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Expression of the Arabidopsis Floral Homeotic Gene *AGAMOUS* Is Restricted to Specific Cell Types Late in Flower Development

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Mutations in the *AGAMOUS* (*AG*) gene cause transformations in two adjacent whorls of the Arabidopsis flower. Petals develop in the third floral whorl rather than the normal stamens, and the cells that would normally develop into the fourth whorl gynoecium behave as if they constituted an *ag* flower primordium. Early in flower development, *AG* RNA is evenly distributed throughout third and fourth whorl organ primordia but is not present in the organ primordia of whorls one and two. In contrast to the early expression pattern, later in flower development, *AG* RNA is restricted to specific cell types within the stamens and carpels as cellular differentiation occurs in those organs. Ectopic *AG* expression patterns in flowers mutant for the floral homeotic gene *APETELA2* (*AP2*), which regulates early *AG* expression, suggest that the late *AG* expression is not directly dependent on *AP2* activity.

INTRODUCTION

Just as each flower consists of a precise pattern of organ types, each individual floral organ consists of several cell types in stereotyped positions. The organs of the flower begin their development as small outgrowths of cells from the floral meristem. At about the time these floral organ primordia arise, their identity is thought to be determined in accordance with their position within the flower, causing them to follow organ type-specific developmental programs. Subsequently, the cells of each of the determined organ primordia must then assess or know their relative positions within the primordia and differentiate into the appropriate cell types. It is this precise pattern of cellular differentiation that results in the different morphological characteristics of each floral organ type. Thus, during flower development, cells must know their position relative to others not only during the specification of organ primordium identity, but also later, during the cellular differentiation of individual floral organs.

Several homeotic mutations have been isolated in Arabidopsis. They cause cells to misinterpret their positions in early flower development. As a consequence, they differentiate into inappropriate cell types (Pruitt et al., 1987; Komaki et al., 1988; Bowman et al., 1988, 1989, 1991; Meyerowitz et al., 1989, 1991; Hill and Lord, 1989; Kunst et al., 1989; Irish and Sussex, 1990). The result is a flower with morphologically normal organ types found in abnormal positions. A model has been proposed, based on a series of genetic experiments, to explain how four homeotic genes, *AGAMOUS* (*AG*), *APETALA2* (*AP2*), *APETALA3* (*AP3*), and *PISTILLATA*, specify the identity of the floral organs by establishing positional cues in the early floral primordium (Bowman et al., 1991). However, genes involved in specifying positional information later in development, within the individual floral organs, have not yet been identified.

Mutations in one of the homeotic genes, *AG*, cause alterations in the third and fourth whorls of the flower. In the third whorl, six petals develop in the positions normally occupied by stamens, and in the fourth whorl, the cells that would normally give rise to the gynoecium behave as if they constituted a flower primordium. This process repeats itself indeterminately, resulting in the formation of >70 floral organs in the pattern (Sepal, Petal, Petal)$_n$ (Bowman et al., 1989). The predicted amino acid sequence of the AG protein contains a domain that is similar to the DNA binding sequence of the mammalian transcription factor SRF (Norman et al., 1988; Yanofsky et al., 1990), to the *MCM1* gene product of yeast (Passmore et al., 1988), and to *DEFICIENS* of snapdragon (Sommer et al., 1990). Hybridization experiments have shown that *AG* RNA is flower specific, being >100-fold enriched in floral

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tissue as compared to vegetative tissue (Yanofsky et al., 1990).

During the stages of flower development in which the third and fourth whorl organ primordia are thought to be specified to become stamens and carpels, *AG* RNA is detected only in the third and fourth whorls, a pattern that is consistent with *AG* having an early role in the specification of organ identity (Drews et al., 1991). *AG* RNA is detected uniformly throughout the third and fourth whorl organ primordia, and is detected even earlier in development in the region of the floral meristem that will later give rise to the third and fourth whorl organ primordia. Uniform expression in the primordia continues until after morphological differentiation of the organ primordia has started. Another of the floral homeotic genes, *AP2*, has been found to be a negative regulator of this early *AG* expression. In an *ap2* mutant background, *AG* RNA is present in all whorls of the flower during the time that floral organ identity is specified (Drews et al., 1991). This confirms a prediction based on observed genetic interactions between *AG* and *AP2* in which *ag* mutations have phenotypic effects in the outer two whorls when in an *ap2* mutant background (Bowman et al., 1991).

In Drosophila, the expression of many homeotic genes that are expressed early, and are responsible for the specification of segmental identity during embryogenesis, is required again, later in development, for proper cellular differentiation within segments (Akam, 1987; Gibson and Gehring, 1988). For example, misexpression of the homeotic selector gene *Antennapedia* during the larval stages of development, after segmental identity has been established, results in homeotic transformations of some tissues (Gibson and Gehring, 1988). The extent to which the Arabidopsis floral homeotic genes are utilized for proper cellular differentiation late in flower development, within the floral organs, has not been examined.

To investigate whether the Arabidopsis floral homeotic gene *AG* could have a role in specifying cell fate late in flower development, in addition to its early role in specifying organ identity, we examined the expression pattern of *AG* late in flower development, during the process of cellular differentiation in the flower. In this paper, we show that the expression of *AG* is dynamic during flower development. In contrast to the uniform expression pattern of *AG* throughout the third and fourth whorl organ primordia during the early stages of flower development (when the identity of the organ primordia is thought to be specified), *AG* RNA is restricted to certain cell types in stamens and carpels at later stages of flower development (after morphological differentiation of the floral organs has started). Examination of late *AG* expression in *ap2* mutant flowers suggests that, in contrast to early *AG* expression, *AP2* does not appear to regulate the cell type-specific pattern of late *AG* expression, and, in addition, that the late *AG* expression patterns seem to be set up independently of early *AG* expression.

RESULTS

Flower development in Arabidopsis has been described in detail and broken down into a series of stages, the first 14 of which are described in Table 1 (Müller, 1961; Smyth et al., 1990). During stages 1 to 6, the organ primordia form and the specification of their identities are thought to take place. We refer to these stages as early flower development. During stages 8 to 12, which we refer to as late flower development, much of the cellular differentiation takes place. Stage 7 is the stage at which morphological differentiation begins to occur in the third and fourth whorls. (The second whorl organ primordia are still morphologically undifferentiated until stage 8 and the first whorl primordia are morphologically differentiated as early as stage 5.) We have summarized some of the landmark events in the development of the ovule and pollen sac in Table 1 (Misra, 1962; Hill and Lord, 1989; Regan and Moffatt, 1990; Webb and Gunning, 1990; Mansfield et al., 1991).

We hybridized an *AG* anti-mRNA probe with tissue sections of stage 8 to 14 flowers to determine whether *AG* RNA is present in stamens and carpels while cellular differentiation of the structures within these organs is taking place. As with early flower development (Drews et al., 1991), hybridization signal is not present above background in sepals and petals. As is shown below, however, *AG* RNA is readily detectable in a limited number of cell types of the stamens and carpels as the maturation of these organs progresses.

AG RNA Becomes Concentrated within Specific Stamen Cell Types Late in Flower Development

Stamen development in Arabidopsis has been described previously (Misra, 1962; Hill and Lord, 1989; Regan and Moffatt, 1990; Smyth et al., 1990). Stamens begin their development when the floral meristem produces six morphologically undifferentiated third whorl primordia (stage 5), and, by stage 7, the regions that will give rise to the filament and the anther become morphologically distinct. Subsequently, the anther region becomes lobed on its adaxial side as the hypodermal cell layer divides, giving rise to the primary parietal cells (preanther wall cells) and the sporogenous cells (prepollen cells; stage 8). The primary parietal cells then give rise to the three anther wall layers (tapetum, middle layer, and endothecium), and the primary sporogenous cells develop into microspore mother cells, which undergo meiosis to form a tetrad of microspores (stage 9). Each microspore develops into a pollen grain (microgametophyte; stage 10). Before dehiscence (stages 11 and 12), two of the cell layers of the anther wall (the middle layer and the tapetum) and the epidermis

Table 1. Stages of Flower Development in Arabidopsis

Stage	Description
1	Flower buttress arises on flank of apical meristem.
2	Flower primordium becomes distinct from main axis by a groove.
3	Sepal primordia arise at flanks of floral primordium; pedicel develops.
4	Medial sepal primordia elongate to overlie floral primordium.
5	Petal and stamen primordia arise; petal primordia are barely visible; medial sepals almost cover bud.
6	Medial and lateral sepals enclose bud; long stamens become distinct from central dome; petal primordia are very small; no visible differentiation of carpels.
7	Stamens become stalked at base; petal primordia are hemispherical; gynoecium begins to differentiate and to form cylinder.
8	Stamens become lobed; petal growth accelerates but petals remain relatively small; pockets of sporogenous tissue are evident in each pollen sac.
9	Petals become stalked at base and form flattened blades; gynoecial cylinder becomes constricted at top; microspore mother cells become separated from each other and the tapetum; microspore mother cells undergo meiosis to form tetrads (isobilateral and tetrahedral) of microspores; ovule primordia arise; ovules grow and become elongated.
10	Petals reach top of short stamens; gynoecial cylinder becomes closed at top; microspores separate from each other to lie freely in pollen sac; microspores round up; ovules become stalked.
11	Gynoecium develops stigmatic papillae; tapetum of pollen sac degenerates; integuments grow around ovule; megaspore mother cell undergoes meiosis to form multiplanar tetrad of megaspores.
12	Petals level with long stamens; endothecium of pollen sac develops fibrous wall thickenings; tapetum of pollen sac is gone; epidermis of pollen sac shows withering; functional megaspore enlarges to become very expanded and highly vacuolated; embryo sac becomes multinucleate; nuclei are visible within embryo sac; ovule begins to show anatropous orientation.
13	Bud opens; petals are visible; anthesis occurs.
14	Long stamens extend above stigma; fertilization occurs.

Scanning electron microscopic observations described by Smyth et al. (1990); ovule and pollen sac development described by Misra (1962), Webb and Gunning (1990), and Mansfield et al. (1991). Stages after 12 described by Müller (1961).

degenerate, leaving only the endothecial layer surrounding the mature pollen grains.

In the early stages of stamen development, up to the stage when the filament and anther become distinct (stage 7), in situ hybridization with an *AG* anti-mRNA probe produced a uniform signal throughout the entire organ primordium (Drews et al., 1991). Figure 1A is a bright-field micrograph of a stamen section at about the time of meiosis (early stage 9). This section contains two pollen sacs, each having a pocket of sporogenous tissue surrounded by the anther wall layers. Figure 1B shows that a signal was present in the cells of the filament, connective, and anther walls. By contrast, the sporogenous tissue exhibited only a background level of hybridization grains as compared to control hybridizations with a sense strand probe (see Methods). Figures 1C and 1D show that ^{3}H-poly(U) produced a much stronger signal in the sporogenous tissue than in the remainder of the anther, indicating that the pattern observed in Figure 1B is not a reflection of poly(A) RNA abundance or probe accessibility within the tissue.

Figure 1E is a bright-field micrograph of a longitudinal stamen section from a stage 11 flower, by which time individual pollen grains are evident. The connective and two pollen sacs, each composed of a locule with pollen grains and surrounded by the anther wall layers, are visible. A nectary is present at the base of the filament. Figure 1F shows that a hybridization signal was concentrated over the connective of the anther and the nectary. In addition, the anther walls and filament contained a lower signal. By contrast, the pollen grains had no hybridization signal above background. Figure 1G is a bright-field micrograph of an anther during stage 13, after most of the anther wall has degenerated but before dehiscence. Figure 1H shows that a signal was associated with the anther wall, which at this stage of development consists of endothecium and withering epidermis (Misra, 1962). No hybridization signal was present above the pollen grains. The grains associated with the external surface of the pollen grains were also observed in control hybridizations with a sense strand probe, and thus represent background. *AG* RNA, therefore, is present in fully differentiated cell types, such as

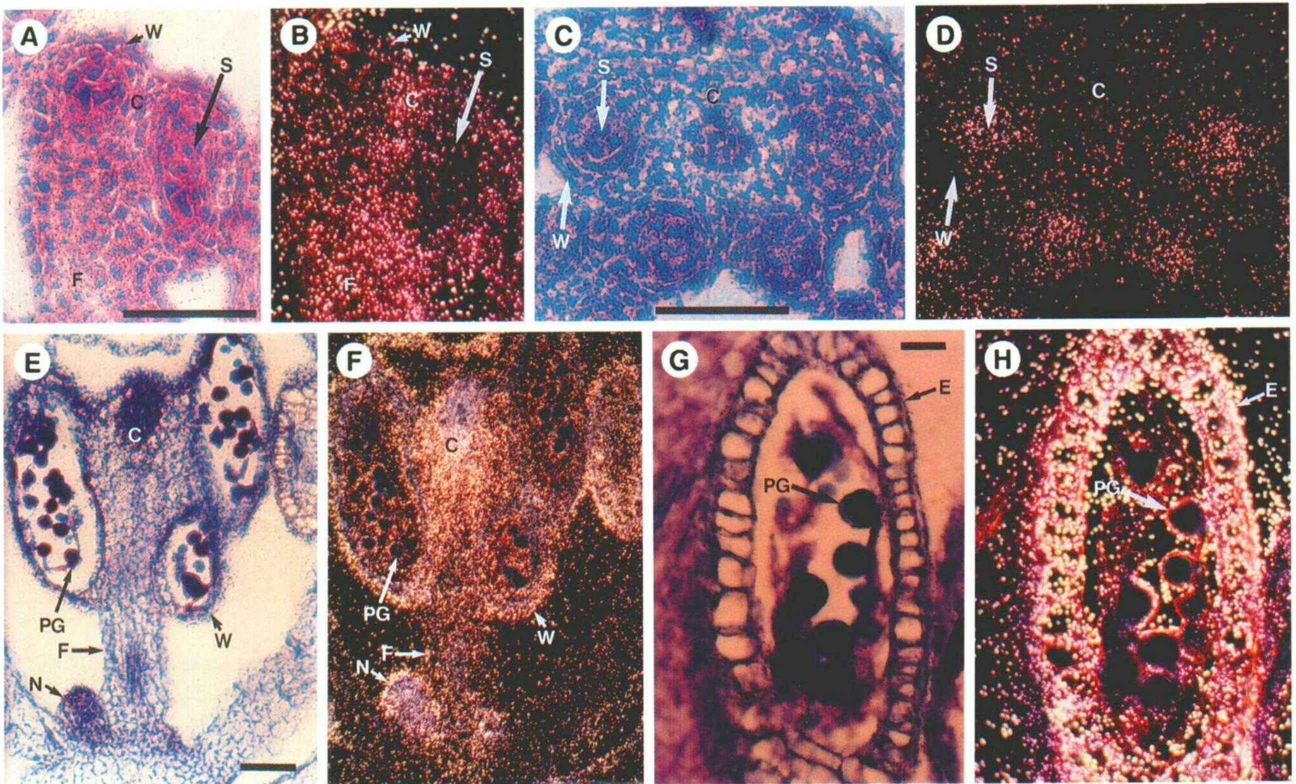

Figure 1. Distribution of *AG* RNA in Developing Stamens.

(A) and **(B)** In situ hybridization of an *AG* anti-mRNA probe with a stage 9 anther. **(A)** Bright-field micrograph. **(B)** Dark-field micrograph.
(C) and **(D)** In situ hybridization of ^{3}H-poly(U) probe with a stage 9 anther. **(C)** Bright-field micrograph. **(D)** Dark-field micrograph.
(E) and **(F)** In situ hybridization of an *AG* anti-mRNA probe with a stage 11 stamen. **(E)** Bright-field micrograph. **(F)** Dark-field micrograph.
(G) and **(H)** In situ hybridization of an *AG* anti-mRNA probe with a stage 13 anther. **(G)** Bright-field micrograph. **(H)** Dark-field micrograph.
Flower structures are given the following abbreviations: C, connective; F, filament; S, sporogenous tissue; N, nectary; PG, pollen grains; W, anther wall layers; E, endothecium.
Bars = 50 μm in **(A)**, **(C)**, and **(E)**; 10 μm in **(G)**.

endothecium and nectaries, but is not detectable in the microspore cell lineage from the time when meiosis takes place.

AG RNA Becomes Concentrated within Specific Carpel Cell Types Late in Flower Development

Carpel development in Arabidopsis has been described previously (Misra, 1962; Smyth et al., 1990; Webb and Gunning, 1990; Mansfield et al., 1991). The first morphological characteristic of the developing gynoecium is the formation of a cylinder during stages 7 and 8. Shortly thereafter (stage 9), ovule primordia arise in four rows from placental tissue on the internal ovary walls of the gynoecial cylinder. During stages 9 to 11, the ovule primordia become stalked, and the development of the two integuments is initiated. As the integuments develop, the ovule curves to assume an anatropous form (stage 12). The hypodermal single-celled archesporium functions directly as the megaspore mother cell that undergoes meiosis to form a multiplanar tetrad of megaspores (stage 11). During stages 12 to 14, the functional megaspore expands, becoming highly vacuolate, and its nucleus undergoes several rounds of mitosis, resulting in an eight-nucleate embryo sac. By this time, the integuments have enclosed the embryo sac, and the inner cell layer of the inner integument has formed the endothelium or integumentary tapetum, a group of cells adjacent to the embryo sac with distinct morphological and staining properties.

Early in carpel development, before the appearance of ovule primordia (through stage 8), in situ hybridization with an *AG* anti-mRNA probe produced a uniform signal throughout the gynoecial tissue (Drews et al., 1991). Figure 2A is a bright-field micrograph of a carpel section at the stage of development when ovule primordia appear. Figure

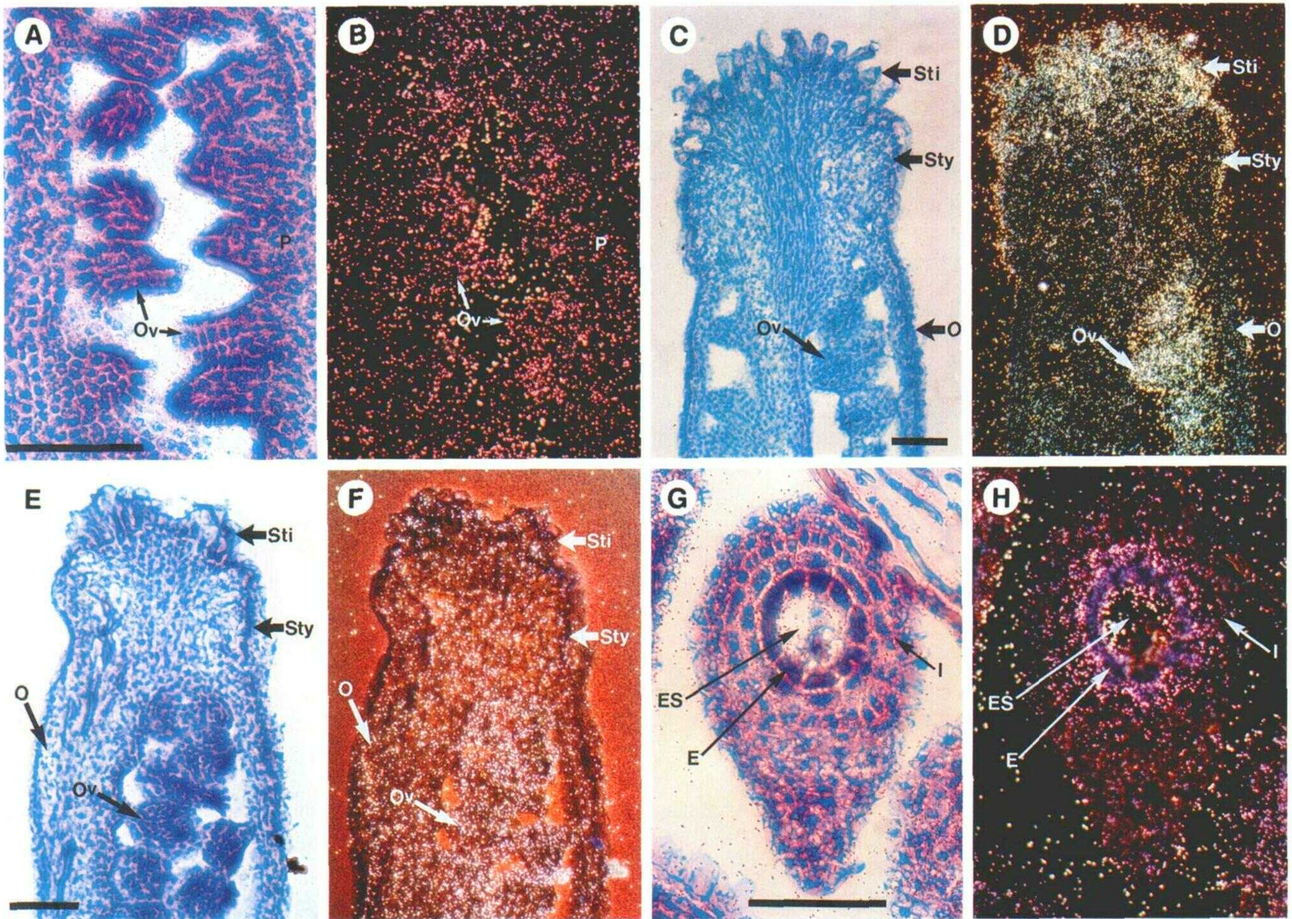

Figure 2. Distribution of *AG* RNA in Developing Carpels.

(A) and **(B)** In situ hybridization of an *AG* anti-mRNA probe with a stage 9 gynoecium. **(A)** Bright-field micrograph. **(B)** Dark-field micrograph.
(C) and **(D)** In situ hybridization of an *AG* anti-mRNA probe with a stage 12 gynoecium. **(C)** Bright-field micrograph. **(D)** Dark-field micrograph.
(E) and **(F)** In situ hybridization of ^{3}H-poly(U) probe with a stage 12 gynoecium. **(E)** Bright-field micrograph. **(F)** Dark-field micrograph.
(G) and **(H)** In situ hybridization of an *AG* anti-mRNA probe with a differentiated ovule. **(G)** Bright-field micrograph. **(H)** Dark-field micrograph.
Flower structures are given the following abbreviations: ES, embryo sac; I, integuments; E, endothelium; O, ovary; Ov, ovule; P, placenta; Sti, stigma; Sty, style.
Bars = 50 μm.

2B shows that a low uniform signal was associated with the tissue of the ovary walls, whereas a higher signal was seen over each ovule primordium. The signal associated with the developing ovule remains spatially uniform throughout the ovule from stage 9 to early stage 12 and increases in intensity through stage 12. Figure 2C is a bright-field micrograph of an early stage 12 gynoecium with several morphologically differentiated tissues such as the stigma, style, and ovary. Figure 2D shows that the stigmatic tissue and ovules had high signals, whereas the style, the ovary walls, and the placenta of the ovary exhibited little or no signal over background. Stigmatic papillae are first visible during stage 11. At this time a hybridization signal was observed in the developing stigmatic tissue at the apex of the gynoecium. In contrast, ^{3}H-poly(U) produced a signal that is relatively uniform, except the signal over the developing ovule is slightly more intense (Figures 2E and 2F).

Figure 2G is a bright-field micrograph of an ovule at about the time of development when fertilization takes place (stage 14 of Smyth et al., 1990). This tissue section shows an embryo sac surrounded by the endothelium and

the other integument layers. Figure 2H demonstrates that the *AG* anti-mRNA probe hybridized strongly with RNA present in the endothelium, the cell layer surrounding the embryo sac. By contrast, the embryo sac and the other integument cell layers had little or no signal above background. We also found that *AG* RNA was present in the stigma of flowers at this stage of development. The distribution of *AG* RNA in the stigma of fertilized flowers was exactly the same as the pattern shown in Figure 2D. Taken together, these data show that *AG* RNA becomes progressively restricted to specific cell types and is present in fully differentiated carpel cells, such as stigmatic papillae and the ovular endothelium.

Expression of *AG* in *ap2* Mutant Flowers

In the early development of the flower, one of the functions of the *AP2* gene product is to suppress *AG* RNA accumulation in the outer two floral whorls (Bowman et al., 1991; Drews et al., 1991; Meyerowitz et al., 1991). This led us to ask whether *AP2* activity also affects the cell type-specific pattern of *AG* RNA accumulation late in flower development. To find out, we determined the location of *AG* RNA in flowers homozygous for the *ap2-2* allele, a null or nearly null allele of this gene.

Homozygous *ap2-2* flowers have fourth whorl carpels and also carpels in the medial first whorl positions. There are usually no second or third whorl organs. As in wild-type carpels, *AG* RNA is distributed uniformly throughout the medial first whorl and fourth whorl organs of *ap2-2* flowers during the initial stages of their development and until the time that they begin to develop as carpels (Drews et al., 1991). Figure 3A is a bright-field micrograph of an *ap2-2* flower showing first (medial) and fourth whorl carpels. Figure 3B shows that the *AG* anti-mRNA probe produced a similar pattern of hybridization grains in these two organs, with highest grain density in stigma and ovules, and lower or no signal above background elsewhere. In both cases, the pattern of hybridization signal resembles very closely the pattern observed in wild-type carpels (Figures 2B and 2D). Figure 3C is a bright-field micrograph of an ovule originating from a first whorl carpel. This tissue section contains an embryo sac surrounded by an endothelium and enclosed by the other integument cell layers. Figure 3D shows that the *AG* anti-mRNA probe hybridized primarily with the endothelium, whereas the embryo sac had little or no signal above background. A very low signal was also observed in the surrounding integument tissue. At a later stage of ovule development, no signal above background was detected in the integuments. Thus, the pattern of *AG* expression in both fourth whorl and ectopic carpels is similar to the progression seen in wild-type carpels: an early, high, uniform signal becoming restricted to the stigmatic tissue and to the endothelium as the ovules mature. *AP2* does not, therefore, appear to be necessary for the cell type-specific pattern of *AG* RNA accumulation in carpels.

As compared with *ap2-2*, *ap2-1* is a weaker allele of the *ap2* gene, having cauline leaflike organs with some carpelloid features (terminal stigmatic tissue and rudimentary ovules) in the first whorl positions. The second whorl organs of *ap2-1* flowers range in phenotype from deformed stamens to slightly staminoid petals; third and fourth whorl organs are stamens and carpels, as in the wild type. Early in *ap2-1* development (through stage 6), *AG* RNA is not present above background in the first whorl organs (Drews et al., 1991). Later in development (after stage 7), the first whorl organs develop carpelloid structures (Bowman et al., 1989). We hybridized the *AG* anti-mRNA probe with sections of *ap2-1* first whorl organs to determine whether *AG* RNA is present in the carpelloid structures in the *ap2-1* outer whorl.

Figure 3E is a bright-field micrograph of a longitudinal section through stage 5, 7, and 10 *ap2-1* flowers. Figure 3F shows, as observed previously (Drews et al., 1991), that the *AG* anti-mRNA probe produced no signal above background in the first whorl organs of the stage 5 flowers. By contrast, the first whorl organs of the stage 7 and stage 10 flowers hybridized strongly with this probe. The first whorl organs of the stage 10 flower in Figures 3E and 3F exhibit some carpelloid characteristics. The *AG* anti-mRNA probe hybridized primarily with the cells at the tips of these organs, where stigmatic papillae are evident, and along the margin over what appears to be a rudimentary ovule. This indicates that high levels of *AG* expression before stage 6 are not a prerequisite for the later cell type-specific RNA accumulation pattern for this gene in the first whorl.

The second whorl organs in *ap2-1* homozygotes are often deformed stamens. We localized *AG* RNA in these organs (data not shown) and in third whorl stamens of *ap2-1* flowers (Figures 3E and 3F), to determine whether absence of wild-type *AP2* gene product has any effect on the late, cell type-specific expression of *AG* in stamens. As with wild-type stamens, a hybridization signal was associated with the cells of the connective, anther walls, and filament, indicating that normal activity of the *AP2* gene product is not necessary for the proper cell type-specific expression of *AG* late in stamen development.

DISCUSSION

Mutations in the floral homeotic gene *AG* cause cells in whorls three and four of the developing Arabidopsis flower to misinterpret their positions and thus to differentiate inappropriately (Bowman et al., 1989, 1991). Consistent with *AG* having a role in floral organ specification, we have previously shown that *AG* RNA is evenly distributed

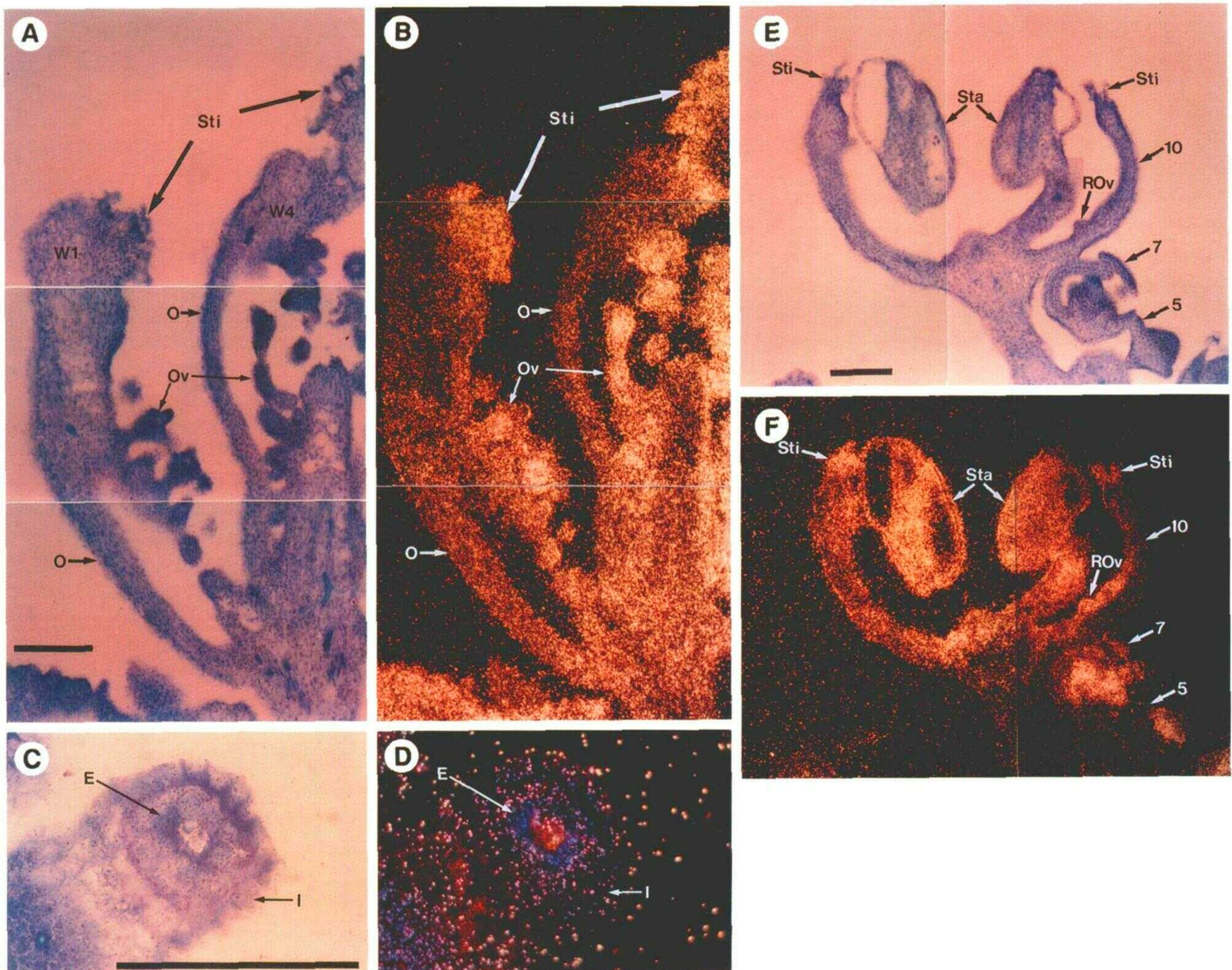

Figure 3. Distribution of *AG* RNA in *apetala2* Flowers.

(A) and **(B)** In situ hybridization of an *AG* anti-mRNA probe with first and fourth whorl carpels of an *ap2-2* flower. **(A)** Bright-field micrograph. **(B)** Dark-field micrograph.

(C) and **(D)** In situ hybridization of an *AG* anti-mRNA probe with a differentiating ovule on a first whorl carpel of an *ap2-2* flower. **(C)** Bright-field micrograph. **(D)** Dark-field micrograph.

(E) and **(F)** In situ hybridization of an *AG* anti-mRNA probe with three *ap2-1* flowers of different developmental stages. Two medial first whorl organs, with stigmatic tissue at their tips, of a stage 10 flower are visible, as well as a stage 7 flower and a stage 5 flower. The arrows identifying the flowers of different stages point to a first whorl organ of each. The two stamens visible in this section are third whorl organs. **(E)** Bright-field micrograph. **(F)** Dark-field micrograph.

Flower structures are given the following abbreviations: I, integuments; E, endothelium; O, ovary; Ov, ovule; ROv, rudimentary ovule; Sta, stamen; Sti, stigma; W1, first whorl; W4, fourth whorl. The numbers refer to the stage of development of the respective flowers.

Bars = 100 μm.

throughout those cells that will give rise to the third and fourth whorl organs during the time when the fates of the floral organ primordia are thought to be specified (Drews et al., 1991). We show here that the expression of *AG* is not limited to early flower development but continues in fully differentiated floral organs. Furthermore, the expression pattern is dynamic, evolving from a uniform distribution throughout stamen and carpel primordia early in development to a restricted pattern with expression in a small number of specific cell types late in flower development.

In stamens, *AG* RNA is present at highest levels in the connective of the anther and at lower levels in the anther walls and the filament (Figures 1F and 1H). In carpels, *AG* RNA is present at its highest levels in stigmatic papillae and developing ovules (Figure 2D). As the ovules mature, *AG* RNA becomes further restricted to just the endothelium (Figure 2H).

AG RNA is not present above background in the pollen grains nor in the sporogenous tissue that gives rise to the pollen grains. Clonal analysis (Satina and Blakeslee, 1941; Derman and Stewart, 1973) and morphological studies (Misra, 1962; Bhandari, 1984) of anther development indicate that the pollen grains are ontogenetically related to the anther wall layers, which, as described above, do contain *AG* RNA. Because *AG* RNA appears to be present in all stamen cells early in development (Drews et al., 1991), one feature of the pollen grain cell lineage is a reduction of *AG* RNA levels.

AG RNA appears to be present in all cells of the ovule early in their development (Figures 2B and 2D). At a later stage of development, when the ovules are fully differentiated, *AG* RNA is localized to the endothelium, which is the cell layer surrounding the embryo sac (Figure 2H). We did not detect *AG* RNA in the embryo sac or the integuments of mature ovules (Figure 2H). Thus, a decline in *AG* RNA level is characteristic of both megagametophyte and microgametophyte development. These data show that as carpel and stamen development proceeds, *AG* RNA becomes progressively restricted to particular cell types, and that *AG* RNA is present in differentiated cell types.

Based on genetic studies, we previously proposed that *AP2* and *AG* negatively regulate each others' activity, such that *AP2* activity is present in all floral whorls in *ag* mutant flowers, and conversely, *AG* activity is present in all floral whorls in *ap2* mutant flowers (Bowman et al., 1991). Drews et al. (1991) showed that *AP2* is a negative regulator of *AG* RNA accumulation in the outer two whorls of the flower; *AG* expression expands to include all floral whorls in an *ap2-2* mutant background. This negative regulation occurs in the early stages of flower development, during the time in which the floral organ primordia are specified.

In *ap2-2* flowers, carpels arise in the medial first whorl positions, which is proposed to be due, at least in part, to the ectopic expression of *AG* RNA in those organ primordia early in development (Bowman et al., 1991; Meyerowitz et al., 1991). Later in development, the *AG* RNA expression pattern observed in these ectopic carpels and in fourth whorl carpels is the same as that observed in the fourth whorl carpels of wild-type flowers; *AG* RNA is expressed at a high level only in specific cell types. The correct temporal and spatial late expression pattern of *AG* RNA observed in *ap2-2* carpels suggests that the factors that regulate the late *AG* expression pattern in wild-type fourth whorl carpels must also be present in *ap2-2* carpels. Thus, the *ap2-2* mutation does not perturb the late expression patterns of *AG*, suggesting strongly that, despite its role as a regulator of early region-specific *AG* RNA expression, *AP2* does not have a direct role in regulating the pattern of cell type-specific expression of *AG* RNA late in carpel development.

AG RNA is not detectable in the first whorl organ primordia of *ap2-1* flowers early in development, during the time in which the organs develop characteristics of leaves, such as stipules and stellate trichomes (Drews et al., 1991). However, *AG* RNA is present in the first whorl organs of *ap2-1* flowers later in development, after they have already begun to develop leaflike characters (>stage 7; Drews et al., 1991). This suggests that the *AG* RNA detected here is equivalent to the *AG* RNA present in specific cells of wild-type stamens and carpels late in development. In agreement with this, *AG* RNA is not distributed evenly throughout the outer whorl organs of *ap2-1* flowers. Rather, it is present in patches of cells (Figures 3E and 3F). This late expression of *AG* could be related to the appearance of carpelloid features of the first whorl organs, such as stigmatic tissue at the tips and rudimentary ovules at the margins, which arise late in development (Bowman et al., 1989). That *AG* RNA is present in carpelloid cell types in the first whorl organs of *ap2-1* flowers late in development, but is not detected in these organs early in flower development, indicates that the late expression patterns of *AG* are not dependent upon wild-type levels of *AG* expression before stage 6 in these organs.

The expression of *AG* in mature stamens and carpels raises the question of whether *AG* has a function late in development. Because *AG* functions in specifying cell fate early in development, this gene might also be involved in specifying cell fate late in development. That the pattern of late *AG* expression is restricted to only a few cell types argues against *AG* having a general role in the maintenance of carpel and stamen fates as the organ primordia differentiate. Rather, a role in the specification of certain cell types of stamens and carpels seems more likely. However, *AG* expression is not an absolute requirement for stigma and ovule specification, inasmuch as these structures develop in the first whorl of double and triple mutant combinations that include *ag-1* (Bowman et al., 1991). Thus, if *AG* is involved in stigma and ovule specification, other factors must be involved as well. One candidate for such a factor is *AGL1*, which is also expressed during ovule development and encodes a putative transcription factor (Ma et al., 1991). Another floral homeotic gene, *DEFICIENS* of snapdragon, is also expressed throughout the development of the organs affected by *deficiens* mutations (Sommer et al., 1990; Schwarz-Sommer et al., 1990). Furthermore, by mosaic analysis, *DEFICIENS* activity has been shown to be capable of controlling the fates of at least some cells late in development (Carpenter and Coen, 1990).

The existing *ag* alleles cannot shed light on the possible late functions of *AG* because in mutants homozygous for

these alleles neither stamens nor carpels develop. Future experiments in which *AG* is expressed early but not late in development will better define the late functions of *AG*. The isolation of weak, conditional alleles of *ag* and the construction of transgenic plants with the *AG* coding region under control of a heterologous inducible promoter will be useful in this regard. Although these tools are not available at present to provide clues to the function of late expression patterns, these data suggest that *AG* has a heretofore unsuspected role late in flower development.

METHODS

Plant Material

The wild-type *Arabidopsis thaliana* variety used in this study was Landsberg *erecta*. The *ap2-2* and *ap2-1* mutations were in the Landsberg *erecta* genetic background. All plants were grown at 25°C. Genetic nomenclature used here is based on recommendations of the Third International Arabidopsis Meeting (East Lansing, Michigan, April 10 to 12, 1987). Wild-type alleles are symbolized in block capitals and italics, and mutant alleles in lower case italics.

In Situ Hybridization

Individual flowers or a cluster of flower buds at stages 1 to 14 of development were dissected and fixed in 3.7% formaldehyde, 5% acetic acid, 50% ethanol. Fixed tissue was dehydrated with ethanol, cleared with xylene, and embedded in paraffin (Paraplast Plus). Embedded tissue was sliced into serial 8- or 10-μm sections with a Sorvall JB-4 microtome and attached to microscope slides that were coated with poly-L-lysine (Sigma). The in situ hybridizations were carried out as described by Cox and Goldberg (1988).

Three probes were utilized in these experiments. The *AG* anti-mRNA probe and control probes were single-stranded ^{35}S-RNA probes derived from sequences within the cDNA clone pCIT565. The *AG* anti-mRNA probe contained nucleotides 240 to 977 of the *AG* sequence published in Yanofsky et al. (1990). This probe did not cross-hybridize with any of the other cloned genes that have significant sequence similarity with *AG* (Yanofsky et al., 1990; Drews et al., 1991; Ma et al., 1991). The control probe contained nucleotides 9 to 977 of the *AG* sequence published in Yanofsky et al. (1990). This probe was synthesized in the opposite orientation of the *AG* anti-mRNA probe and thus contained sequences identical to *AG* mRNA. The control probe was used to measure background hybridization. The third probe was ^{3}H-poly(U), which was purchased from Amersham (TRK.481). The poly(U) probe was used to describe the distribution of total poly(A) RNA in floral tissue. This probe also allowed us to assess whether the RNA within a cell was accessible to the in situ hybridization probes.

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