7-2006

AINTEGUMENTA Contributes to Organ Polarity and Regulates Growth of Lateral Organs in Combination with *YABBY* Genes

Staci Nole-Wilson
University of South Carolina - Columbia

Beth A. Krizek
University of South Carolina - Columbia, krizek@sc.edu

Publication Info

Published in *Plant Physiology*, ed. Donald R. Ort, Volume 141, Issue 3, 2006, pages 977-987.

AINTEGUMENTA Contributes to Organ Polarity and Regulates Growth of Lateral Organs in Combination with *YABBY* Genes[1]

Staci Nole-Wilson[2] and Beth A. Krizek*

Department of Biological Sciences, University of South Carolina, Columbia, South Carolina 29208

Lateral organs in flowering plants display polarity along their adaxial-abaxial axis with distinct cell types forming at different positions along this axis. Members of three classes of transcription factors in Arabidopsis (*Arabidopsis thaliana*; the Class III homeodomain/leucine zipper [HD-ZIP] proteins, KANADI proteins, and YABBY proteins) are expressed in either the adaxial or abaxial domain of organ primordia where they confer these respective identities. Little is known about the factors that act upstream of these polarity-determining genes to regulate their expression. We have investigated the relationship between *AINTEGUMENTA* (*ANT*), a gene that promotes initiation and growth of lateral organ primordia, and polarity genes. Although *ant* single mutants do not display any obvious defects in organ polarity, loss of *ANT* activity in combination with mutations in one or more *YABBY* genes results in polarity defects greater than those observed in the *yabby* mutants alone. Our results suggest that *ANT* acts in combination with the *YABBY* gene *FILAMENTOUS FLOWER* (*FIL*) to promote organ polarity by up-regulating the expression of the adaxial-specifying HD-ZIP gene *PHABULOSA*. Furthermore, we show that *ANT* acts with *FIL* to up-regulate expression of the floral homeotic gene *APETALA3*. Our work defines new roles for *ANT* in the development of lateral organs.

In flowering plants, leaves and floral organs are produced on the periphery of apical meristems. These lateral organs possess an inherent asymmetry with regard to the meristem in that their adaxial side is adjacent and close to the meristem, while their abaxial side is located further from the meristem. This asymmetry gives rise to a polarity that is readily apparent at the cellular and whole organ level and which can have important functional consequences. For example, cells within the adaxial region of a leaf are specialized for light capture, while those in the abaxial region are specialized for gas exchange. In addition, outgrowth of the leaf lamina is dependent on the juxtaposition of cells with adaxial and abaxial identities (Waites and Hudson, 1995). A similar mechanism may be responsible for the outgrowth of flattened floral organs such as sepals and petals.

Members of three classes of transcription factors contribute to the establishment of adaxial and abaxial cell fates in lateral organs of Arabidopsis (*Arabidopsis thaliana*; for review, see Engstrom et al., 2004). Class III homeodomain/Leu zipper (HD-ZIP) proteins specify adaxial identity, while KANADI and YABBY proteins specify abaxial identity. Three HD-ZIP genes, *PHABULOSA* (*PHB*), *PHAVOLUTA* (*PHV*), and *REVOLUTA* (*REV*), are expressed in the adaxial domain of lateral organ primordia (McConnell et al., 2001; Emery et al., 2003). Dominant mutations in *PHB* or *PHV* result in transformation of abaxial cells to adaxial fates and the production of radially symmetric lateral organs (McConnell and Barton, 1998; McConnell et al., 2001). *phb phv rev* triple mutants produce just a single abaxialized and radialized cotyledon in the most severe case (Emery et al., 2003).

At least three members of the *KANADI* gene family (*KAN1*, *KAN2*, and *KAN3*) redundantly specify abaxial identity (Eshed et al., 2001; Kerstetter et al., 2001). Transformation of abaxial cell types into adaxial cell types is observed with increasing severity in *kan* double and triple mutants, while ectopic expression of *KAN* genes results in the development of abaxial tissues in adaxial regions and the radialization of lateral organs (Eshed et al., 2001, 2004; Kerstetter et al., 2001). *KAN1* is expressed in the abaxial domain of developing lateral organs, complementary to the expression of *PHB*-like genes in the adaxial domain (Kerstetter et al., 2001). Members of the *YABBY* gene family also contribute to the specification of abaxial identity. Three *YABBY* genes, *FILAMENTOUS FLOWER* (*FIL*), *YABBY2* (*YAB2*), and *YABBY3* (*YAB3*), are expressed in the abaxial half of all lateral organ primordia (Siegfried et al., 1999). Ectopic expression of these *YABBY* genes can convert some adaxial cell types into abaxial cells (Sawa et al., 1999; Siegfried et al., 1999). Although loss of both *FIL* and *YAB3* activity does not result in conversion of abaxial cells into adaxial cells,

[1] This work was supported by the U.S. Department of Energy (grant no. 98ER20312).

[2] Present address: Department of Genetics, North Carolina State University, Raleigh, NC 27695.

* Corresponding author; e-mail krizek@sc.edu; fax 803–777–4002.

The author responsible for the distribution of materials integral to the finding presented in this article in accordance with the policy described in the Instructions for Authors (www.plantphysiol.org) is: Beth A. Krizek (krizek@sc.edu).

Article, publication date, and citation information can be found at www.plantphysiol.org/cgi/doi/10.1104/pp.106.076604.

increased adaxialization of lateral organs occurs in *kan1/+ kan2* plants upon loss of *FIL* and *YAB3* activity (Eshed et al., 2004). KANADI proteins are members of the GARP family of transcriptional regulators, while YABBY proteins are zinc finger-containing proteins with an HMG box-like domain (Bowman and Smyth, 1999; Sawa et al., 1999; Eshed et al., 2001; Kerstetter et al., 2001).

It has been proposed that a meristem-derived signal is responsible for the establishment of adaxial-abaxial polarity in lateral organs (for review, see Bowman et al., 2002). Cells closest to the meristem, those in the adaxial domain, perceive this signal, while cells further away do not. In response, PHB-like proteins are activated in cells of the adaxial domain. Because of the antagonism between *PHB*-like and *KANADI* genes, *KANADI* gene expression is subsequently inhibited in the adaxial region and becomes restricted to the abaxial domain (Eshed et al., 2004). Thus, an initial asymmetry due to the meristem-derived signal is thought to be maintained by antagonism between the *PHB*-like and *KANADI* genes and results in restriction of *PHB*-like gene expression to the adaxial domain and *KANADI* gene expression to the abaxial domain. Additionally, the mutual repression between the *PHB*-like and *KANADI* genes leads to the abaxial specific expression of the *YABBY* genes (Eshed et al., 2004).

We are interested in the relationship between factors that promote the initiation and growth of lateral organ primordia, such as AINTEGUMENTA (ANT), and factors that act within lateral organ primordia to establish their polarity, such as PHB-like, KANADI, and YABBY proteins. *ANT* expression is up-regulated in leaf and flower founder cells in apical meristems and is one of the earliest markers of lateral organ specification (Elliott et al., 1996; Long and Barton, 2000). It has been suggested that *YABBY* genes could initially be activated by proteins that promote primordia initiation (such as *ANT*), while other factors act later to restrict *YABBY* gene expression to abaxial regions (Bowman, 2000). ANT encodes a transcription factor of the APETALA2/ethylene-responsive element binding factor family that binds to 5′-gCAC(A/G)N(A/T)TcCC(a/g)ANG(c/t)-3′ DNA sequences (Nole-Wilson and Krizek, 2000). Although we find that ANT can bind in vitro to such a sequence within the *FIL* and *YAB3* promoters, ANT is not required for normal levels of *FIL* or *YAB3* expression. Characterization of *fil-8 ant-4* and *fil-8 yab3-2 ant-4* double and triple mutants does suggest a role for ANT in the establishment of adaxial-abaxial polarity in leaves and floral organs.

RESULTS

ANT Binds to a Conserved Element in the *FIL* and *YAB3* Promoters

FIL and *YAB3* are expressed in largely overlapping domains and share sequence similarity within an approximately 300-bp 5′ regulatory region, part of which is shown in Figure 1A (Siegfried et al., 1999; Watanabe and Okada, 2003). Deletion analysis of the *FIL* promoter identified two cis-acting regulatory elements required for proper *FIL* expression (Watanabe and Okada, 2003). A region proximal to the *FIL* coding sequence (−1,742 to −1,547) is required for expression in both adaxial and abaxial domains, while a 12-bp (−1,748 to −1,737) sequence is required for the abaxial-specific expression of the *FIL* gene (Fig. 1A). A sequence with similarity to the ANT consensus binding site is present within the former *FIL* region and within the *YAB3* promoter. The putative ANT binding sites in the *FIL* and *YAB3* promoters match the in vitro-determined ANT consensus binding site in 10 of 14 conserved positions (Fig. 1A). Gel mobility shifts revealed that ANT binds in vitro to fragments of both promoters that contain these sequences (Fig. 1B). The observed binding to either of these sites is weaker than that of ANT to the consensus binding site (BS15; Fig. 1B). Binding to the *FIL* and *YAB3* promoter sites was competed by unlabeled BS15 but not by a mutated version of this binding site (data not shown).

FIL and *YAB3* Expression Is Normal in *ant* Flowers

To probe the potential role of ANT in *FIL* and *YAB3* regulation, we examined the expression of *FIL* and *YAB3* in an *ant* mutant background. If ANT is a positive regulator of *FIL* and/or *YAB3*, we might expect *FIL* and *YAB3* expression to be reduced in *ant* mutants. *FIL* expression was examined in *ant-4* flowers by in situ hybridization.

A similar level and pattern of *FIL* expression was observed in Landsberg *erecta* (L*er*) and *ant-4* flowers (Fig. 2, A–D). *YAB3* mRNA was examined in L*er* and *ant-4* inflorescences by real-time reverse transcription (RT)-PCR. Similar levels of *YAB3* mRNA were present in both genotypes (Fig. 2E). These results suggest that ANT activity is not required for activation of *YAB3* or *FIL* in flowers.

fil ant and *fil yab3 ant* Plants Are Reduced in Size

To gain insight into the relationship between *ANT* and the two *YABBY* genes, we generated *fil-8 ant-4* double mutants and *fil-8 yab3-2 ant-4* triple mutants. The double and triple mutant plants were dwarfed and exhibited severe alterations in organ development during both vegetative and reproductive development (Figs. 3, A–D, and 5, A and B). While the leaves of *yab3-2*, *fil-8*, and *ant-4* single mutants were not dramatically different in size from those of wild type (Kumaran et al., 2002), the leaves of *fil-8 yab3-2*, *fil-8 ant-4*, and *fil-8 yab3-2 ant-4* were considerably smaller than those of wild type (Fig. 3, E–H; Table I). The decreased leaf area of the double and triple mutants resulted from reductions in both length and width (Table I). In the triple *fil-8 yab3-2 ant-4* mutant, there was a dramatic reduction in lamina expansion such that the petioles of *fil-8 yab3-2 ant-4* leaves were often not clearly distinguishable from the lamina (Fig. 3, H and L).

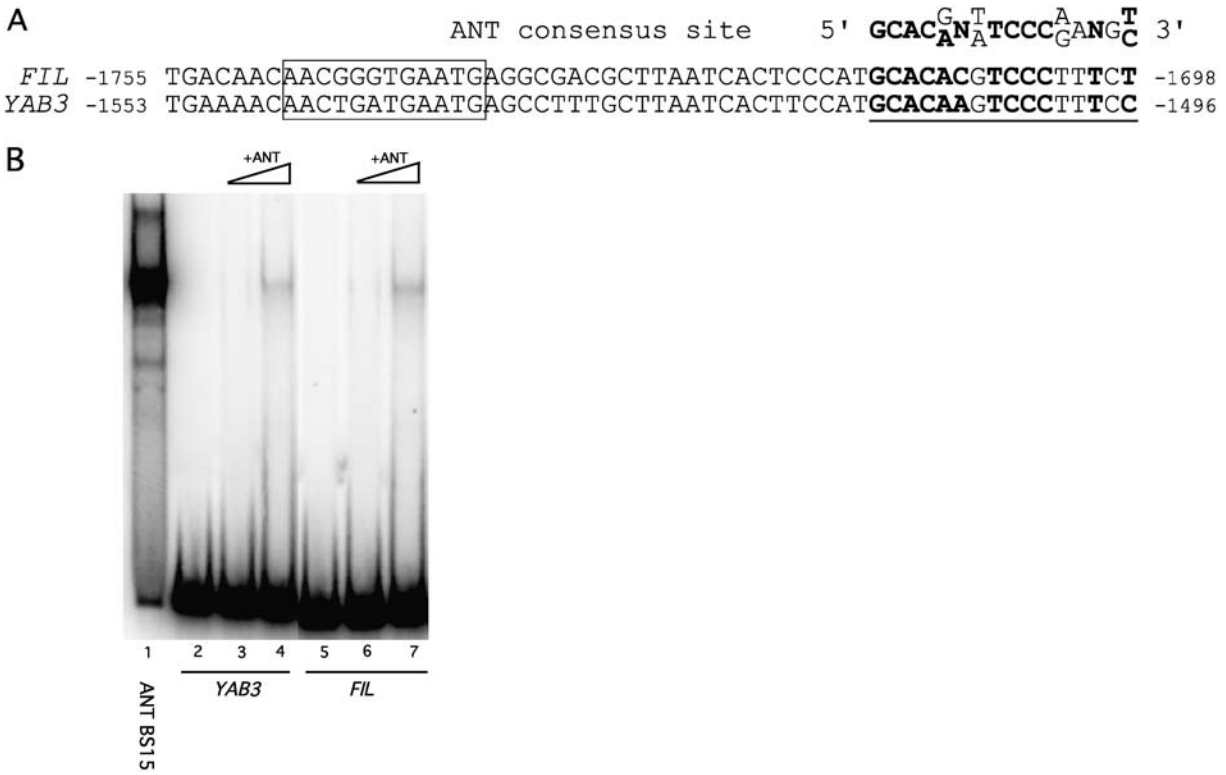

Figure 1. ANT binds to a sequence within the *FIL* and *YAB3* promoters. A, Alignment of a conserved element within the 5′ regulatory region of *FIL* and *YAB3* to the ANT consensus site. The putative ANT binding site is underlined. Nucleotides shared between the ANT consensus binding site and the *FIL* and *YAB3* promoters are shown in bold. A putative Kruppel binding site (−1,748 to −1,737), required for repression of *FIL* in the adaxial domain, is boxed. Numbers indicate positions relative to the start codons. B, Gel shift showing binding of ANT to the consensus binding site (BS15), the *YAB3* promoter site, and the *FIL* promoter site. The *YAB3* promoter fragment is a 106-bp sequence corresponding to nucleotides −1,561 to −1,456 and the *FIL* promoter fragment is a 131-bp sequence corresponding to nucleotides −1,763 to −1,633. Lanes 2 and 5 contain no protein. Increasing amounts of ANT protein are shown in lanes 3 and 4 (and 6 and 7). The same amount of ANT protein was used in lanes 1, 4, and 7.

To determine the basis for the smaller leaf size in *fil-8 ant-4* and *fil-8 yab3-2 ant-4* plants, the size of mature leaf epidermal cells was examined using scanning electron microscopy (SEM). Epidermal cells were larger in *fil-8 ant-4* and *fil-8 yab3-2 ant-4* plants compared to L*er* (Fig. 4, A, B, and I–L). This indicates that the smaller leaf blades of the double and triple mutants are due to the presence of fewer cells. Similarly, the reduced height of the double and triple mutant plants (Fig. 5A; Table II) was due to fewer cells in the stem (data not shown).

fil ant and *fil yab ant* Mutants Show Disruptions in Leaf Polarity

A juxtaposition of adaxial and abaxial cell types is thought to be required for leaf blade expansion (for review, see Bowman, 2000; Bowman et al., 2002). To determine whether the reduced expansion of leaf blades in *fil-8 ant-4* and *fil-8 yab3-2 ant-4* plants might be a consequence of reduced leaf polarity, epidermal cell morphologies were examined. The adaxial and abaxial surfaces of wild-type leaves are distinct. The adaxial epidermis is flat, while the abaxial epidermis is undulating (Fig. 4, A and B). In addition, adaxial epidermal cells are fairly uniform in size, while abaxial epidermal cells are variably sized and puzzle shaped (Fig. 4, A and B). The adaxial and abaxial surfaces of *ant-4* and *fil-8* leaves are normal (Fig. 4, C–F). The adaxial surface of *fil-8 yab3-2* leaves is normal (Fig. 4G). However, the abaxial surface of *fil-8 yab3-2* leaves was altered slightly from wild type, indicating a partial loss of abaxial identity (Fig. 4H; Siegfried et al., 1999).

More dramatic changes in adaxial and abaxial identities were observed in *fil-8 ant-4* and *fil-8 yab3-2 ant-4* plants. Adaxial epidermal cells of *fil-8 ant-4* leaves were variable in size and sometimes puzzle shaped, slightly resembling abaxial epidermal cells (Fig. 4I). In addition, the abaxial surface was flatter than wild type and the cells larger than wild type (Fig. 4J). This suggests a partial loss of both adaxial and abaxial identities in *fil-8 ant-4* plants. Thus, at least some of the reduced growth of *fil-8 ant-4* leaves may result from a loss of polarity. The more dramatic reduction in lamina expansion in the triple mutant was correlated with a more complete loss of polarity. In *fil-8 yab3-2 ant-4* leaves, adaxial and abaxial epidermal cells

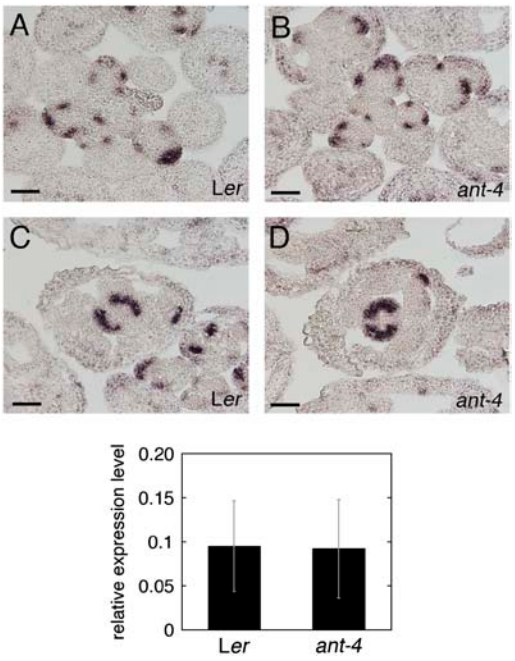

Figure 2. *FIL* and *YAB3* expression in wild-type and *ant-4* plants. A, *FIL* mRNA is present in floral primordia and sepal primordia in this transverse L*er* inflorescence section. B, *FIL* mRNA in an *ant-4* inflorescence. C, *FIL* expression in a stage 8 L*er* flower. D, *FIL* expression in a stage 8 *ant-4* flower. Size bars correspond to 50 μm in A to D. Bottom section, Relative expression levels (compared to *ACTIN2*) of *YAB3* in L*er* and *ant-4* inflorescences. The average of two experiments is shown. The bars show SD.

closely resembled each other with neither the adaxial or abaxial surface displaying its characteristic appearance (Fig. 4, K and L). Loss of both adaxial and abaxial identities distinguishes the *fil ant* and *fil yab ant* mutants from mutations in either the *KAN* or *PHB*-like genes, where there is replacement of one identity with the other identity (i.e. replacement of abaxial cell fates with adaxial identities in *kan* mutants).

fil-8 ant-4 and *fil-8 yab3-2 ant-4* leaves also exhibit alterations in their vascular patterning. Vascular tissue in wild-type leaves exhibits a reticulate pattern with minor veins branching from the major vein (Fig. 3I). There was a marked decrease in vascular branching in the leaves of *fil-8 ant-4* plants (Fig. 3K). This phenotype is similar to that reported previously for *yab3-1 fil-5* leaves (Siegfried et al., 1999) and *fil-8 yab3-2* (Fig. 3J). *fil-8 yab3-2 ant-4* leaves often have just a single vein running the length of the leaf (Fig. 3L). In some cases, one to several shorter veins branch from this central vein.

fil ant and *fil yab ant* Mutants Show Disruptions in Floral Organ Identity and Polarity

fil-8 ant-4 and *fil-8 yab3-2 ant-4* plants exhibit inflorescence defects similar to those observed in *fil-8* plants with the inflorescence meristem switching between the production of flowers and filaments (Sawa et al., 1999). In *fil-8 ant-4*, around 12 flowers were produced before the inflorescence meristem started to produce filaments (Fig. 6A). This is similar to the number of individual flowers initiated by the inflorescence meristem of *fil-8* plants prior to filament production. Fewer flowers were produced prior to filament production in *fil-8 yab3-2 ant-4* plants (Fig. 5J). After producing some filaments, *fil-8 ant-4* and *fil-8 yab3-2 ant-4* inflorescence meristems switched to producing a mixture of flower-like structures and filaments (Fig. 6). This was subsequently followed by termination of the inflorescence meristem. After termination of the primary inflorescence, secondary and axillary inflorescences grew out, resulting in the production of short and bushy *fil-8 ant-4* and *fil-8 yab3-2 ant-4* plants (Fig. 5).

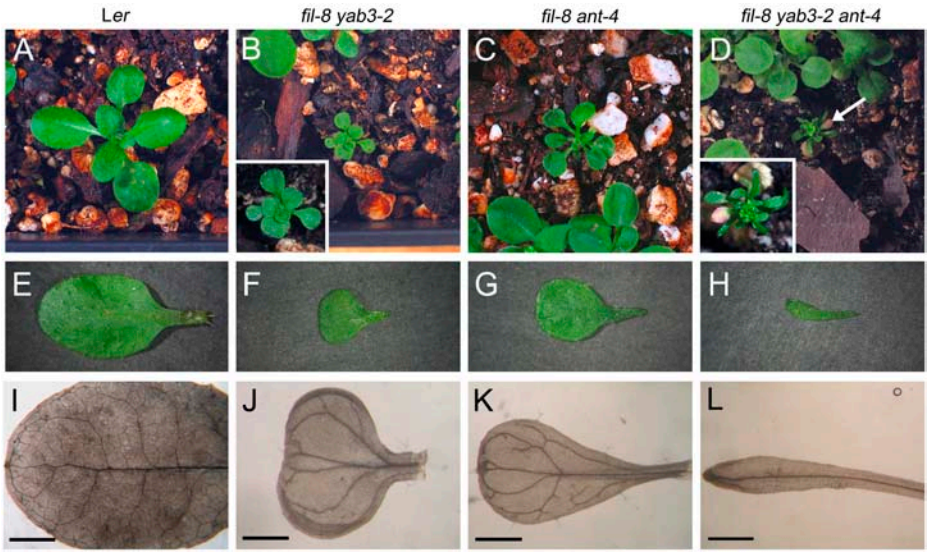

Figure 3. Wild-type, *fil-8 yab3-2*, *fil-8 ant-4*, and *fil-8 yab3-2 ant-4* leaves. A to D, Mature rosettes just prior to bolting: L*er* (A), *fil-8 yab3-2* (B), *fil-8 ant-4* (C), and *fil-8 yab3-2 ant-4* (D). The pictures in A to D are taken at the same magnification. Insets in B and D show closer views. E to H, Fully expanded leaves from L*er* (E), *fil-8 yab3-2* (F), *fil-8 ant-4* (G), and *fil-8 yab3-2 ant-4* (H) plants. The pictures in E to H are taken at the same magnification. I to L, Vascular patterns of L*er* (I), *fil-8 yab3-2* (J), *fil-8 ant-4* (K), and *fil-8 yab3-2 ant-4* (L) fully expanded leaves. The pictures in I to L are taken at the same magnification. Leaves shown in E to L were from position 5 or 6 of the rosette. Size bars correspond to 1 mm in I to L.

Table I. *Leaf size in wild-type and mutant plants*

The data are indicated as averages ± SD. *, Values that are significantly different from wild type (Student's *t* test, $P < 0.01$).

	Leaf Area	Leaf Width	Leaf Length
	mm²	*mm*	*mm*
L*er*	55 ± 8.4	6.8 ± 0.52	12.5 ± 1.1
ant-4	48 ± 5.7*	7.0 ± 0.49	9.3 ± 0.64*
fil-8	54 ± 14	7.3 ± 0.94	11.5 ± 2.0
fil-8 yab3-2	10 ± 2.3*	3.2 ± 0.60*	5.3 ± 0.85*
fil-8 ant-4	27 ± 13*	5.0 ± 1.1*	8.9 ± 1.9*
fil-8 yab3-2 ant-4	4.1 ± 1.1*	1.6 ± 0.48*	3.7 ± 1.9*

The flowers produced by *fil-8 ant-4* and *fil-8 yab3-2 ant-4* plants were much smaller than flowers of wild type, *fil-8*, or *fil-8 yab3-2* (Fig. 5B). In addition, they exhibited loss of floral identity as demonstrated by the presence of flowers with subtending leaves and by a loss of floral organ identity. *fil-8 ant-4* and *fil-8 yab3-2 ant-4* flowers typically consisted of narrow, flat, green organs; filaments; and carpelloid organs (Figs. 5, G and I, and 6, B and C). Because these organs lack most recognizable features of floral organs, we examined their development and cell types by SEM to better characterize them. Because *fil-8 ant-4* and *fil-8 yab3-2 ant-4* flowers were quite similar, we present a detailed SEM analysis of just *fil-8 ant-4* flowers.

fil-8 ant-4 flowers typically produced two or three whorls of floral organs with variable numbers and positions of organs within each whorl (Fig. 6, D–F). Flowers arising later on the inflorescence typically produced a fewer number of organs. The flat, outermost organs from early arising *fil-8 ant-4* flowers had epidermal cells resembling those of sepals (Fig. 6G). In later-arising flowers, these organs became thinner and more pointed. SEM analysis showed that these later-arising outer whorl organs were mosaics containing both leaf-like and sepal-like cells (Fig. 6H). Filamentous organs present in the outer two whorls of *fil-8 ant-4* flowers were variable in appearance. Those in the outermost whorl were typically dark green and had epidermal cells resembling those of sepals (Fig. 6I), while filaments in the second whorl were light green or white in color with more regular cells in files (Fig. 6J). In addition, filaments in the outer whorl tended to be thicker than those in the second whorl. The flat innermost organs of *fil-8 ant-4* flowers resembled unfused or partially fused carpels. Style-like cells were found at the top of these organs (Fig. 6, K and L), and valve-like ovary cells were present along the rest of their length. Stigmatic papillae were sometimes present, although not necessarily at the apex of these organs (Fig. 6C). The carpel-like organs arose as distinct primordia rather than the fused ring of tissue that develops in wild-type flowers (Fig. 6D). These primordia gave rise to either distinct organs (Fig. 6E) or partially fused organs (Fig. 6F). Internal tissues present within a normal gynoecium (septum, transmitting tract, placenta, and ovules) were almost completely lacking in *fil-8 ant-4* flowers (Fig. 6, B and C).

In a few early arising *fil-8 ant-4* flowers, narrow white organs, and/or yellow stamen-like organs were present (Figs. 5H and 6M). SEM examination indicated that petal epidermal cells were present on the surface of some of these white organs (Fig. 6N). In some cases, these organs exhibited polarity defects, as petals cells with both adaxial and abaxial morphologies were present on the abaxial surface of these organs (Fig. 6O). Epidermal cells characteristic of stamens were present on the stamen-like organs (Fig. 6, P and Q). A small amount of internal carpel tissue was occasionally present in early arising *fil-8 ant-4* flowers (Fig. 6P). *fil-8 yab3-2 ant-4* flowers exhibit a slightly more severe phenotype than *fil-8 ant-4* flowers in that organs with

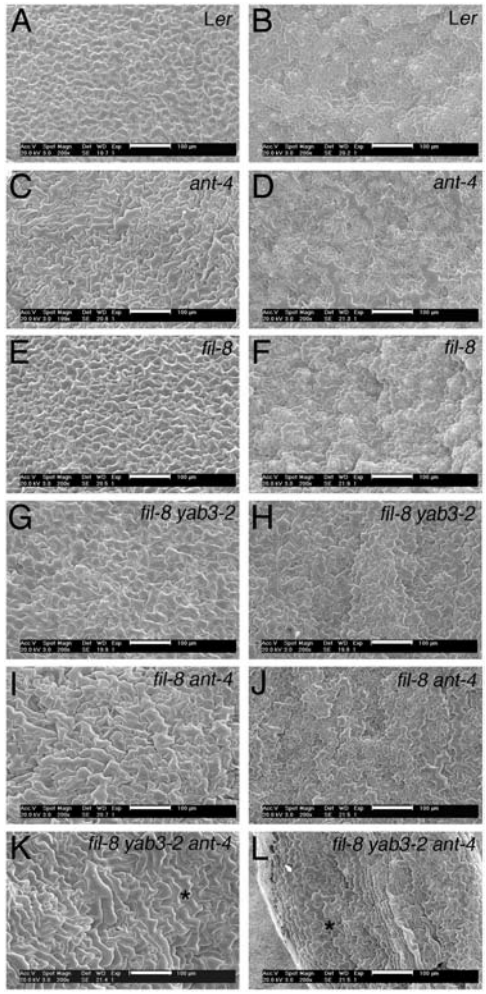

Figure 4. Adaxial and abaxial surfaces of leaves from wild-type, *ant-4*, *fil-8*, *fil-8 yab3-2*, *fil-8 ant-4*, and *fil-8 yab3-2 ant-4* plants. Shown are adaxial leaf surfaces of L*er* (A), *ant-4* (C), *fil-8* (E), *fil-8 yab3-2* (G), *fil-8 ant-4* (I), and *fil-8 yab3-2 ant-4* (K). Also shown are abaxial leaf surfaces of L*er* (B), *ant-4* (D), *fil-8* (F), *fil-8 yab3-2* (H), *fil-8 ant-4* (J), and *fil-8 yab3-2 ant-4* (L). Two cells with similar morphologies in K and L are noted with *. Size bars correspond to 100 μM.

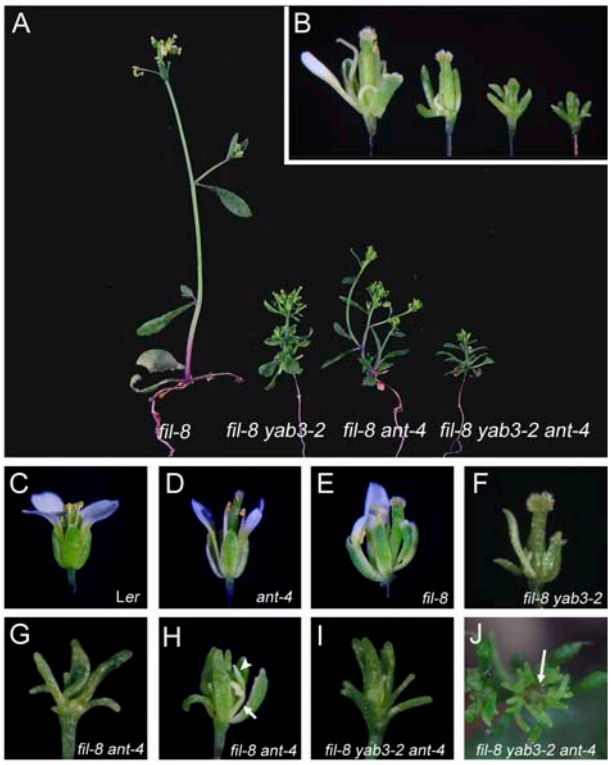

Figure 5. Flowers from wild-type, *ant-4*, *fil-8*, *fil-8 yab3-2*, *fil-8 ant-4*, and *fil-8 yab3-2 ant-4* plants. A, Side view of *fil-8*, *fil-8 yab3-2*, *fil-8 ant-4*, and *fil-8 yab3-2 ant-4* plants of approximately 3 weeks of age. B, A single image showing the side views of *fil-8*, *fil-8 yab3-2*, *fil-8 ant-4*, and *fil-8 yab3-2 ant-4* flowers (from left to right) and the relative sizes of the flowers. C, L*er* flower. D, *ant-4* flower. E, *fil-8* flower. F, *fil-8 yab3-2* flower. G, *fil-8 ant-4* flower. H, Early arising *fil-8 ant-4* flower. Arrow points to a staminoid organ and arrowhead points to a white petaloid organ. I, *fil-8 yab3-2 ant-4* flower. J, *fil-8 yab3-2 ant-4* inflorescence meristem that has initiated filament production. Arrow points to the inflorescence meristem.

petal and stamen-like cells were never observed in *fil-8 yab3-2 ant-4* flowers.

fil-8 ant-4 and *fil-8 yab3-2 ant-4* flowers have a more severe phenotype than *fil-8 yab3-2* flowers (Fig. 5, F, G, and I). *fil-8 yab3-2* flowers consist of radialized or flat sepal-like organs, no petals, small stamen filaments lacking anthers, and a carpel with a larger style and no replum (Siegfried et al., 1999; Kumaran et al., 2002). In comparison, *fil-8 ant-4* and *fil-8 yab3-2 ant-4* flowers consisted of fewer floral organs and show increased radialization of outer whorl organs and loss of carpel adaxial tissue.

fil ant Flowers Show Altered Expression of Floral Organ Identity Genes

Because of the greatly reduced floral organ identity in *fil-8 ant-4* flowers, we examined the expression patterns of the floral homeotic genes *APETALA3* (*AP3*) and *AGAMOUS* (*AG*) by in situ hybridization. *AP3* is a B class floral homeotic gene involved in the specification of petal and stamen identities, and *AG* is a C function gene involved in the specification of stamen and carpel identities (Yanofsky et al., 1990; Jack et al., 1992). *AP3* expression is greatly reduced or absent in young *fil-8 ant-4* flowers as compared with wild type (Fig. 7, A–D). When *AP3* mRNA was detected in young *fil-8 ant-4* flowers, it was present in a normal spatial pattern (Fig. 7B). *AP3* mRNA was only rarely observed in organs of older *fil-8 ant-4* flowers (Fig. 7, E and F). *AP3* mRNA was often observed in a few cells located between the inner and outer whorls of *fil-8 ant-4* flowers (Fig. 7G). Weak patches of *AP3* expression were rarely observed in filaments produced in place of flowers (Fig. 7H).

In wild-type plants, *AG* mRNA was first detected in the center of the floral meristem of stage 3 flowers (Fig. 7I). In *fil-8 ant-4* plants, *AG* was misexpressed in the inflorescence meristem (Fig. 7J). *AG* mRNA was also detected in stage one and two floral meristems, earlier than the first appearance of *AG* mRNA in L*er* flowers (data not shown). *AG* was expressed at high levels in the center of young *fil-8 ant-4* floral meristems, similar to the pattern seen in young L*er* stage 3 floral meristems (Fig. 7, K and L). In some cases, this *AG* expression domain was broader in stage 4 *fil-8 ant-4* flowers than stage 4 L*er* flowers and extended into the outermost organ primordia (Fig. 7M). In L*er* flowers, *AG* is expressed throughout developing stamens and carpels until late stages of flower development (Fig. 7N). In older *fil-8 ant-4* flowers, *AG* mRNA was detected on the inner surface of carpel-like organs (Fig. 7O). *AG* mRNA was also present in the center of filamentous structures produced by the inflorescence meristem (Fig. 7P).

fil ant Flowers Show Reduced Floral Expression of the Adaxial Cell Fate Regulator *PHB*

The radialization of *fil-8 ant-4* and *fil-8 yab3-2 ant-4* floral organs suggests that these organs have lost adaxial-abaxial polarity. To further investigate this possibility, we examined the expression of the adaxial cell fate regulator *PHB* in *fil-8 ant-4* flowers. *PHB* mRNA is present in the inflorescence meristem and throughout young floral meristems of L*er* flowers (Fig. 8A). In stage 4 flowers, *PHB* mRNA was detected in the center of the floral meristem and in the adaxial half of developing sepal primordia (Fig. 8B). The

Table II. *Height of wild-type and mutant plants*

The data are indicated as averages ± SD. *, Values that are significantly different from wild type (Student's *t* test, $P < 0.01$).

	Height
	cm
L*er*	14.2 ± 2.52
ant-4	11.9 ± 1.87
fil-8	10.4 ± 1.85
fil-8 yab3-2	1.8 ± 0.492*
fil-8 ant-4	3.8 ± 0.898*
fil-8 yab3-2 ant-4	1.1 ± 0.084*

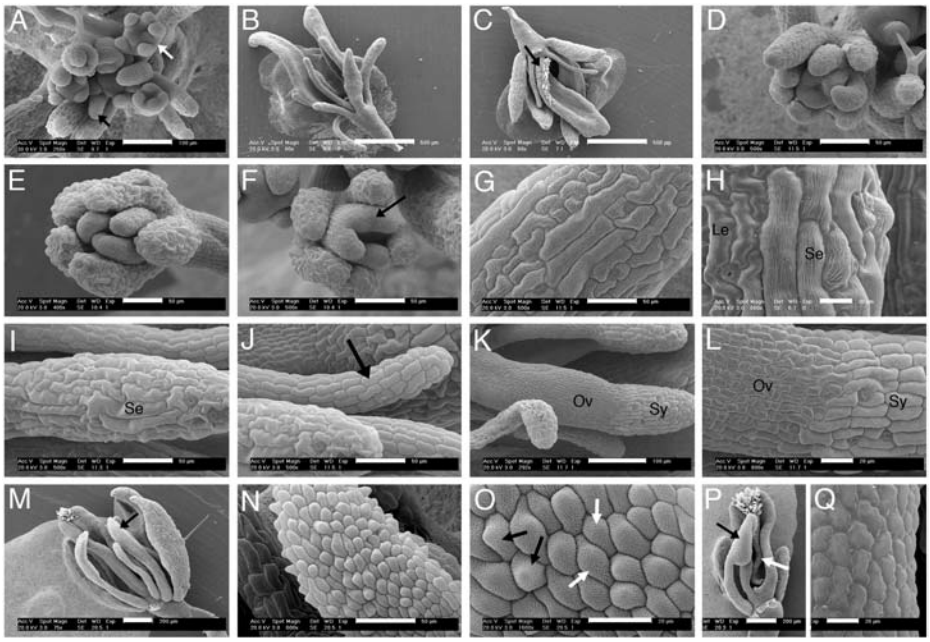

Figure 6. SEM analyses of *fil-8 ant-4* flowers. A, *fil-8 ant-4* inflorescence meristem producing a mixture of flowers (white arrow) and filamentous structures (black arrow). B, *fil-8 ant-4* flower. C, *fil-8 ant-4* flower. Stigmatic papillae are indicated with an arrow. D, Stage 7 *fil-8 ant-4* flower showing three whorls of organ primordia. E, *fil-8 ant-4* flower with two visible whorls of organs. The inner whorl contains three unfused organ primordia. F, Stage 8 *fil-8 ant-4* flower with two visible whorls of organs. A black arrow points to a region of fusion between the inner whorl organ primordia. G, An outer whorl organ with sepal-like cells. H, An outer whorl organs with leaf-like (Le) and sepal-like (Se) cells. I, An outer whorl filamentous organ with sepal-like (Se) cells. J, An inner whorl filamentous organ (arrow). K, An inner whorl carpel-like organ with ovary valve-like (Ov) cells at the base and style-like (Sy) cells at the top. L, Close-up of organ shown in K. M, An early arising *fil-8 ant-4* flower. A black arrow indicates a petal-like organ. N, Close-up of the petal-like organ in M showing cells with petal morphologies. O, Close-up of the organ in M. Petal cells with adaxial (black arrows) and abaxial (white arrows) morphologies are present on the abaxial surface of this *fil-8 ant-4* organ. The adaxial petal epidermal cells are conical in shape with epicuticular thickenings oriented along the cone axis. The abaxial epidermal petal cells are flatter with more zigzagged epicuticular thickenings. P, Staminoid organ (black arrow) present in an early arising *fil-8 ant-4* flower. The white arrow points to adaxial carpel tissue. Q, Close-up of the anther-like region of the staminoid organ in P. Size bars correspond to 20 μm in H, L, O, and Q; 50 μm in D to G, I, J, and N; 100 μm in A and K; 200 μm in M and P; and 500 μm in B and C.

expression pattern and levels of *PHB* mRNA were similar in *fil-8* and *ant-4* single mutants as compared with L*er* (Fig. 8, C–F). In *fil-8 ant-4* plants, *PHB* mRNA was typically present at lower levels in the inflorescence meristem and stage 1 and 2 floral meristems as compared with wild type (Fig. 8G). *PHB* mRNA was usually absent from the outer whorl organs of *fil-8 ant-4* flowers and was present in reduced amounts in the floral meristem of young stage 3 and 4 *fil-8 ant-4* flowers (Fig. 8H).

DISCUSSION

ANT is an important regulator of lateral organ development. *ANT* expression marks cells that will leave the meristem to form lateral organs, and it is required for proper initiation and growth of lateral organs. Mutations in *ANT* result in the production of fewer and smaller floral organs (Elliott et al., 1996; Klucher et al., 1996). Despite its importance in lateral organ development, little is known about the genes regulated by this transcription factor. Our results here suggest that ANT acts with FIL to regulate organogenesis and to up-regulate genes establishing organ polarity and those specifying organ identity.

ANT Regulates Organ Polarity

While lateral organs in *ant* single mutants exhibit normal polarity, defects along the adaxial/abaxial axis were visible at the whole organ, cellular, and molecular level in *fil ant* and *fil yab ant* plants. *fil yab ant* leaves showed dramatic reductions in lamina growth and loss of both adaxial and abaxial epidermal cell identities. *fil ant* and *fil yab ant* floral organs were severely radialized with many floral organs replaced by filaments or very narrow organs. In some floral organs, adaxial cell types were found in abaxial positions. These defects are more severe than those observed in either *fil* or *fil yab* mutants.

To investigate whether the role of ANT in polarity establishment involves regulation of known adaxial and/or abaxial identity factors, we examined the

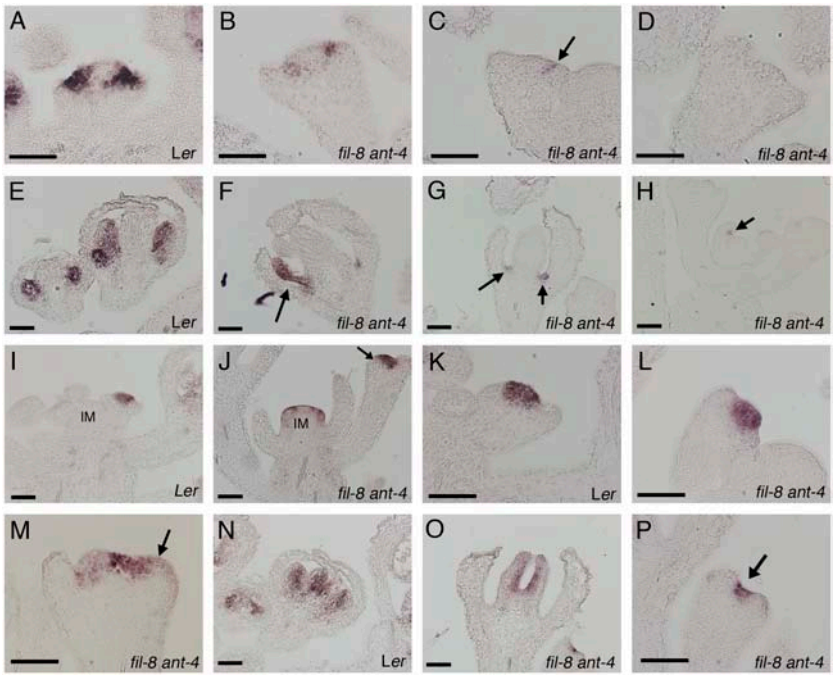

Figure 7. *AP3* and *AG* expression in wild-type and *fil-8 ant-4* flowers. *AP3* expression is shown in A to H, and *AG* expression is shown in I to P. Size bars correspond to 50 μm in A to P. A, *AP3* mRNA in a L*er* stage 3 flower. B, *AP3* mRNA in a stage 3 *fil-8 ant-4* flower. C, Arrow points to region of *AP3* expression in a stage 3 *fil-8 ant-4* flower. D, No *AP3* mRNA was detected in this *fil-8 ant-4* flower. E, *AP3* mRNA is detected in the second and third whorls of stage 5 (left) and stage 8 (right) flowers. F, *AP3* mRNA is detected in a stamen-like organ of a *fil-8 ant-4* flower. G, *AP3* mRNA is present in a few cells located between the outer and inner whorl in older *fil-8 ant-4* flowers. H, *AP3* mRNA was rarely detected in filaments (arrow) initiated by the inflorescence meristem of *fil-8 ant-4* plants. I, *AG* expression is first detected in a stage 3 flower in wild type. J, *AG* is expressed in the inflorescence meristem of this *fil-8 ant-4* plant. K, *AG* mRNA is detected in the central part of the floral meristem in a stage 3 L*er* flower. L, *AG* expression in a stage 3 *fil-8 ant-4* flower. M, *AG* expression in the outer organ primordia (arrow) of a stage 4 *fil-8 ant-4* flower. N, *AG* is expressed in the stamens and carpels of a stage 8 L*er* flower. O, *AG* mRNA is detected on the adaxial surface of the central carpel-like organs in a *fil-8 ant-4* flower. P, *AG* mRNA is detected in the central region of filamentous structures (arrow) produced by a *fil-8 ant-4* inflorescence meristem.

expression of *FIL*, *YAB3*, and *PHB* in *ant* mutants. While the expression of *FIL*, *YAB3*, and *PHB* are normal in *ant-4* flowers, *PHB* expression was reduced in *fil ant* double mutants. This suggests that ANT and FIL together are required for normal levels of *PHB* mRNA. Examination of the *PHB* promoter revealed a sequence matching the ANT consensus binding site in 12 of 14 conserved positions including one gap (S. Nole-Wilson and R. Franks, personal communication). We were unable to detect binding of ANT to this site in vitro, suggesting that ANT is not a direct regulator of *PHB* expression, its role in *PHB* regulation involves additional factors, and/or that ANT binds to a different DNA sequence within the context of the *PHB* promoter.

It is curious that an ANT binding site is present within a conserved region of the *FIL* and *YAB3* promoters but that ANT is not required for *FIL* or *YAB3* expression. Seven *AINTEGUMENTA*-like (*AIL*) genes are present within the Arabidopsis genome, and several of these genes are expressed in young floral primordia in overlapping domains with ANT (Nole-Wilson et al., 2005). Sequence conservation within the DNA-binding APETALA2 repeat regions of AIL proteins suggest that they may have similar DNA-binding specificities. It is possible that redundancy within the AIL family masks any effect of loss of ANT activity on *FIL* and *YAB3* expression. It will be necessary to generate double, triple, and perhaps higher order mutants within members of the *AIL* gene family to investigate this possibility.

As both adaxial and abaxial identities are partially lost in *fil yab ant* mutants and *ANT* is expressed throughout lateral organs, our results suggest that ANT is a positive regulator of both genes specifying adaxial fates and those specifying abaxial fates. ANT may function as a general activator of *PHB*-like and *YABBY* genes throughout organ primordia with their region-specific expression resulting from mutually repressive interactions between the *PHB*-like and *KAN* genes. Supporting our view that ANT is a positive regulator of genes specifying either adaxial or abaxial fates, preliminary examination of *ant rev* double mutants revealed enhanced carpel phenotypes

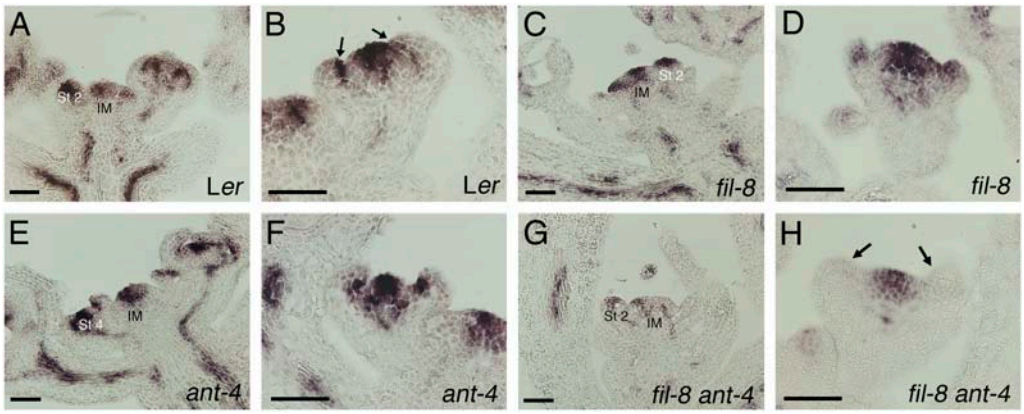

Figure 8. *PHB* expression in wild-type, *fil-8*, *ant-4*, and *fil-8 ant-4* flowers. Size bars correspond to 50 μm in A to H. A, *PHB* expression in a wild-type inflorescence. B, *PHB* is expressed in the adaxial region of sepal primordia (arrows) and in the floral meristem dome in a stage 4 L*er* flower. C, *PHB* expression in a *fil-8* inflorescence. D, *PHB* expression in a stage 3 *fil-8* flower. E, *PHB* expression in an *ant-4* inflorescence. F, *PHB* expression in a stage 4 *ant-4* flower. G, *PHB* mRNA is present at reduced levels in *fil-8 ant-4* inflorescences. H, *PHB* is expressed in the floral meristem dome but not in the outer whorl organ primordia of this stage 3 *fil-8 ant-4* flower. St 2/4, Stage 2 and stage 4 flowers; IM, inflorescence stem.

including enhanced loss of some adaxial tissues relative to either single mutant (S. Nole-Wilson and R. Franks, personal communication).

ANT May Act with SEUSS and LEUNIG to Regulate Expression of *PHB*

SEUSS (*SEU*) and *LEUNIG* (*LUG*) have been proposed to promote polarity along the adaxial/abaxial axis in petals by positively regulating *PHB* and *FIL* expression (Franks et al., 2006). Similar effects on leaf lamina/petal blade expansion and vascular development are observed in *ag seu lug* petals as reported here for *fil yab ant* leaves (Franks et al., 2006). These similarities are particularly intriguing as *SEU*, *LUG*, and *ANT* share other functions during flower development. All three proteins act as negative regulators of the floral homeotic gene *AG* (Liu and Meyerowitz, 1995; Krizek et al., 2000; Franks et al., 2002). Interestingly, *FIL* also acts as an *AG* repressor in whorls one and two (Chen et al., 1999). Similar carpel growth defects result from the combined loss of *LUG* and *ANT* or the combined loss of *FIL* and *ANT*. In both *fil ant* and *lug ant* flowers, the inner whorl consists of unfused or partially fused valve-like structures with style cells at their tips and an almost complete loss of adaxial tissues (placenta, ovules, and septa; Krizek et al., 2000; Liu et al., 2000).

The similarities in these phenotypes suggest that ANT, FIL, SEU, and LUG have overlapping and partially redundant functions. These proteins might form a complex in which the SEU-LUG transcriptional corepressor (Sridhar et al., 2004) is recruited to promoter sequences via interaction with either of the DNA-binding proteins ANT or FIL (Nole-Wilson and Krizek, 2000; Kanaya et al., 2002). The Antirrhinum LUG ortholog STYLOSA has been shown to physically interact with YABBY proteins (Navarro et al., 2004). *ANT* and *FIL* could share some functions such that severe phenotypes only result in the absence of both *ANT* and *FIL*.

ANT and *YABBY* Genes Promote Lamina Expansion and Floral Organ Identity

Besides contributing to the specification of abaxial identity, *YABBY* genes are important regulators of lamina expansion. Polar expression of *YABBY* genes has been proposed to regulate signaling events between the adaxial and abaxial domains that control cell division in each domain and expansion of the leaf lamina (Eshed et al., 2004). Although *ant* mutants show only slight reductions in leaf area, the narrower lamina of *fil yab ant* leaves as compared with *fil yab* leaves indicates that *ANT* plays an important growth promotion role in leaves as well as flowers. Consistent with a role in lamina expansion, *ANT* expression in developing leaves becomes refined to the central and marginal regions in a pattern that is quite similar to the *Solanum tuberosum YABBY* gene *StYABBY1* (Long and Barton, 2000; Eshed et al., 2004).

While petals and stamens are present in *fil* and *ant* single mutants, organs with petal or stamen characteristics are rare in *fil ant* double mutants. The loss of floral organ identify in *fil ant* double mutants was correlated with altered floral homeotic gene expression. *AP3* expression was reduced in *fil ant* flowers while the pattern of *AG* expression was altered. Thus, ANT acts as a positive regulator of the class B gene *AP3* and acts to prevent *AG* expression in inflorescences and flowers prior to stage 3. A role for ANT in *AG* repression has been noted previously (Krizek et al., 2000; Liu et al., 2000). Similar losses in floral organ identity have been observed in other genotypes with polarity defects. For example, *kan1 kan2 fil yab* flowers consist of carpels and radialized organs that lack

cell types characteristic of sepals, petals, or stamens (Eshed et al., 2004). These results suggest a connection between the establishment of polarity and the specification of organ identity during flower development. Further studies will be needed to probe this relationship and to better understand the diverse processes that ANT regulates during lateral organ development.

MATERIALS AND METHODS

Protein Expression

Full-length *ANT* lacking a stop codon was cloned into pQE12 (Qiagen) and expressed by induction with 1 mM isopropyl-β-D-thiogalactoside in XL1-Blue MRF′ Tet cells (Stratagene) at 30°C. Cells were harvested between 6 and 8 h after induction. ANT was purified using Ni-NTA (Qiagen) under denaturing conditions according to the manufacturer's instructions. ANT refolded upon dilution in the DNA-binding reactions.

Gel Mobility Shift Assays

Gel mobility shift assays were carried out as described previously (Nole-Wilson and Krizek, 2000), except that binding reactions were incubated for 4 h at room temperature or overnight at 4°C. The *YAB3* and *FIL* binding sites were created by PCR amplification of Columbia genomic sequence using YAB-12 (5′-CTCGAGATTAAGTGTGAAAACAACTGAT-3′) and YAB-13 (5′-GAATTCAAAGGACGCAAAGTTCGATG-3′) or FIL-3 (5′-TACACTCGAGTTAAGGAATGACAACAACGGG-3′) and YAB-9 (5′-TACCGGATCCGAATTCGCAGTTCCCAATGGA-3′), respectively. These fragments correspond to nucleotides at positions 1,561 to 1,456 upstream of the *YAB3* start codon and nucleotides at positions 1,763 to 1,633 upstream of the *FIL* start codon. The PCR products were cloned into pCRScript (Stratagene) and the probes were prepared as described previously (Nole-Wilson and Krizek, 2000).

Plant Growth Conditions

Arabidopsis (*Arabidopsis thaliana*) ecotype L*er* was used as the wild type. Plants were grown in a soil mixture of Fafard 4P:perlite:vermiculite in a ratio of 4:1:1 under continuous light (70–100 μmol m^{-2} s^{-1}) at a temperature of 22°C. Plants were fertilized once at 1 to 2 weeks postgermination.

Real-Time RT-PCR

Inflorescences were collected from 3- to 4-week-old L*er* and *ant-4* plants grown on soil under continuous light at 22°C. Total RNA was extracted and DNase treated as described previously (Nole-Wilson et al., 2005). Approximately 5 μg of total RNA was reverse transcribed using the Superscript First-Strand Synthesis system for RT-PCR (Invitrogen). Real-time RT-PCR was performed as described previously except that *ACTIN2* (*ACT2*) was used for normalization purposes (Nole-Wilson et al., 2005). The *ACT2* primers were ACT-3 (5′-CCTTTGTTGCTGTTGACT-3′) and ACT-4 (5′-GAACAAGACTTCTGGGCATCT-3′). The *YAB3* primers were YAB3-10 (5′-GCGGAGGGCAGAATATAAAC-3′) and YAB3-11 (5′-CACTGATCTTCCGTTGCGA-3′).

In Situ Hybridization

Inflorescences were fixed, embedded, sectioned, hybridized, and washed as described previously (Krizek, 1999). Digoxigenin-labeled RNA probes were synthesized by in vitro transcription using T7 RNA polymerase (*AP3*, *AG*, and *FIL* probes) or T3 RNA polymerase (*PHB* probe) and the appropriate linearized plasmids. The *AP3*, *AG*, and *FIL* plasmids used for probe production have been described before (Yanofsky et al., 1990; Jack et al., 1992; Siegfried et al., 1999). The *PHB* probe corresponds to nucleotides 609 to 2,559 of PHB and was made after linearization of PHB/pBSKS with *Xba*I.

Generation of *fil-8 ant-4* and *fil-8 yab3-2 ant-4* Plants

yab3-2 fil-8/+ seeds were obtained from John Bowman. These alleles have been described previously (Kumaran et al., 1999, 2002). Both *yab3-2* and *fil-8* mutants are Ds insertion lines in the L*er* background. *YAB3* transcripts are not detectable in *yab3-2* (Kumaran et al., 2002). *ant-4* seeds were obtained from Charles Gasser (Baker et al., 1997). *ant-4* is in the L*er* background and contains a T-to-A transversion at nucleotide 1,335, altering the donor splice site of the fourth intron. These alleles were chosen as they are all strong alleles in the L*er* background. The double and triple mutants were generated by pollinating putative *fil-8 yab3-2*/+ plants with *ant-4* pollen. F_2 and subsequent generations were observed for segregation of plants with novel phenotypes. *fil-8 ant-4* double mutants and *fil-8 yab3-2 ant-4* triple mutants were confirmed by PCR genotyping.

PCR Genotyping

yab3-2

Green leaf tissue was prepared as described previously (Klimyuk et al., 1993) and subjected to PCR. PCR reactions using YAB35I-F (5′-GCCCTCCTCTCTCTCTTACTC-3′) and YAB35I-R2 (5′-TCTGACCGTCACCGTCTTGA-3′) verified the absence of the wild-type *YAB3* allele and PCR reactions using Ds5I-1 (5′-CCGTTTACCGTTTTGTATATCCCG-3′) and YAB35I-R2 verified the presence of the *yab3-2* mutant allele.

fil-8

Green leaf tissue was prepared as described above and subjected to PCR. PCR reactions using AFO-FW3 (5′-AGATTCCTAAAGCACCACCC-3′) and YAB1R (5′-GATACGTTGGATCTCCTCCC-3′) verified the absence of the wild-type *FIL* allele and PCR reactions using AFO-FW3 and Ds5I-1 verified the presence of the *fil-8* allele.

ant-4

DNA was isolated from green leaf tissue by grinding in 200 mM Tris, pH 7.5, 250 mM NaCl, 25 mM EDTA, 0.5% SDS, and precipitation with isopropanol. The DNA was then PCR amplified using ANT-6 (5′-TCAAGGATCCACTTTTGGACAACGAACTTCT-3′) and ANT-33 (5′-TCTTGGATCCTGCAACATATTCTTGTCTAGT-3′). The PCR product was gel purified and cloned into the *Bam*HI site of pGEM3Z (Promega). Sequencing of multiple clones confirmed the presence of only the *ant-4* allele in putative *fil-8 ant-4* and *fil-8 yab3-2 ant-4* plants.

Leaf Area and Plant Height Measurements

For each genotype, the largest rosette leaf was removed from six different plants at the time of bolting. Leaf surface area was measured with a LI-COR LI-3000 portable area meter. The length and width of rosette leaves were measured using an ocular micrometer or a ruler. Plant heights were determined for six different plants of each genotype at the time when their primary inflorescences were starting to senesce. Bolting time was approximately the same for all genotypes.

Leaf Vascular Staining

Leaves were fixed overnight at room temperature in a 3:1 solution of ethanol:acetic acid. The tissue was mounted in 70% ethanol and examined using a dissecting microscope with illumination from below.

SEM

Tissue for SEM was fixed, dried, dissected, and coated as described previously (Krizek, 1999). SEM analysis was performed on a FEI XL30 ESEM (Hillsboro).

ACKNOWLEDGMENTS

We thank John Bowman for the *fil-8 yab3-2*/+ seeds and the *FIL* in situ plasmid, Charles Gasser for the *ant-4* seeds, David Lincoln for help with leaf area measurements, John Herr for assistance with the xylem staining, and Mike Prigge and Steve Clark for the *PHB* in situ plasmid. We also thank Bob

Franks for sharing unpublished data and providing valuable comments on the manuscript.

Received January 2, 2006; revised May 5, 2006; accepted May 12, 2006; published May 19, 2006.

LITERATURE CITED

Baker SC, Robinson-Beers K, Villanueva JM, Gaiser JC, Gasser CS (1997) Interactions among genes regulating ovule development in *Arabidopsis thaliana*. Genetics **145:** 1109–1124

Bowman JL (2000) The YABBY gene family and abaxial cell fate. Curr Opin Plant Biol **3:** 17–22

Bowman JL, Eshed Y, Baum SF (2002) Establishment of polarity in angiosperm lateral organs. Trends Genet **18:** 134–141

Bowman JL, Smyth DR (1999) *CRABS CLAW*, a gene that regulates carpel and nectary development in Arabidopsis, encodes a novel protein with zinc finger and helix-loop-helix domains. Development **126:** 2387–2396

Chen Q, Atkinson A, Otsuga D, Christensen T, Reynolds L, Drews GN (1999) The Arabidopsis *FILAMENTOUS FLOWER* gene is required for flower formation. Development **126:** 2715–2726

Elliott RC, Betzner AS, Huttner E, Oakes MP, Tucker WQJ, Gerentes D, Perez P, Smyth DR (1996) *AINTEGUMENTA*, an *APETALA2*-like gene of Arabidopsis with pleiotropic roles in ovule development and floral organ growth. Plant Cell **8:** 155–168

Emery JF, Floyd SK, Alvarez J, Eshed Y, Hawker NP, Izhaki A, Baum SF, Bowman JL (2003) Radial patterning of Arabidopsis shoots by class III HD-ZIP and KANADI genes. Curr Biol **13:** 1768–1774

Engstrom EM, Izhaki A, Bowman JL (2004) Promoter bashing, microRNAs, and KNOX genes. New insights, regulators, and targets-of-regulation in the establishment of lateral organ polarity in Arabidopsis. Plant Physiol **135:** 685–694

Eshed Y, Baum SF, Perea JV, Bowman JL (2001) Establishment of polarity in lateral organs of plants. Curr Biol **11:** 1251–1260

Eshed Y, Izhaki A, Baum SF, Floyd SK, Bowman JL (2004) Asymmetric leaf development and blade expansion in Arabidopsis are mediated by KANADI and YABBY activities. Development **131:** 2997–3006

Franks RG, Liu Z, Fischer RL (2006) *SEUSS* and *LEUNIG* regulate cell proliferation, vascular development and organ polarity in Arabidopsis petals. Planta (in press)

Franks RG, Wang C, Levin JZ, Liu Z (2002) *SEUSS*, a member of a novel family of plant regulatory proteins, represses floral homeotic gene expression with *LEUNIG*. Development **129:** 253–263

Jack T, Brockman LL, Meyerowitz EM (1992) The homeotic gene *APETALA3* of *Arabidopsis thaliana* encodes a MADS box and is expressed in petals and stamens. Cell **68:** 683–687

Kanaya E, Nakajima N, Okada K (2002) Non-sequence-specific DNA binding by the FILAMENTOUS FLOWER protein from *Arabidopsis thaliana* in reduced by EDTA. J Biol Chem **227:** 11957–11964

Kerstetter RA, Bollman K, Taylor RA, Bomblies K, Poethig RS (2001) *KANADI* regulates organ polarity in Arabidopsis. Nature **411:** 706–709

Klimyuk VI, Carroll BJ, Thomas CM, Jones JDG (1993) Alkali treatment for rapid preparation of plant material for reliable PCR analysis. Plant J **3:** 493–494

Klucher KM, Chow H, Reiser L, Fischer RL (1996) The *AINTEGUMENTA* gene of Arabidopsis required for ovule and female gametophyte development is related to the floral homeotic gene *APETALA2*. Plant Cell **8:** 137–153

Krizek BA (1999) Ectopic expression of *AINTEGUMENTA* in *Arabidopsis* plants results in increased growth of floral organs. Dev Genet **25:** 224–236

Krizek BA, Prost V, Macias A (2000) *AINTEGUMENTA* promotes petal identity and acts as a negative regulator of *AGAMOUS*. Plant Cell **12:** 1357–1366

Kumaran MK, Bowman JL, Sundaresan V (2002) *YABBY* polarity genes mediate the repression of *KNOX* homeobox genes in Arabidopsis. Plant Cell **14:** 2761–2770

Kumaran MK, Ye D, Yang W-C, Griffith ME, Chaudhury AM, Sundaresan V (1999) Molecular cloning of *ABNORMAL FLORAL ORGANS*: a gene required for flower development in Arabidopsis. Sex Plant Reprod **12:** 118–122

Liu Z, Franks RG, Klink VP (2000) Regulation of gynoecium marginal tissue formation by *LEUNIG* and *AINTEGUMENTA*. Plant Cell **12:** 1879–1891

Liu Z, Meyerowitz EM (1995) *LEUNIG* regulates *AGAMOUS* expression in *Arabidopsis* flowers. Development **121:** 975–991

Long J, Barton MK (2000) Initiation of axillary and floral meristems in Arabidopsis. Dev Biol **218:** 341–353

McConnell JR, Barton MK (1998) Leaf polarity and meristem formation in Arabidopsis. Development **125:** 2935–2942

McConnell JR, Emery J, Eshed Y, Bao N, Bowman J, Barton MK (2001) Role of *PHABULOSA* and *PHAVOLUTA* in determining radial patterning in shoots. Nature **411:** 709–713

Navarro C, Efremova N, Golz JF, Rubiera R, Kuckenberg M, Castillo R, Tietz O, Saedler H, Schwarz-Sommer Z (2004) Molecular and genetic interactions between *STYLOSA* and *GRAMINIFOLIA* in the control of Antirrhinum vegetative and reproductive development. Development **131:** 3649–3659

Nole-Wilson S, Krizek BA (2000) DNA binding properties of the *Arabidopsis* floral development protein AINTEGUMENTA. Nucleic Acids Res **28:** 4076–4082

Nole-Wilson S, Tranby T, Krizek BA (2005) *AINTEGUMENTA*-like (*AIL*) genes are expressed in young tissues and may specify meristematic or division-competent states. Plant Mol Biol **57:** 613–628

Sawa S, Watanabe K, Goto K, Kanaya E, Morita EM, Okada K (1999) *FILAMENTOUS FLOWER*, a meristem and organ identity gene of Arabidopsis, encodes a protein with a zinc finger and HMG-related domains. Genes Dev **13:** 1079–1088

Siegfried KR, Eshed Y, Baum SF, Otsuga D, Drews GN, Bowman JL (1999) Members of the *YABBY* gene family specify abaxial cell fate in Arabidopsis. Development **126:** 4117–4128

Sridhar VV, Surendrarao A, Gonzalez D, Conlan RS, Liu Z (2004) Transcriptional repression of target genes by LEUNIG and SEUSS, two interacting regulatory proteins for Arabidopsis flower development. Proc Natl Acad Sci USA **101:** 11494–11499

Waites R, Hudson A (1995) *phantastica*: a gene required for dorsoventrality of leaves in *Antirrhinum majus*. Development **121:** 2143–2154

Watanabe K, Okada K (2003) Two discrete cis elements control the abaxial side-specific expression of the FILAMENTOUS FLOWER gene in Arabidopsis. Plant Cell **15:** 2592–2602

Yanofsky MF, Ma H, Bowman JL, Drews GN, Feldman KA, Meyerowitz EM (1990) The protein encoded by the *Arabidopsis* homeotic gene *AGAMOUS* resembles transcription factors. Nature **346:** 35–39