescence architecture by *UFO* and *LFY*

A model (Fig. 4) in which LFY and UFO act co-operatively in the IM to promote floral-meristem identity and bract suppression in a manner analogous to their co-regulation of floral-organ type (Lee et al. 1997; Parcy et al. 1998; Samach et al. 1999; Honma and Goto 2000; reviewed in Lohmann and Weigel 2002) is favored. Several pieces of evidence suggest that this may be true. First, expression of both genes occurs in the IM, and precedes both the emergence of stage 1 primordia and activation of *AP1* expression (this study, Lee et al. 1997; Samach et al. 1999; Weigel et al. 1992; Blázquez et al. 1997; Hempel et al. 1997). Second, *lfy* and *ufo* inflorescences have very similar defects in structure and morphology. For example, *lfy* mutants possess flat triangular-shaped stage 1 primordia (Fig. 1g; see also Weigel et al. 1992) and show *ufo*-like defects in inflorescence architecture (Schultz and Haughn 1991; Huala and Sussex 1992; Weigel et al. 1992).

Interestingly, neither *UFO* (Lee et al. 1997; Samach et al. 1999) nor *LFY* (this study) is expressed in the cryptic bract suggesting that they control bract development in a non-cell autonomous manner. Consistent with this hypothesis, Nilsson et al. (1998) demonstrated that the ablation of floral meristems, by placing the gene for diphtheria toxin under the control of the *LFY* promoter, results in the replacement of flowers by leaves. It is unlikely that UFO and LFY control bract development indirectly through their promotion of floral development. The *35S::AP1* transgene restores floral development but is unable to suppress bracts in a *lfy* mutant background (Liljegren et al. 1999) indicating that floral development alone is insufficient to suppress bracts in the absence of LFY. Therefore UFO and LFY probably control bract suppression from the floral anlagen by a mechanism independent of floral promotion and AP1 function (Fig. 4). The mechanism by which UFO co-regulates LFY function (Lee et al. 1997) remains unclear. The UFO protein has homology with F-box proteins, which provide substrate specificity for large protein complexes called SCFs, that have an E3 ubitiqutin ligase activity and that target proteins for degradation via the ubiquitin/proteosome pathway (Patton et al. 1998; Samach et al. 1999; Wang et al. 2003; Ni et al. 2004). Protein targets for an UFO-containing SCF complex have yet to be found, but in other systems comprise two major classes: cell cycle regulators and transcription factors (Patton et al. 1998). Therefore UFO may promote floral-meristem development through the control of transcription factor activity,

localized cell proliferation, or both. It is possible that UFO enhances LFY activity either directly or indirectly by promoting degradation of an inhibitor of LFY transcription factor activity.

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