# Transcriptional program controlled by the floral homeotic gene *AGAMOUS* during early organogenesis

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## Summary

**Floral organs, whose identity is determined by specific combinations of homeotic genes, originate from a group of undifferentiated cells called the floral meristem. In *Arabidopsis*, the homeotic gene *AGAMOUS* (*AG*) terminates meristem activity and promotes development of stamens and carpels. To understand the program of gene expression activated by *AG*, we followed genome-wide expression during early stamen and carpel development. The *AG* target genes included most genes for which mutant screens revealed a function downstream of *AG*. Novel targets were validated by in situ hybridisation and binding to AG in vitro and in vivo. Transcription factors formed a large fraction of AG targets, suggesting that during early organogenesis, much of the genetic program is concerned with elaborating gene expression patterns. The results also suggest that AG and other homeotic proteins with which it interacts (SEPALLATA3, APETALA3, PISTILLATA) are coordinately regulated in a positive-feedback loop to maintain their own expression, and that AG activates biosynthesis of gibberellin, which has been proposed to promote the shift from meristem identity to differentiation.**

Key words: Homeotic genes, Floral development, Transcription, AGAMOUS

## Introduction

The genetic control of floral organ identity is one of the most remarkable examples of how regulatory genes determine plant structure (reviewed by Ferrario et al., 2004; Zik and Irish, 2003a). A flower starts its development as a group of undifferentiated cells (the floral meristem), which arises on the flank of the shoot apical meristem. The floral meristem gives rise to organ primordia, which develop into each of the four types of floral organs: sepals, petals, stamens and carpels. The identity of these organs is specified by homeotic genes, most of which encode MADS-domain transcription factors. The homeotic genes are expressed in different but partially overlapping domains in the floral meristem, and the specific combination of homeotic genes active in each organ primordium directs the development of its organ type. These partially overlapping expression domains are set up by genes that are active in the meristem, but subsequently the expression and function of the homeotic genes is maintained throughout organ development.

The molecular basis for the combinatorial action of homeotic genes may be that in each case, the corresponding proteins are assembled into a different protein complex. For example, stamen development requires combination of the homeotic genes *AGAMOUS* (*AG*), *APETALA3* (*AP3*), *PISTILLATA* (*PI*) and at least one of the *SEPALLATA* (*SEP1*, *SEP2* and *SEP3*) genes, whereas carpel development occurs when *AG* and *SEP* are expressed, but not *AP3*/*PI* (Bowman and Meyerowitz, 1991; Honma and Goto, 2001; Jack et al., 1992; Krizek and Meyerowitz, 1996; Pelaz et al., 2000; Yanofsky et al., 1990). Based on protein-protein interactions in yeast and on co-immunoprecipitation, it has been proposed that stamen development is directed by a protein complex in which SEP3 bridges the interaction between AG and the AP3/PI heterodimer; similarly, the direct interaction between SEP3 and AG in yeast and suggests that these two proteins associate to control carpel development (Honma and Goto, 2001).

Presumably each of the complexes containing homeotic proteins selects a different set of downstream target genes that participate in the development of a specific organ type, although the exact composition of these complexes in vivo, and how they select different target genes, remains unknown (Jack, 2001). To understand how the activity of homeotic genes is combined and translated into the patterns of cell division and differentiation that actually shape the floral organs, it is necessary to identify these downstream targets. However, very little is known about the genes that function downstream of the floral homeotic genes.

Genetic analysis has revealed some intermediate regulatory genes that control specific aspects of floral organ development. For example, AG activates *SPOROCYTELESS* (*SPL*), which controls sporogenesis in both stamens and carpels (Ito et al., 2004). *SUPERMAN* (*SUP*) controls cell proliferation in stamen and carpel primordia and its expression depends on *AG*, *AP3* and *PI* (Sakai et al., 2000; Sakai et al., 1995). The *SHATTERPROOF* genes (*SHP1* and *SHP2*) are required in the carpel margins for differentiation of the dehiscence zone, where later the fruit splits open to release the seeds (Liljegren

et al., 2000). *SPATULA* (*SPT*) controls cell differentiation at the carpel margins and in the transmitting tract (the tissue that guides the growth of pollen tubes towards the ovules) (Bowman and Smyth, 1999; Heisler et al., 2001), and *CRABS CLAW* (*CRC*) participates in directing the development of tissues derived from the abaxial side of the carpel primordium (e.g. the outer epidermis) (Eshed et al., 1999).

A more comprehensive view of gene expression in floral organs came from transcript profiling experiments comparing wild type and homeotic mutants (Wellmer et al., 2004; Zik and Irish, 2003b). These experiments revealed hundreds of genes that are preferentially expressed in different organs, but these were mostly expressed at late stages of development and were probably only indirectly dependent on the floral homeotic genes (Wellmer et al., 2004). To fully understand the program of gene expression controlled by the floral homeotic genes, it is necessary to know how it unfolds from organ initiation to maturity. Here, we report the results of a global analysis of the program of gene expression triggered by AG, from the onset of organogenesis to early stages of reproductive organ development.

## Materials and methods

### Plant material

Plants were grown on a mix of vermiculite:soil:sand at 18°C with 16-hour light/8-hour dark cycles. All mutants (*ag-3*, *ap1-1*, *ap1-1 cal-1* and *ag-3 ap1-1*) and *AGGR* were in a L*er* background, which was used as the wild type.

Dexamethasone (Sigma, stock solution 10 mM in ethanol) was used at a final concentration of 10 μM in Silwet L-77 0.015%, applied directly on the inflorescence tips; for mock treatments, the solution contained the same amount of ethanol (0.1%) and Silwet L-77. After treatment, RNA was extracted from inflorescence apices and stored at –70°C until activation of AGGR was confirmed (2 weeks later).

### Scanning electron microscopy (SEM)

Plants were fixed in 2.5% glutaraldehyde in phosphate-buffered saline (PBS) at 4°C overnight, dehydrated in an ethanol series, critical-point dried in liquid $CO_2$, sputter-coated with gold palladium, analysed and photographed with a Philips XL 30 FEG SEM.

### RNA isolation

Total RNA was extracted using TRI reagent (Sigma) according to the manufacturer's instructions. For array hybridisation, the RNA was cleaned up with RNeasy columns (Qiagen) and precipitated to increase final concentration.

### Array hybridisation and analysis of expression data

Gene Chip arrays were hybridised as in the manufacturer's protocol (Affymetrix). To calculate *P*-values for increase or decrease in expression, the Wilcoxon signed-rank test (Hubbell et al., 2002; Liu et al., 2002) was applied to each pair of chips after normalisation across all probe sets, using Micro Array Suite 5.0 (Affymetrix). To calculate fold differences in expression, raw expression levels were imported from Micro Array Suite 5.0 into Gene Spring 5.1 (Silicon Genetics) and normalised first to the fiftieth percentile of each chip, then across all chips before further analysis.

### Reverse transcription-PCR (RT-PCR)

Total RNA (2 μg) was treated with RNase-free DNase, and first strand cDNA was synthesised using oligo(dT) primer (Invitrogen) and Superscript RT (Invitrogen). Aliquots of the cDNA were used as template for PCR with gene specific primers (see Table S3 in supplementary material).

### In situ hybridisation

RNA was hybridised in situ (Fobert et al., 1996), using digoxigenin-labelled probes transcribed with T7 polymerase from linearised plasmid (pGEM-T easy, Promega) containing 3′ cDNA fragments. Colour detection was performed with BCIP/NBT according to the manufacturer's instructions (Boehringer).

### Production of recombinant AG protein

To produce AG protein, the AG ORF was PCR-amplified from pCIT1516 vector (Yanofsky et al., 1990) and cloned into pRSET-A (Invitrogen). BL21(DE3) pLysE cells were transformed with the construct, and His-AG proteins were expressed under the control of the T7 promoter. To prepare recombinant His-AG, inclusion bodies were purified using the BugBuster HT Protein Extraction Reagent (Novagen), according to the manufacturer's instructions, dissolved in dialysis buffer (20 mM Tris, 100 mM KCl, 1 mM EDTA, 1 mM DTT, 1 mM PMSF, 12% glycerol, pH 8.0) containing 6M urea, dialysed overnight against the same buffer without urea and stored at –20°C.

### Electrophoretic mobility shift assays (EMSA)

Probes were made from complementary oligonucleotides (see Table S3 in supplementary material), annealed in 20 mM Tris (pH 8.0), 50 mM NaCl, 1 mM EDTA, labelled with $^{32}P$ by filling in with DNA polymerase I (Klenow fragment), and gel-purified prior to use. DNA-binding assays and gel electrophoresis were essentially as described previously (Riechmann et al., 1996).

### Chromatin immunoprecipitation (ChIP)

The procedure was adapted from Ito et al. and Wang et al. (Ito et al., 1997; Wang et al., 2002). Inflorescence tissue (~1 g) of Col-0 plants was fixed with 1% formaldehyde in MC buffer [10 mM sodium phosphate (pH 7.0), 50 mM NaCl, 0.1 M sucrose) for 1 hour under vacuum. Fixation was stopped with 0.125 M glycine, followed by three washes with MC. The tissue was ground in liquid nitrogen, the powder was suspended in M1 buffer [10 mM sodium phosphate (pH 7.0), 0.1 M NaCl, 1 M 2-methyl 2,4-pentanediol, 10 mM β-mercaptoethanol, Complete™ Protease Inhibitor Cocktail (Roche Diagnostics GmbH, Mannhein, Germany)], the slurry was filtrated through 55 μm mesh and centrifuged at 1000 ***g*** for 10 minutes. Subsequent steps were at 4°C unless indicated otherwise. Filtration and centrifugation were repeated twice, then the pellet was washed five times with M2 buffer (M1 buffer with 10 mM $MgCl_2$, 0.5% Triton X-100) and once with M3 buffer (M1 without 2-methyl 2,4-pentanediol). The nuclear pellet was resuspended in 1 ml Sonic buffer [10 mM sodium phosphate (pH 7.0), 0.1 M NaCl, 0.5% Sarkosyl, 10 mM EDTA, Complete™ Protease Inhibitor Cocktail (Roche Diagnostics GmbH), 1 mM PMSF]. Chromatin was solubilised on ice with a probe sonicator (MSE, Soniprep 150) by 25 cycles of 15-second pulses of half maximal power with 30 seconds cooling time between pulses. After sonication, the suspension was centrifuged (microcentrifuge, top speed) for 5 minutes and the supernatant was mixed with one volume of IP buffer [50 mM Hepes (pH 7.5), 150 mM KCl, 5 mM $MgCl_2$, 10 μM $ZnSO_4$, 1% Triton X-100, 0.05% SDS]. The solubilised chromatin was pre-adsorbed overnight with 7.5 μl antiserum against CLAVATA3 (CLV3) (*sc-12598*, Santa Cruz Biotechnology, Santa Cruz, CA) (used as AG-negative serum due to the lack of pre-immune serum). After centrifugation, the supernatant was mixed with 40 μl of protein G-Sepharose [Sigma, 50% slurry in 10 mM Tris (pH 7.5), 150 mM NaCl] and incubated on a rotating wheel for 1 hour. After centrifuging, the supernatant was equally divided over two tubes with 2.5 μl AG antiserum (*sc-12697*, Santa Cruz Biotechnology) or 2.5 μl CLV3 serum (control). After 1 hour on a rotating wheel and centrifugation, the supernatant was mixed with 20 μl protein G-Sepharose (Sigma) before incubation for another hour

on the rotating wheel. The protein G-Sepharose beads were washed five times with 1 ml IP buffer for 10 minutes at room temperature. Elution with 0.1 M glycine, 0.5 M NaCl, 0.05% Tween-20 (pH 2.8) was as described (Wang et al., 2002). The eluate was treated with 1 µl RNase A (10 mg/ml) and proteinase K (to final of 0.5 mg/ml). After overnight incubation, a second aliquot of proteinase K was added and incubated at 65°C for 6 hours. After phenol/cloroform, then chloroform extraction, DNA was precipitated with 2.5 volumes of ethanol, one-tenth volume 3M NaAc (pH 5.4) and 1 µl glycogen, and resuspended in 10 µl of 10 mM Tris (pH 8.0).

ChIP PCR was performed to reveal if a specific DNA fragment was enriched in the immunoprecipitated DNA sample compared with the pre-immune DNA sample. Primers were designed around the consensus AG binding sites and control primers were made for regions lacking the consensus AG binding site. Template ChIP DNA was diluted, amplified for 35 to 40 cycles (see Fig. 5), and analysed on a 1.5% agarose gel, followed by scanning with a Molecular Imager FX-PRO Plus (Bio-Rad Laboratories, Hercules, CA). The primer sequences were (5′ to 3′):

*CRC*, TGGATGCATGAATAATGGGTAG and CGTGGACTAGAAATAATGAGACGA;

*AP3*, CGGAGCTCCGTTAATAAATTGACG and TTTGGTGGAGAGGACAAGAGA;

*AP3* exon 7, AACATGTTTTGGTGAATTAGGAA and GCACCAGCAAACCTTTTAGC;

*GA4*, TTGTCCCTTTATATACGCATTAATCA and GAGACCAAGAGGAGGCAAAA;

*AG*, TGGTCTGCCTTCTACGATCC and CAACAACCCATTAACACATTGG;

*SEP3*, CGGCCATATCCACTTTTACG and TTTTTGGGATAATTTTACTTTCCAC; and

*EIF4A1* control, TCTTGGTGAAGCGTGATGAG and GCTGAGTTGGGAGATCGAAG.

## Results

### Activation of AG in *cal-1, ap1-1* plants induced synchronised stamen and carpel primordia

To follow changes in gene expression after stamen and carpel initiation, we generated plants with AG under external control. Plants were transformed with a construct in which the 35S promoter directed a fusion between AG and part of the rat glucocorticoid receptor (GR), as reported previously (Ito et al., 2004); for simplicity, we will refer to the *35S:AG-GR* construct as *AGGR*. In the loss-of-function *ag-3* mutants, *AGGR* rescued development of stamens and carpels only when the plants were treated daily with the steroid dexamethasone (DEX), confirming that the AG-GR fusion could replace AG function (Fig. 1A-D).

To focus on early organogenesis, *AGGR* was combined with the *ap1-1* and *cal-1* mutations. *AP1* and *CAL* act redundantly to specify floral meristem identity. The double mutant accumulates indeterminate lateral meristems that fail to initiate floral organs (Kempin et al., 1995) (Fig. 1E,G), although the defect can become less severe late in development, allowing flowers to form (Ferrandiz et al., 2000). Expression of AG under the 35S promoter in *ap1-1, cal-1* plants restored robust stamen and carpel development (Mizukami and Ma, 1997). In *AGGR*, *ap1-1, cal-1* plants, DEX treatment induced stamen and carpel formation, whereas mock-treated controls remained meristematic (Fig. 1E,F). A single DEX treatment was sufficient for full stamen and carpel development, which followed a time course comparable to wild-type development (Smyth et al., 1990) (Fig. 1F). However, in *AGGR, ap1-1, cal-1* plants that were also homozygous for the *ag-3* mutation, organ development required daily DEX treatments (not shown), implying that a single DEX treatment initiated stamen and carpel development that was subsequently sustained by the endogenous *AG*. Thus, although an artificial construct was used to trigger organogenesis, subsequent development was controlled by the endogenous gene, followed the normal time course and yielded fully functional organs.

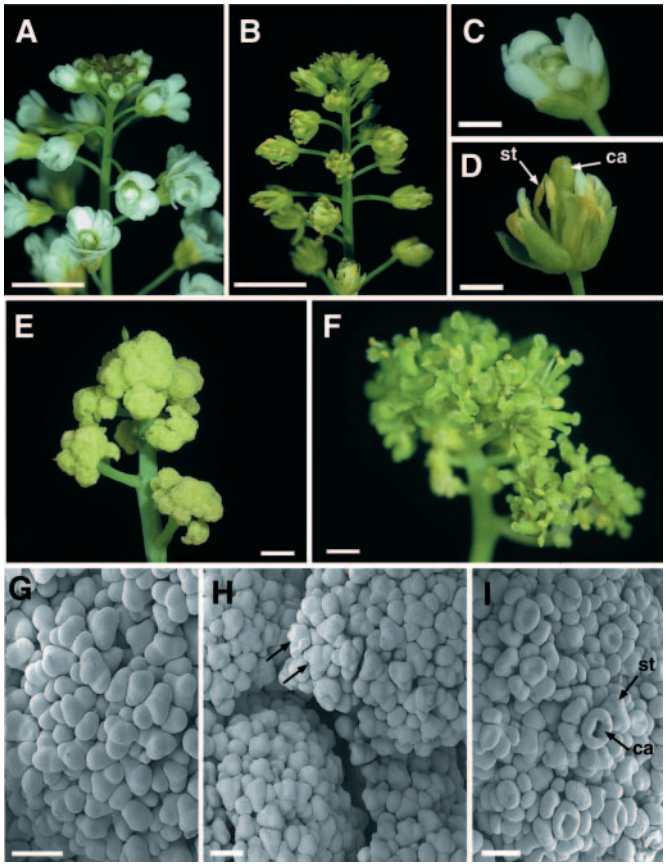


**Fig. 1.** Steroid-inducible stamen and carpel development. (A,B) *ag-3, AGGR* inflorescences, mock treated (A) or treated with dexamethasone (DEX; B). (C,D) Close-up view of flowers from the plants shown in A,B; while the mock-treated plant shows an indeterminate number of sepals and petals (C), DEX treatment has induced stamen (st) and carpel (ca) development (D). (E,F) *cal-1, ap1-1, AGGR* inflorescences, two weeks after mock treatment (E) or treatment with DEX (F). Note the mass of meristems in E (similar to those shown at high magnification in G) compared with the mature stamens and carpels in F. (G-I) Scanning electron micrographs of *cal-1, ap1-1, AGGR* inflorescences 1 day (G), 3 days (H) and 7 days (I) after DEX treatment. The arrows in H indicate meristems that are beginning to produce organ primordia; in I, developing stamens (st) and carpels (ca) are morphologically identifiable. Scale bars: 5 mm in A,B; 1 mm in C-F; 100 µm in G-I.

In plants treated in parallel with solution lacking DEX, organogenesis was not seen, whereas the frequency of DEX-induced organogenesis after a single DEX treatment ranged from between 30% and 100% of plants in different experiments. Individual DEX-treated plants showed an all-or-nothing response (i.e. either robust organ induction in all treated inflorescence apices, or no induction). *AGGR* was still

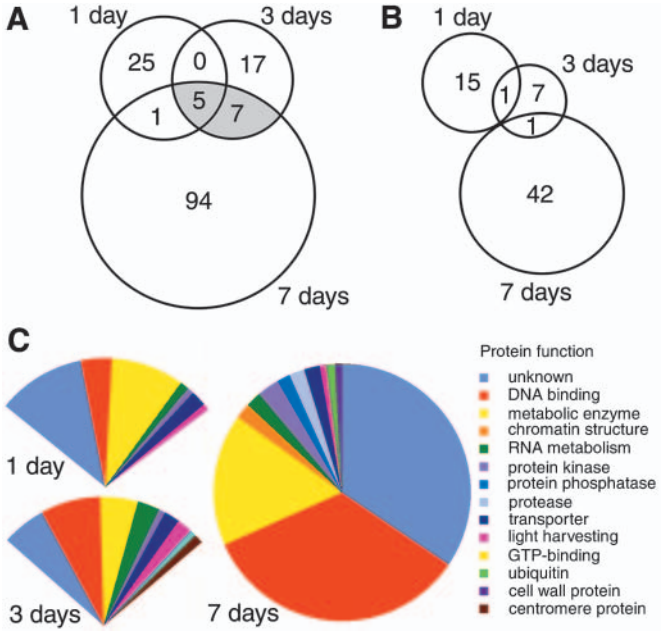


**Fig. 2.** Summary of changes in gene expression after AGGR activation. (A,B) Venn diagrams showing the number of genes significantly activated (A) or repressed (B), 1, 3 or 7 days after DEX treatment. The grey area contains the 12 genes chosen for more detailed analysis because their activation was sustained during the time course. (C) Predicted functions of the proteins encoded by the genes shown in A. The ratio between the number of genes and the area of the coloured sections is the same across the diagrams for 1, 3 and 7 days after AGGR activation.

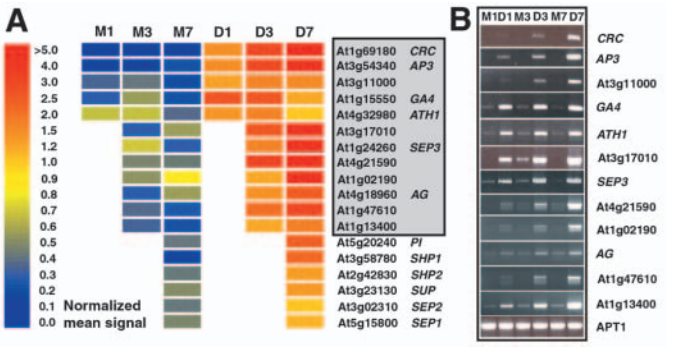


**Fig. 3.** Expression levels of selected genes after AGGR activation. (A) Expression detected on the oligonucleotide array. M1 to M7 and D1 to D7 indicate 1, 3 and 7 days after mock treatment and DEX treatment, respectively. The coloured rectangles show normalised mean expression according to the colour scale on the left; the levels are shown only when the difference between mock and DEX treatment was statistically significant. The 12 genes in the grey box showed sustained activation and correspond to the grey area in Fig. 2A. Additional genes with previously characterised roles in stamen or carpel development and with significant activation at day 7 only are also shown. (B) Activation of the 12 genes in the grey box in Fig. 2A, confirmed by RT-PCR (in the case of AG, the primers used did not amplify *AGGR*). M1-7 and D1-7 are as described in A; APT1 (adenosine phosphotransferase) was used as a constitutive control.

expressed in plants that failed to initiate organs in response to DEX (not shown), so transgene silencing was unlikely to be the cause of the variable organ induction. The all-or-nothing response suggested that organ induction was a bistable switch (see Discussion).

## Global analysis of gene expression during AG-induced organogenesis

To screen for genes whose expression changed in *ap1-1, cal-1* meristems after *AG* activation, we used the Arabidopsis ATH1 high-density oligonucleotide array (Affymetrix). Three time points were chosen after a single DEX treatment of *35S::AG-GR, cal-1, ap1-1* meristems: one day, when no morphological changes were visible, three days, when the earliest signs of organ primordia were seen, and seven days, when stamen and carpel primordia were recognisable (Fig. 1G-I). To ensure that the samples came from plants in which organogenesis had been induced, a few treated meristems were left on each plant and organ development was checked after two weeks. For each time point, two independent samples with AGGR activated were compared with two mock-treated controls, giving four possible combinations of treatment versus control. Genes up- or downregulated were defined independently for each time point as those with a statistically significant change in all four treatment/control pairs (Wilcoxon signed-rank test, $P<0.05$) (Hubbell et al., 2002; Liu et al., 2002) and a mean change of at least twofold.

Using these filtering criteria, 149 of the 22,810 genes represented on the array were upregulated in at least one of the three time points (Fig. 2A, and Tables S1 and S2 in supplementary material). Based on their predicted molecular function, the majority of these genes fell into three classes: unknown function (50), DNA-binding proteins (38) and metabolic enzymes (30) (Fig. 2C). The set of upregulated genes contained most of the known genes with a specific role in stamen and/or carpel development, including *AG* itself (Yanofsky et al., 1990), *AP3* (Jack et al., 1992), *PI* (Goto and Meyerowitz, 1994), *SEP1*, *SEP2* and *SEP3* (Pelaz et al., 2000), *SUP* (Sakai et al., 1995), *CRC* (Bowman and Smyth, 1999) and *SHP1*, *SHP2* (Liljegren et al., 2000). *JAGGED* (*JAG*) (Dinneny et al., 2004; Ohno et al., 2004), which controls the development of leaves and floral organs, was also activated, in addition to four (At2g01520, At3g04960, At4g21590, At5g57720) out of ten uncharacterised genes whose expression correlated with that of floral homeotic genes during floral induction (Schmid et al., 2003). Thus our array experiment independently detected many of the genes expected to function downstream of *AG*, based on previous genetic and array-based experiments.

The set of downregulated genes was smaller (16 on day 1, 9 on day 3, 43 on day 7; Fig. 2B, and Tables S1 and S2 in supplementary material) and included only one gene with a well-known role in floral development. *UFO* is expressed in meristems, functions upstream of the floral homeotic genes to set the pattern of *AP3* expression and is only expressed at the earliest stages of reproductive organ development (Ingram et al., 1995; Lee et al., 1997; Levin and Meyerowitz, 1995). Accordingly, *UFO* appeared among the genes that were repressed at day 7 of organ development.

A set of 1453 genes expressed mostly at relatively late stages in specific floral organs has been identified by comparing the transcripts in wild-type and homeotic mutant flowers (Wellmer et al., 2004). The overlap between these genes and our list of

*AG*-regulated genes is relatively small (20 of the 149 AG-activated genes and six of the AG-repressed genes; see Tables S1 and S2 in supplementary material), suggesting that the transcriptional program in early organogenesis is distinct from that in late organs.

### Genes that showed sustained activation are expressed in wild-type carpel and stamen development

To confirm independently of the array data that we have identified genes controlled by AG, we focused on genes that were activated at multiple time points after AG induction. A set of twelve genes were upregulated on day 1 or 3 and then remained activated until day 7 (Fig. 3A). This set includes four well-known regulators of stamen or carpel development (*AP3*, *CRC, AG*, *SEP3*), and two genes implicated in the biosynthesis of the growth regulator, gibberellin: *GA4* encodes an enzyme that catalyses the production of bioactive gibberellin (William et al., 2004; Williams et al., 1998) and *ATH1* encodes a homeodomain protein proposed to regulate gibberellin biosynthetic genes (Garcia-Martinez and Gil, 2001). The remaining six genes encode a B3 domain protein (At3g17010), a zinc-finger protein (At1g13400) related to *SUP*, a homologue (At3g11000) of a protein implicated in somatic embryogenesis in carrot (Schrader et al., 1997), a predicted bifunctional nuclease (At4g21590), a WD-domain protein (At1g47610) and a protein (At1g02190) similar to CER1, which is involved in the synthesis of epicuticular wax and in pollen development (Aarts et al., 1995).

Activation of all 12 genes in *cal-1, ap1-1, AGGR* plants was verified by RT-PCR using a new set of RNA samples collected 1, 3 and 7 days after treatment (Fig. 3B). Genes controlled by AG should also be active during stamen or carpel development in wild-type flowers. This has already been shown for *AP3*, *AG*, *SEP3* and *CRC*; for other genes in the set, expression was analysed by RNA in situ hybridisation (Fig. 4). At4g21590 was expressed in the centre of the floral meristem, in a pattern similar to that of AG, and continued to be expressed at later stages of stamen development (Fig. 4A,B). At3g17010 and At1g13400 were expressed in emerging stamen primordia and later in part of the developing carpels; expression of At3g17010, but not At1g13400, remained high in the sporogenous tissue of stamens and in the carpel ovary (Fig. 4C-E). Both genes implicated in gibberellin biosynthesis were expressed at very low levels in developing stamens: *ATH1* expression was seen in the early organs, while *GA4* was only detectable in the stamen filaments (Fig. 4F-H). Expression of At3g11000, At1g47610 and At1g02190 was below detection levels by in situ hybridisation. In all in situ hybridisation experiments, sense control probes showed only uniform background signal (not shown).

### Binding to AG in vitro and in vivo

We next tested whether the 12 genes in the 'core' set contained AG binding sites. We scanned sequences upstream of the start codon for the CArG box bound by AG in vitro, TT(A/T/G)CC(A/T)$_6$GG(A/T/C)AA (Shiraishi et al., 1993), accepting a maximum of two nucleotide mismatches, except when the mismatches eliminated either the CC or GG sequences flanking the A/T core. This level of stringency was calibrated using the well-characterised CArG boxes present in the AP3 promoter and in the second intron of AG (Hill et al., 1998; Hong et al., 2003; Tilly et al., 1998). Of the remaining 10 genes, eight had at least one CArG box match within 3 kb upstream of the start codon (Fig. 5A); in all cases, binding to AG was confirmed in vitro (Fig. 5B).

One caveat of detecting AG binding sites is that the frequency of CArG boxes in *Arabidopsis* genes is high: our search criteria detected at least one match in 49% of 27,186 upstream 3 kb sequences (www.arabidopsis.org/cgi-bin/patmatch/nph-patmatch.pl). The likelihood of finding a match in eight out of ten genes, however, is relatively low (4.8%, assuming binomial distribution and 49% likelihood for any single gene). Thus our subset of 12 genes was enriched for AG binding sites. A comparable enrichment was not seen for the complete set of AG-

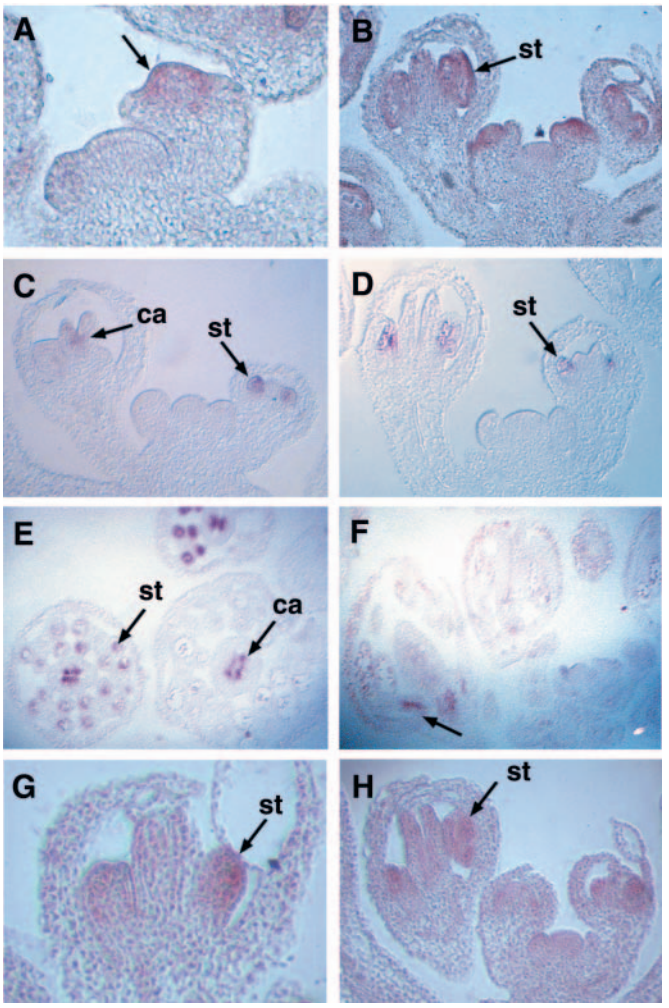


**Fig. 4.** RNA in situ hybridisation of selected genes during wild-type floral development. (A,B) At4g21590. The arrows show expression in the centre of a stage 3 bud (A), and later in developing stamens (B). (C) At1g13400. Arrows indicate expression in emerging stamen primordia (st), and in the placental region in early carpels (ca). (D,E) At3g17010. Expression in emerging stamen primordia (st) is indicated in D; arrows in E indicate expression in the sporogenous tissue of stamens (st) and in the placental region of carpels (ca). (F) Expression of GA4 in stamen filaments (arrow). (G,H) Expression of *ATH1* in developing stamens (st). For better contrast, the sections in A, B, G and H were photographed in aqueous medium, before the tissues were permanently mounted.

activated or repressed genes (matches were found in the upstream 3 kb sequences for 61% of the upregulated genes and 56% of downregulated genes), possibly because the complete set includes indirect AG targets.

Another caveat of the in vitro binding results is that multiple MADS domain proteins recognise similar sequences in vitro (Riechmann et al., 1996), so the CArG boxes might be targeted in vivo by MADS domain proteins other than AG. To confirm binding to AG in vivo, we used chromatin immunoprecipitation (ChIP) for a subset of genes of particular interest: *AG*, *AP3*, *SEP3* and *CRC* (which suggested that *AG* activated itself and most of the other regulators of stamen and carpel identity); and *GA4* (which suggested that another role of *AG* is to promote gibberellin biosynthesis). Fragments of these genes containing the in vitro-detected AG binding sites were enriched in immunoprecipitates obtained with antibodies against AG, but not with an unrelated antibody (Fig. 5C). By contrast, fragments that lacked AG binding sequences, such exon 4 of EIF4A1 (Fig. 5C) and exon 7 of *AP3* (not shown), were detected to the same background levels with both antibodies. Thus AG interacted in vivo with predicted regulatory sequences of *AG*, *AP3*, *CRC*, *SEP3* and *GA4*.

### AG and AP1 maintain AP3 expression during organogenesis

The activation of AP3 by AG was not predicted by previous genetic and molecular analysis, particularly because *AP3* is expressed normally in *ag* mutants (Jack et al., 1992) (Fig. 6B). This, however, could be due to redundant activation by *AP1* (Lamb et al., 2002; Ng and Yanofsky, 2001), which is normally repressed by *AG* in the centre of the floral bud (Gustafson-Brown et al., 1994) and could take over *AP3* activation in the innermost organs of *ag* mutant flowers. To test this idea, we compared *AP3* expression in the *ag-3* mutant, in the *ap1-1* mutant and in the double mutant (Fig. 6). In the *ag-3* mutant, stamens and carpels are replaced by additional whorls of sepals and petals (Yanofsky et al., 1990) (Fig. 6A). As expected, *AP3* expression was readily detected in stage 3 buds and persisted throughout the development of both normal and ectopic petals of the *ag-3* mutant (Jack et al., 1992) (Fig. 6B). In the *ap1-1* mutant, petals are mostly absent and sepals are replaced by leaf-like organs that often subtend ectopic flowers (Mandel et al., 1992) (Fig. 6C). In this mutant, *AP3* expression was normal in stage 3 and continued throughout stamen development (Fig. 6D). Like *ag-3*, the *ag3-3, ap1-1* double mutant flower produced an indeterminate number of organs, which were leaf-like and subtended secondary flowers (Fig. 6E), similar to the first whorl organs of *ap1-1*. In the double mutant, early *AP3* expression showed the normal pattern in both the primary and secondary flowers, while expression in later organ development was abolished (Fig. 6F).

We conclude that early *AP3* expression did not require *AG* or *AP1*, and was probably due to activation by other regulatory genes, such as *LEAFY* in combination with *UFO* (Lamb et al., 2002; Parcy et al., 1998). Maintenance of *AP3* expression in later stages of floral development, however, required either *AG* or *AP1*.

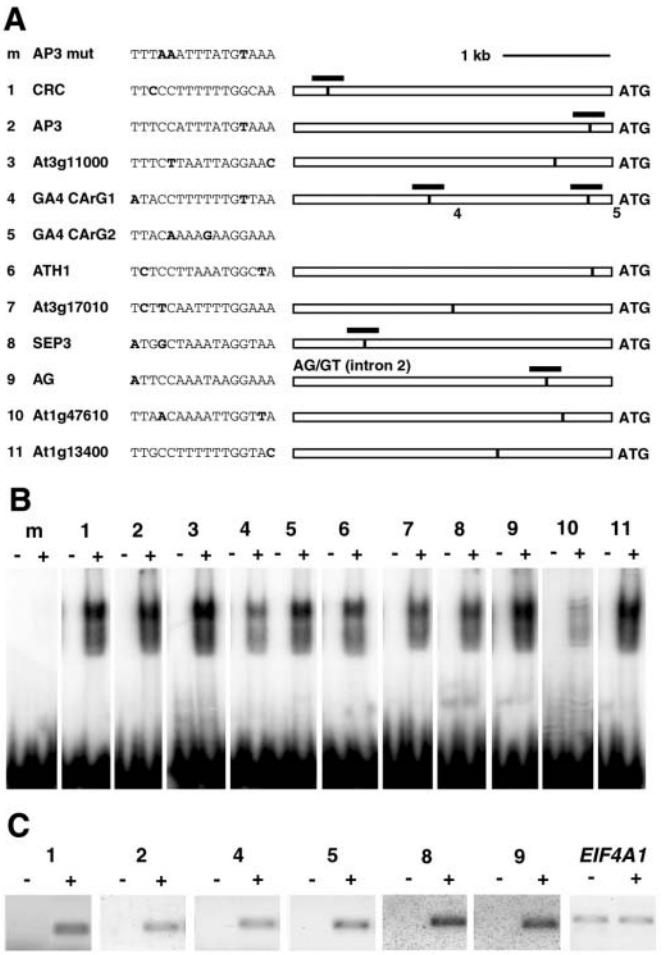


**Fig. 5.** AG binds to candidate target genes. (A) Binding sites identified by sequence analysis. 'm' is a mutated version of the binding site in AP3, used as a negative control. Next to each numbered binding site, the corresponding gene and sequence are shown, with mismatches to the consensus AG binding site TT(A/T/G)CC(A/T)$_6$GG(A/T/C)AA (Shiraishi et al., 1993) marked in boldface. The white boxes represent sequences upstream of the start codon (except for *AG*, where the reference point is the 5′ splice site of the second intron), with vertical lines indicating the position of the AG binding sites. The horizontal bars above some of the binding sites indicate the fragment amplified in the ChIP experiment (C). (B) Binding to AG in vitro, shown by electrophoretic mobility shift assays (EMSA). The probes contained the binding sites numbered in A. Each probe was incubated with extract from bacteria induced to express AG (+) or an empty expression vector (–). In all experiments, the same amount of labelled probe was used and a lane with probe 2 (not shown) was included to adjust the exposure to comparable levels. (C) Binding to AG in vivo, shown by ChIP. Numbers correspond to the binding sites shown in A; in each panel, PCR amplification (35 cycles) of sequences containing the binding site (black bars in A) is compared in immunoprecipitates obtained with antiserum against AG (+) or CLV3 (–). In the last panel on the left, the fourth exon of *EIF4A1* was used as a negative control lacking AG binding sequences: with 35 cycles (not shown), no band was seen; with 40 cycles (panel), similar levels of contaminating template were amplified. The results shown were replicated in two fully independent ChIP experiments.

## Discussion

### Regulators of floral organ identity function in an auto-regulatory module

Positive auto-regulatory loops are a common device to stabilise

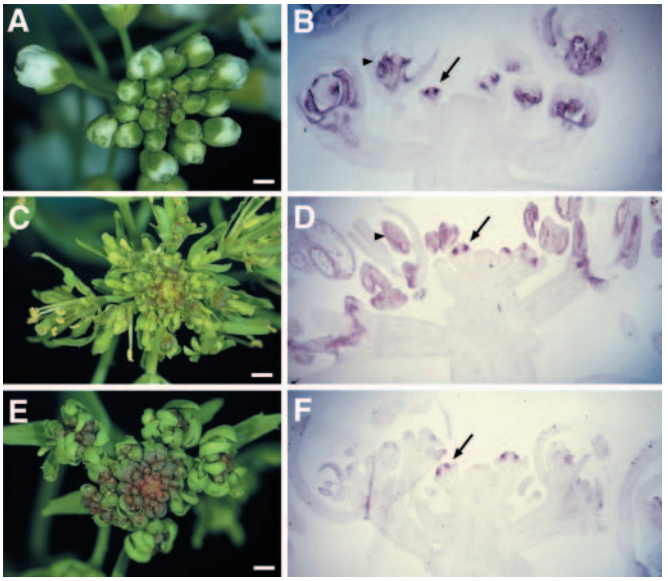


**Fig. 6.** Maintenance of *AP3* expression requires either AG or AP1. (A,C,E) Top view of inflorescence in *ag-3* (A), *ap1-1* (C), and in the *ag-3, ap1-1* double mutant (E). (B,D,F) RNA in situ hybridisation with the AP3 probe hybridised to longitudinal sections through the inflorescence apex of *ag-3* (B), *ap1-1* (D), and the *ag-3, ap1-1* double mutant (F). Arrows point at stage 3 buds, where AP3 expression is initiated, and arrowheads indicate later expression during petal (B) and stamen (D) development. The sections of the three genotypes were hybridised in parallel on the same slides, to allow comparison of the expression levels. Scale bars: 1 mm in A,C,E.

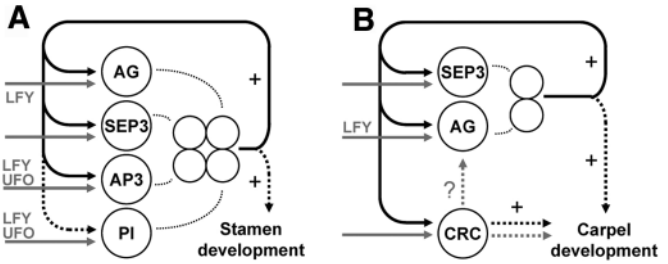


**Fig. 7.** Model for the co-ordinated regulation of floral organ identity regulators. (A) In stamen development, *AG*, *AP3*, *PI* and *SEP3* are initially activated independently (grey arrows) (Ferrario et al., 2004; Zik and Irish, 2003a). LFY is responsible for the initial activation of *AG*, whereas early activation of *AP3*/*PI* occurs in areas of the meristem that express both LFY and UFO (Parcy et al., 1998). The AG, AP3, PI and SEP3 proteins (circles) function together in a complex to promote stamen development (Honma and Goto, 2001), and to amplify and maintain their own expression. Solid black arrows indicate direct interactions supported by ChIP; feedback activation of PI may be indirect (dashed arrow) (Honma and Goto, 2000). (B) The protein complex proposed to control carpel development contains AG and SEP3 (Honma and Goto, 2001). As in A, the initially independent expression of AG and SEP3 is maintained by a positive-feedback loop. In addition, CRC expression is reinforced, although CRC expression can promote carpel development independently of AG (indicated by the parallel grey arrow) (Alvarez and Smyth, 1999). As in A, interactions supported by ChIP are indicated by solid black arrows. The possibility that CRC might also promote AG activity is indicated by the dashed arrow with a question mark.

expression patterns that arise from transient inputs during development (Davidson et al., 2002). Our results suggest that *AG*, *AP3*, *PI* and *SEP3* are part of such an auto-regulatory loop: in *ap1-1, cal-1* plants, transient AG activation was sufficient to trigger self-maintaining stamen and carpel development, during which *AG*, *AP3*, *PI* and *SEP3* were activated; in addition, AG interacted directly with the *AG*, *AP3* and *SEP3* genes in vitro and in vivo.

Previously, auto-regulation of floral homeotic genes was known only for *AP3* and *PI*, and their orthologues in snapdragon, *DEF*/*GLO*. In early buds, these genes are activated independently of each other, and, where they overlap, a positive-feedback loop is established that maintains their expression during petal and stamen development (Jack et al., 1994; Schwarz-Sommer et al., 1992). Activation of *AP3* by AP3/PI is likely to be direct, whereas activation of *PI* requires an intermediate protein synthesis step (Honma and Goto, 2000). In the case of *AP3*, the auto-regulatory loop is required only in stamens: *AP3* expression is still maintained in the sepal-like organs that replace petals in the *pi-1* mutant (Jack et al., 1992). This is an important point, because it shows that *AP3* expression can be uncoupled from the organ identity directed by *AP3*/*PI*, and therefore the absence of *AP3* expression in the developing organs of *ag-3, ap1-1* double mutants was not a trivial consequence of the fact that these organs were neither petals nor stamens. The requirement of *AG* to maintain *AP3* expression when this role cannot be fulfilled by *AP1* supported the idea that *AG* also participates in *AP3*/*PI* regulation.

The co-ordinated regulation of *AG*, *AP3*, *PI* and *SEP3* would be expected if, as proposed by recent models, these proteins function together in the same protein complexes (Honma and Goto, 2001; Jack, 2001). In particular, if the predicted protein complexes are correct, the *AP3*/*PI* auto-regulatory loop should also require either *AG* or *AP1* (which has also been proposed to form a complex with AP3/PI and SEP during petal development) (Honma and Goto, 2001). Our results confirmed this prediction.

However, if AG can only function when complexed with other MADS-domain proteins, then initiation of organogenesis by *AGGR* must have relied on partner proteins already present in the *cal-1, ap1-1* meristems. One possibility is that a low level of AG-independent expression of *SEP*, *AP3* and *PI* genes provided the required partners. This initial expression could be controlled by the same mechanism that activates these genes independently of each other in early wild-type buds. The need to establish a regulatory loop to amplify initially limiting levels of its partners may be the reason why a single activation of *AGGR* in *cal-1, ap1-1* meristems resulted either in no response, or in robust organogenesis in an apparently random fashion.

In addition to *AP3*, *CRC* was strongly activated by *AG*. This was not expected because of the genetic evidence that *CRC* can function in the absence of *AG*. In the *ag-1, ap2-2, pi-1* triple mutant, in spite of the loss of *AG* function, the floral organs develop several carpelloid features, such as stigmatic cells and ectopic ovules. In this background, loss of *CRC* function caused a clear reduction of these carpelloid features, showing that *CRC* does not require *AG* to direct carpel development (Alvarez and Smyth, 1999). Our results suggest that although independently activated, *CRC* expression is reinforced by *AG*. Previous genetic results suggest that this reinforcement may be

mutual: loss of *CRC* weakens *AG* function, causing the heterozygous *ag-1/AG* plants, which normally have a wild-type phenotype, to show a partial *ag* loss-of-function phenotype (Alvarez and Smyth, 1999). It remains to be tested whether this occurs because *CRC* also activates *AG*, participating in the auto-regulatory loop.

We also saw that, at least in the *cal-1, ap1-1* backgound, *AG* activated its own transcription. This could be inferred independently of the array experiments, from the fact that the endogenous *AG* was required for organogenesis in *cal-1, ap1-1* plants after transient activation of *AGGR*, and was supported by the chromatin immunoprecipitation results. One difficulty with the idea that *AG* auto-regulates, however, is that *AG* is still expressed in the inner organs of *ag-1* mutant flowers (Gustafson-Brown et al., 1994). Thus if *AG* activates itself during normal development, this activity must be redundant. As discussed above, if *CRC* participates in the *AG* regulatory loop, then *CRC* activity might account for the continued *AG* expression in *ag* flowers.

Combined with the published data, our results suggest a model for how AG and other floral organ identity genes are co-ordinately regulated (Fig. 7). In stamen development, AG, AP3, SEP3 and PI are initially expressed independently of each other. Where their expression overlaps, the predicted AG/SEP3/AP3/PI MADS protein complex (Honma and Goto, 2001; Jack, 2001) maintains and amplifies their expression. In carpel development, the predicted AG/SEP3 complex may establish a similar feedback loop, which also reinforces *CRC* expression.

### Interaction between AG and gibberellin

A link between gibberellin and homeotic genes has been shown previously via regulation of *LEAFY* (*LFY*), which activates homeotic genes in the early stages of floral development (Blazquez et al., 1998). More recently, gibberellin has been reported to activate floral homeotic genes at later stages of development, when *LFY* is no longer active (Yu et al., 2004). Our results suggest that the reverse is also true, that is, homeotic genes positively regulate the gibberellin pathway. *GA4* is part of a small family of genes that encode GA3-β-hydroxylases, which catalyse the last step in the biosynthesis of gibberellin and have a regulatory role in the pathway (Hedden and Phillips, 2000; Itoh et al., 1999; Talon et al., 1990), so *GA4* activation suggested that *AG* induced gibberellin biosynthesis during organogenesis.

Activation of *GA4* by *AG* may be another branch of the homeotic gene autoregulatory loop. There may be, however, additional functions for gibberellin in floral organogenesis. Another gibberellin biosynthetic gene, encoding GA20-oxidase, is repressed by genes that maintain undifferentiated cells in the meristem, and activated in the leaf primordia that emerge from the meristem (Hay et al., 2002; Sakamoto et al., 2001). This suggests that gibberellin may have a more general role in the transition from meristem identity to organogenesis. This idea seems inconsistent with the fact that organ emergence is normal in gibberellin-deficient mutants, both during the vegetative phase and in flowers (the floral defects in *ga1-3* become visible only at later stages of development) (Goto and Pharis, 1999; Wilson et al., 1992). However, even severe mutants such as *ga1-3*, still produce low levels of gibberellin (Hedden and Phillips, 2000), which might be sufficient for the proposed functions in early organ development. Although it is not clear what these functions might be, the known role of gibberellin in controlling cell growth and division (Yang et al., 1996) suggests that it might play a role in the localised changes in growth that drive the emergence of organ primordia from the meristem. If this is true in the floral organ primordia, then gibberellin could be part of the link between homeotic genes and the cellular behaviour that shapes floral organs.

### Global view of gene expression in stamen and carpel primordia

From the predicted protein functions of the 149 genes that were upregulated by AG, two prominent features emerged (Fig. 2). First, genes expected to function in transcriptional control were over-represented (26%), compared with their total frequency in the genome (5.9%) (Riechmann and Ratcliffe, 2000). The fraction of regulatory genes increased over the time course from 13% (day 1) to 28% (day 3) to 34% (day 7). This contrasts with more mature organs, where the frequency of regulatory genes was 5.5%, similar to their representation in the genome (Wellmer et al., 2004), and suggests that up to 7 days after organ initiation much of the program of gene expression downstream of AG was concerned with refining patterns of gene expression. Complex cascades of transcription factors, as seen in early development of *Drosophila* and sea urchin, also cause delayed responses to initial inputs and have been proposed to function as timing devices during development (Rosenfeld and Alon, 2003).

Second, of the 36 predicted DNA-binding proteins that were upregulated at day 7, 53% belonged to two transcription factor families (10 B3 domain, PFAM profile PF02362, and 9 MADS domain, PF00319). MADS domain proteins play a prominent role in floral development and the diversification of this family correlates with the evolution of plant reproductive structures (Theissen et al., 2000). Our data suggests that the B3 domain family has undergone a comparable diversification of roles in reproductive development.

Developmental genetics has identified many regulatory genes whose expression determines where and when a specific structure or organ develops. The problem of understanding how regulatory gene expression is translated into complex multicellular structures is universal, and has led to a number of attempts to describe the gene expression programs controlled by these regulators (Furlong et al., 2001; Livesey et al., 2000; Michaut et al., 2003). Like other global descriptions of changes in gene expression during development, however, our view of gene expression under AG has two limitations. First, it is unlikely to be complete, because we cannot guarantee that genes with very low or localised expression were not missed, and because of the difficulties associated with detecting downregulation if it occurs only in a subset of the cells. Second, the set of genes controlled by AG probably cannot be organised within a single network of interactions, because they may represent the overlap of multiple programs of gene expression that run in parallel in different regions and cell types of organ primordia.

In spite of these limitations, the list of genes controlled by AG will provide a basis for the functional analysis of intermediate regulators of early organogenesis, and will provide target promoters that are needed to test current

models for the molecular basis of how homeotic genes act combinatorially.

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### Supplementary material

Supplementary material for this article is available at
http://dev.biologists.org/cgi/content/full/132/3/429/DC1

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**Table S1. Genes upregulated 1, 3 and 7 days after AGGR activation**

| Affymetrix code | AGI code | Description | 1 day dex | 1 day mock | 1 day fold | 3 days dex | 3 days mock | 3 days fold | 7 days dex | 7 days mock | 7 days fold | Predicted function | (Wellmer et al., 2004) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 251898_at | ***At3g54340*** | APETALA3 (AP3) | 1.8 | 0.062 | 28.89 | 3.741 | 0.121 | 30.71 | 13.96 | 0.282 | 49.386 | DNA binding | |
| 260355_at | ***At1g69180*** | CRABS CLAW (CRC) | 1.766 | 0.069 | 25.41 | 3.759 | 0.15 | 24.97 | 16.11 | 0.044 | 359.51 | DNA binding | Carpel |
| 254823_at | At4g12580 | Unknown protein | 1.014 | 0.06 | 16.66 | | | | | | | Unknown | |
| 254197_at | At4g24040 | Trehalase-like protein | 1.518 | 0.093 | 16.25 | | | | | | | Enzyme | Carpel |
| 252948_at | At4g38610 | Putative beta-catenin-like NLS receptor - PFAM PF00514 | 0.999 | 0.068 | 14.52 | | | | | | | Transporter | |
| 256349_at | At1g54890 | Similar to late embryogenesis abundant protein | 0.878 | 0.066 | 13.17 | | | | | | | Unknown | |
| 255229_at | At4g05490 | Contains PF00646 F-box domain | 0.906 | 0.072 | 12.58 | | | | | | | Enzyme | |
| 260276_at | At1g80450 | Unknown protein | 1.177 | 0.109 | 10.71 | | | | | | | Unknown | |
| 261768_at | **At1g15550** | Gibberellin 3 beta-hydroxylase GA4 | 3.077 | 0.357 | 8.617 | 3.19 | 0.666 | 4.787 | 1.487 | 0.271 | 5.472 | Enzyme | |
| 263735_s_at | At1g60040 | Contains MADS box | 2.678 | 0.33 | 8.109 | | | | | | | DNA-binding | |
| 248993_at | At5g45240 | Contains similarity to disease resistance protein | 0.469 | 0.074 | 6.289 | | | | | | | Protein kinase | |
| 265387_at | At2g20670 | Unknown protein | 0.772 | 0.125 | 6.149 | | | | | | | Unknown | |
| 254976_at | At4g10510 | Subtilisin-like serine protease | 0.82 | 0.133 | 6.146 | | | | | | | Enzyme | |
| 255695_at | At4g00080 | Contains PFAM PF04043 Plant invertase/pectin methylesterase | 0.566 | 0.115 | 4.903 | | | | | | | Enzyme | |
| 262575_at | At1g15210 | Putative ABC transporter | 2.467 | 0.527 | 4.676 | | | | | | | Transporter | |
| 252700_at | At3g43690 | Unknown protein | 1.477 | 0.405 | 3.64 | | | | | | | Unknown | |
| 257579_at | **At3g11000** | B2, GDA-2 homologue | 1.478 | 0.417 | 3.544 | 1.973 | 0.5 | 3.94 | 1.782 | 0.263 | 6.776 | Unknown | |
| 248137_at | At5g54950 | Contains similarity to cytoplasmic aconitate hydratase | 2.283 | 0.68 | 3.356 | | | | | | | Enzyme | |
| 244983_at | rpl16.chloroplast | Ribosomal protein L16 | 1.264 | 0.427 | 2.96 | | | | | | | RNA metabolism | |
| 252332_at | At3g48810 | Contains PF01535 PPR repeat | 0.317 | 0.108 | 2.917 | | | | | | | Unknown | |
| 259017_at | At3g07310 | Unknown protein | 1.066 | 0.393 | 2.711 | | | | | | | Unknown | |
| 256569_at | At3g19550 | Unknown protein | 1.862 | 0.692 | 2.689 | | | | | | | Unknown | |
| 250960_at | At5g02940 | Unknown protein | 2.345 | 0.969 | 2.419 | | | | | | | Unknown | |
| 249755_at | At5g24580 | Unknown protein | 1.019 | 0.428 | 2.376 | | | | | | | Unknown | |
| 263265_at | At2g38820 | Unknown protein | 1.274 | 0.539 | 2.363 | | | | | | | Unknown | |
| 251119_at | At3g63510 | Contains PFAM PF01207 Dihydrouridine synthase | 1.475 | 0.665 | 2.218 | | | | | | | Enzyme | |
| 255445_at | At4g02740 | Contains PF00646 F-box domain | 0.765 | 0.347 | 2.201 | | | | | | | Enzyme | |
| 248296_at | At5g53090 | FEY3 (forever young) | 1.812 | 0.833 | 2.174 | | | | | | | Enzyme | |
| 245023_at | psbI | PSII I protein | 1.057 | 0.493 | 2.144 | | | | | | | Light harvesting | |
| 250007_at | At5g18670 | Beta-amylase-like protein | 0.945 | 0.446 | 2.119 | | | | | | | Enzyme | |
| 253411_at | **At4g32980** | Homeobox gene ATH1 | 1.663 | 0.808 | 2.057 | 1.892 | 0.792 | 2.388 | 1.149 | 0.432 | 2.656 | DNA-binding | |
| 263752_at | At2g21310 | Similar to retroelement pol polyprotein | | | | 1.922 | 0.114 | 16.87 | | | | Enzyme | |
| 245505_at | At4g15690 | Glutaredoxin | | | | 0.303 | 0.03 | 10.03 | | | | Unknown | |
| 257930_at | **At3g17010** | Contains B3 DNA-binding domain (PFAM PF02362) | | | | 3.024 | 0.361 | 8.356 | 8.294 | 0.664 | 12.485 | DNA binding | Carpel |
| 254391_at | **At4g21590** | Putative bifunctional nuclease | | | | 4.116 | 0.57 | 7.221 | 8.151 | 0.52 | 15.65 | RNA metabolism | |
| 265591_at | At2g20150 | Unknown protein | | | | 1.142 | 0.159 | 7.172 | | | | Transporter | |
| 250716_at | At5g06170 | Sucrose transporter protein | | | | 0.465 | 0.07 | 6.595 | | | | Light harvesting | |
| 244973_at | psbT | PSII T protein | | | | 1.329 | 0.202 | 6.579 | | | | RNA metabolism | |
| 245049_at | rps16 | Ribosomal protein S16 | | | | 3.96 | 0.656 | 6.035 | | | | Unknown | |
| 251568_at | At3g58280 | Unknown protein | | | | 2.038 | 0.365 | 5.576 | | | | Enzyme | |
| 262436_at | **At1g47610** | Contains WD domain, G-beta repeat (PFAM PF00400) | | | | 2.338 | 0.441 | 5.291 | 3.714 | 0.31 | 11.964 | Unknown | |

| | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 266271_at | At2g29440 | Putative glutathione S-transferase | | | | 1.864 | 0.371 | 5.016 | | | | Transporter | |
| 248354_at | At5g52330 | Contains PFAM PF00917 MATH domain | | | | 1.033 | 0.211 | 4.887 | | | | RNA metabolism | Stamen |
| 254595_at | ***At4g18960*** | AGAMOUS (AG) | | | | 3.19 | 0.666 | 4.787 | 2.926 | 0.656 | 4.46 | DNA-binding | |
| 252144_at | At3g51190 | Ribosomal protein L8 homologue | | | | 1.652 | 0.374 | 4.414 | | | | Protein kinase | Stamen |
| 257028_at | At3g19230 | Contains PFAM PF00560 LRR:Leucine Rich Repeat | | | | 1.601 | 0.367 | 4.36 | | | | Light harvesting | |
| 259386_at | **At1g13400** | Zinc finger protein | | | | 1.398 | 0.41 | 3.411 | 2.025 | 0.283 | 7.137 | DNA binding | |
| 245017_at | psaI | PSII T protein | | | | 2.387 | 0.851 | 2.804 | | | | Enzyme | |
| 261158_at | At1g34500 | Wax-synthase-like | | | | 1.564 | 0.559 | 2.797 | | | | GTP-binding | |
| 256127_at | At1g18200 | Small GTP-binding protein | | | | 1.642 | 0.683 | 2.404 | | | | Unknown | |
| 264872_at | ***At1g24260*** | SEPALLATA3 (SEP3) | | | | 1.895 | 0.832 | 2.276 | 5.805 | 0.336 | 17.264 | DNA binding | |
| 244993_s_at | ycf1.1 | Unknown protein | | | | 1.223 | 0.54 | 2.263 | | | | DNA binding | |
| 263683_at | At1g26870 | NAC domain protein | | | | 2.247 | 0.993 | 2.262 | | | | Centromere protein | |
| 264180_at | **At1g02190** | CER1-like; contains similarity to receptor-like protein glossy1 | | | | 1.327 | 0.623 | 2.128 | 5.532 | 0.994 | 5.564 | Enzyme | |
| 253532_at | At4g31570 | Similar tocentromere protein E PIR1:S28261 | | | | 0.479 | 0.231 | 2.071 | | | | | |
| 251555_at | *At3g58780* | SHATTERPROOF 1 (SHP1) | | | | | | | 2.556 | 0.047 | 54.263 | DNA binding | Carpel |
| 255609_s_at | At4g01180 | Contains HMG DNA-binding domain (PFAM PF00505) | | | | | | | 0.746 | 0.025 | 29.374 | DNA binding | |
| 247324_at | At5g64190 | Unknown protein | | | | | | | 0.384 | 0.02 | 18.864 | Unknown | |
| 253100_at | At4g37400 | Cytochrome P450 monooxygenase-like protein | | | | | | | 1.152 | 0.082 | 13.891 | Enzyme | Carpel |
| 260077_at | At1g73620 | Contains PFAM profile: PF00314 thaumatin family | | | | | | | 2.591 | 0.205 | 12.584 | Unknown | |
| 255427_at | At4g03380 | Unknown protein | | | | | | | 0.63 | 0.055 | 11.298 | Unknown | |
| 261815_at | At1g08325 | Leucine zipper protein | | | | | | | 2.116 | 0.231 | 9.156 | DNA binding | |
| 266319_s_at | At2g46720 | Putative beta-ketoacyl-CoA synthase | | | | | | | 7.256 | 0.846 | 8.577 | Enzyme | |
| 263203_at | At1g05490 | Unknown protein | | | | | | | 1.088 | 0.132 | 8.193 | Unknown | |
| 264347_at | At1g12040 | Putative extensin | | | | | | | 0.336 | 0.041 | 8.101 | Cell wall protein | |
| 253684_at | At4g29690 | Nucleotide pyrophosphatase-like protein | | | | | | | 1.377 | 0.179 | 7.67 | Enzyme | |
| 261568_at | At1g01030 | Contains B3 DNA-binding domain (PFAM PF02362), similar to RAV1 | | | | | | | 0.664 | 0.087 | 7.556 | DNA binding | |
| 250258_at | At5g13790 | MADS protein AGL15 | | | | | | | 0.289 | 0.038 | 7.426 | DNA binding | |
| 247875_at | At5g57720 | Contains B3 DNA-binding domain (PFAM PF02362) | | | | | | | 1.827 | 0.256 | 7.129 | DNA binding | |
| 252495_at | At3g46770 | Contains B3 DNA-binding domain (PFAM PF02362) | | | | | | | 3.175 | 0.463 | 6.853 | DNA binding | Carpel |
| 256569_at | At3g19550 | Unknown protein | | | | | | | 1.193 | 0.179 | 6.665 | Unknown | |
| 255768_at | At1g16705 | Unknown protein | | | | | | | 1.639 | 0.279 | 5.865 | Unknown | |
| 254201_at | At4g24130 | Unknown protein | | | | | | | 0.679 | 0.117 | 5.772 | Unknown | |
| 254002_at | At4g26280 | Steroid sulfotransferase-like protein | | | | | | | 4.744 | 0.822 | 5.765 | Enzyme | Carpel |
| 260263_at | *At1g68480* | JAGGED, C2H2-type zinc finger protein family, contains Pfam PF00096 zinc finger, C2H2 type | | | | | | | 1.359 | 0.236 | 5.738 | DNA binding | |
| 257859_at | At3g12955 | ARG7 auxin responsive gene | | | | | | | 0.711 | 0.123 | 5.737 | Unknown | |
| 246072_at | *At5g20240* | PISTILLATA (PI) | | | | | | | 2.743 | 0.516 | 5.315 | DNA binding | |
| 266353_at | At2g01520 | Major latex-like protein | | | | | | | 1.289 | 0.243 | 5.304 | Unknown | |
| 263204_at | At1g05480 | Contains PFAM DEAD/H helicases (PF00271) and SNF2 N-terminal domain (PF00176) | | | | | | | 2.5 | 0.507 | 4.928 | Chromatin structure | Carpel |
| 264342_at | At1g12080 | Unknown protein | | | | | | | 1.326 | 0.277 | 4.776 | Unknown | |
| 248752_at | At5g47600 | Contains PFAM PF00011 Hsp20/alpha crystallin family | | | | | | | 3.569 | 0.802 | 4.447 | Unknown | |
| 257899_at | At3g28345 | Contains PF00005 ABC transporter | | | | | | | 0.87 | 0.204 | 4.254 | Transporter | |

| | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 262994_at | At1g54420 | Unknown protein | | | | | | | 0.181 | 0.045 | 4.02 | Unknown | |
| 266760_at | At2g46870 | Contains B3 DNA-binding domain (PFAM PF02362), similar to RAV1 | | | | | | | 1.104 | 0.276 | 3.995 | DNA-binding | |
| 259089_at | At3g04960 | Contains ATP_GTP_A (P-loop) :IPR001687, Tropomyosin:IPR000533, NLS_BP:IPR001472 | | | | | | | 1.952 | 0.49 | 3.982 | Unknown | |
| 247023_at | At5g67060 | Putative bHLH transcription factor | | | | | | | 1.485 | 0.379 | 3.914 | DNA-binding | |
| 256062_at | At1g07090 | Unknown protein | | | | | | | 2.743 | 0.717 | 3.826 | Unknown | |
| 250071_at | At5g18000 | Contains B3 DNA-binding domain (PFAM PF02362) | | | | | | | 0.336 | 0.095 | 3.5 | DNA-binding | |
| 258321_at | At3g22840 | Early light-induced protein, contains PFAM PF00504 Chlorophyll A-B binding protein | | | | | | | 2.62 | 0.755 | 3.467 | Unknown | |
| 258856_at | At3g02040 | Contains PFAM PF03009 glycerophosphoryl diester phosphodiesterase | | | | | | | 2.025 | 0.584 | 3.465 | Enzyme | |
| 263907_at | At2g36270 | ABSCISIC ACID INSENSITIVE 5 (ABI5) | | | | | | | 2.774 | 0.815 | 3.399 | DNA-binding | |
| 260041_at | At1g68780 | Contains PFAM PF00560 Leucine Rich Repeat | | | | | | | 1.746 | 0.546 | 3.197 | Protein kinase | |
| 254203_at | At4g24150 | Similar to transcription activator (GRL5) | | | | | | | 3 | 0.946 | 3.171 | DNA-binding | |
| 253845_at | At4g27980 | Contains Tropomyosin:IPR000533(3); NLS_BP:IPR001472(1) | | | | | | | 1.836 | 0.582 | 3.153 | Unknown | |
| 245035_at | At2g26400 | Contains PFAM PF03079 ARD/ARD' family | | | | | | | 1.92 | 0.619 | 3.099 | Unknown | |
| 249939_at | At5g22430 | Unknown protein | | | | | | | 1.716 | 0.554 | 3.095 | Unknown | |
| 263988_at | *At2g42830* | SHATTERPROOF 2 (SHP2) | | | | | | | 1.515 | 0.502 | 3.013 | DNA-binding | Carpel |
| 266540_at | At2g35310 | Contains B3 DNA-binding domain (PFAM PF02362) | | | | | | | 1.773 | 0.593 | 2.987 | DNA-binding | Carpel |
| 248496_at | At5g50790 | MtN3-like protein | | | | | | | 3.118 | 1.046 | 2.98 | Unknown | |
| 263092_at | At2g16210 | Contains B3 DNA-binding domain (PFAM PF02362) | | | | | | | 1.72 | 0.577 | 2.977 | DNA-binding | |
| 251223_at | At3g62610 | Putative transcription factor MYB11 | | | | | | | 1.613 | 0.547 | 2.948 | DNA-binding | |
| 250136_at | At5g15380 | DRM1 DOMAINS REARRANGED METHYLASE (DRM) | | | | | | | 1.111 | 0.384 | 2.892 | Chromatin structure | |
| 251986_at | At3g53310 | Contains B3 DNA-binding domain (PFAM PF02362) | | | | | | | 2.068 | 0.74 | 2.793 | DNA-binding | |
| 250208_at | At5g14000 | NAC domain protein | | | | | | | 1.496 | 0.537 | 2.785 | DNA-binding | Carpel |
| 250491_at | At5g09780 | Contains B3 DNA-binding domain (PFAM PF02362) | | | | | | | 1.326 | 0.488 | 2.713 | DNA-binding | |
| 247084_at | At5g66300 | NAC domain protein | | | | | | | 0.823 | 0.306 | 2.69 | DNA-binding | |
| 257915_at | *At3g23130* | SUPERMAN (SUP) | | | | | | | 1.575 | 0.591 | 2.665 | DNA-binding | Carpel |
| 264497_at | At1g30840 | Putative purine permease | | | | | | | 3.086 | 1.169 | 2.639 | Unknown | Carpel |
| 248430_at | At5g51800 | Contains PFAM PF00069 Protein kinase domain | | | | | | | 1.372 | 0.526 | 2.607 | Protein kinase | |
| 262516_at | At1g17190 | Putative glutathione transferase | | | | | | | 1.473 | 0.566 | 2.601 | Enzyme | |
| 264066_at | At2g27880 | ARGONAUTE (AGO1)-like protein | | | | | | | 2.425 | 0.953 | 2.542 | RNA metabolism | Carpel |
| 260199_at | At1g67590 | Contains PFAM PF03763 Remorin, C-terminal region | | | | | | | 0.904 | 0.355 | 2.54 | DNA-binding | |
| 252076_at | At3g51660 | LS1-like protein; contains PFAM PF01187 Macrophage migration inhibitory factor | | | | | | | 0.797 | 0.316 | 2.518 | Unknown | |
| 266756_at | At2g46950 | Putative cytochrome P450 | | | | | | | 1.074 | 0.428 | 2.508 | Enzyme | Stamen |
| 258589_at | At3g04290 | Contains Pfam profile: lipase/acylhydrolase with GDSL-like motif | | | | | | | 3.513 | 1.426 | 2.463 | Enzyme | |
| 265625_at | At2g27240 | Unknown protein | | | | | | | 0.998 | 0.411 | 2.428 | Unknown | |
| 251017_at | At5g02760 | Protein phosphatase 2C homolog | | | | | | | 1.052 | 0.434 | 2.42 | Protein phosphatase | |
| 262196_at | At1g77870 | Contains PFAM PF00240 Ubiquitin family | | | | | | | 1.103 | 0.462 | 2.384 | Ubiquitin | |
| 263306_at | At2g12480 | Putative serine carboxypeptidase II | | | | | | | 0.881 | 0.37 | 2.377 | Protease | Carpel |
| 253769_at | At4g28560 | LRR protein kinase | | | | | | | 1.134 | 0.477 | 2.375 | Protein kinase | |
| 245571_at | At4g14695 | Light induced protein like | | | | | | | 1.726 | 0.73 | 2.362 | Unknown | |

| | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 258299_at | At3g23410 | Alcohol oxidase (FAO3) | | | | | | | 0.173 | 0.073 | 2.361 | Enzyme | |
| 262121_at | At1g02800 | Similar to endo-1,4-beta-glucanase precursor | | | | | | | 2.254 | 0.961 | 2.345 | Enzyme | |
| 245076_at | At2g23170 | Auxin-regulated protein GH3 homologue | | | | | | | 1.733 | 0.742 | 2.334 | Unknown | |
| 249860_at | At5g22860 | Prolylcarboxypeptidase-like protein | | | | | | | 1.213 | 0.52 | 2.331 | Protease | |
| 262369_at | At1g73010 | Putative acid phosphatase | | | | | | | 1.817 | 0.779 | 2.331 | Enzyme | |
| 262236_at | At1g48330 | Unknown protein | | | | | | | 1.904 | 0.819 | 2.325 | Unknown | |
| 266965_at | At2g39510 | Nodulin-like protein | | | | | | | 1.613 | 0.697 | 2.314 | Unknown | |
| 251394_at | At3g60900 | Similar to endosperm specific protein SC3, Zea mays, PIR:T04348 | | | | | | | 2.211 | 0.96 | 2.303 | Unknown | |
| 259966_at | At1g76500 | Contains AT-hook DNA-binding domain | | | | | | | 1.279 | 0.555 | 2.301 | DNA-binding | |
| 252363_at | At3g48460 | Lipase-like protein | | | | | | | 1.004 | 0.437 | 2.298 | Enzyme | |
| 265025_at | At1g24575 | Unknown protein | | | | | | | 1.953 | 0.85 | 2.297 | Unknown | |
| 249788_at | At5g24330 | Contains PFAM PF00856 SET domain, PF00628 PHD-finger, PF00097 Zinc finger, C3HC4 type (RING finger) | | | | | | | 0.491 | 0.214 | 2.295 | DNA-binding | |
| 246531_at | *At5g15800* | SEPALLATA1 (SEP1) | | | | | | | 1.273 | 0.56 | 2.272 | DNA-binding | |
| 256175_at | At1g51670 | Unknown protein | | | | | | | 1.387 | 0.618 | 2.241 | Unknown | |
| 262388_at | At1g49320 | Unknown protein | | | | | | | 1.043 | 0.467 | 2.229 | Unknown | |
| 245560_at | At4g15480 | Similar to indole-3-acetate beta-glucosyltransferase | | | | | | | 2.624 | 1.191 | 2.202 | Enzyme | Stamen |
| 248467_at | At5g50800 | MtN3-like protein | | | | | | | 0.953 | 0.433 | 2.202 | Unknown | Stamen |
| 251181_at | At3g62820 | Pectinesterase homologue | | | | | | | 1.104 | 0.503 | 2.196 | Enzyme | |
| 254670_at | At4g18390 | Teosinte branched1-like protein | | | | | | | 1.854 | 0.847 | 2.187 | DNA-binding | |
| 259124_at | *At3g02310* | SEPALLATA2 (SEP2) | | | | | | | 1.137 | 0.523 | 2.172 | DNA-binding | |
| 258167_at | At3g21560 | Similar to UDP-glucose:indole-3-acetate beta-D-glucosyltransferase | | | | | | | 3.824 | 1.774 | 2.154 | Enzyme | |
| 259301_at | At3g05110 | Unknown protein | | | | | | | 1.369 | 0.644 | 2.124 | Unknown | |
| 267410_at | At2g34920 | Contains PFAM PF00097 Zinc finger, C3HC4 type (RING finger) | | | | | | | 1.729 | 0.825 | 2.094 | Unknown | |
| 247915_at | At5g57570 | Unknown protein | | | | | | | 0.916 | 0.439 | 2.085 | Unknown | |
| 253408_at | At4g32950 | Contains PFAM PF00481 Protein phosphatase 2C | | | | | | | 1.916 | 0.918 | 2.085 | Protein phosphatase | |
| 266358_at | At2g32280 | Unknown protein | | | | | | | 1.459 | 0.706 | 2.067 | Unknown | |
| 250261_at | At5g13400 | Contains PFAM PF00854 POT family, peptide transporter | | | | | | | 1.129 | 0.552 | 2.044 | Transporter | |
| 248968_at | At5g45280 | Pectin acetylesterase | | | | | | | 1.21 | 0.593 | 2.038 | Enzyme | |
| 265066_at | At1g03870 | Contains PFAM PF02469 Fasciclin domain | | | | | | | 1.749 | 0.864 | 2.025 | Unknown | |

Numbers show average normalized intensity and fold increase, and are only presented when the difference was statistically significant (details in the manuscript).
Genes studied in detail in the manuscript are marked in boldface; genes previously shown to have a role in carpel or stamen development are italicized; genes reported to be expressed in carpels or stamens by Wellmer et al. (Wellmer et al., 2004) are underlined.

**Table S2. Genes downregulated 1, 3 and 7 days after AGGR activation**

| Affymetrix code | AGI code | Description | 1 day dex | 1 day mock | 1 day fold | 3 days dex | 3 days mock | 3 days fold | 7 days dex | 7 days mock | 7 days fold | Wellmer et al., 2004 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | | | |
| 246465_at | At5g17000 | Quinone oxidoreductase-like protein | 0.113 | 2.204 | 19.385 | | | | | | | |
| 254540_s_at | At4g19770 | Chitinase homologue | 0.065 | 0.928 | 14.143 | | | | | | | |
| 253952_at | At4g26840 | Ubiquitin-like protein SMT3 | 0.43 | 1.86 | 4.324 | 0.38 | 0.831 | 2.187 | | | | |
| 247717_at | At5g59320 | Lipid-transfer protein LPT3 | 0.606 | 1.92 | 3.167 | | | | | | | Carpel |
| 257599_at | At3g24830 | 60S ribosomal protein L13a like protein | 0.733 | 2.061 | 2.809 | | | | | | | |
| 266421_at | At2g38540 | Putative nonspecific lipid-transfer protein | 0.596 | 1.582 | 2.651 | | | | | | | |
| 252912_at | At4g39200 | Ribosomal protein S25 | 0.605 | 1.596 | 2.635 | | | | | | | |
| 254386_at | At4g21960 | Peroxidase prxr1 | 0.618 | 1.6 | 2.587 | | | | | | | |
| 258287_at | At3g15990 | Putative sulfate transporter (ATST1) | 0.67 | 1.615 | 2.408 | | | | | | | |
| 263691_at | At1g26880 | 60s ribosomal protein L34 | 0.781 | 1.823 | 2.333 | | | | | | | |
| 249581_at | At5g37600 | Glutamate-ammonia ligase | 0.484 | 1.107 | 2.283 | | | | | | | |
| 248062_at | At5g55450 | Lipid transfer protein, contains PFAM PF00234 (protease inhibitor) | 0.45 | 0.984 | 2.184 | | | | | | | |
| 262050_at | At1g80130 | Unknown protein | 0.809 | 1.756 | 2.168 | | | | | | | |
| 266709_at | At2g03120 | Unknown protein | 0.781 | 1.626 | 2.081 | | | | | | | |
| 261408_s_at | At1g07820 | Histone H4 | 0.846 | 1.744 | 2.06 | | | | | | | |
| 263821_s_at | At2g09990 | 40S ribosomal protein S16 | 0.696 | 1.413 | 2.029 | | | | | | | |
| 255347_at | At4g03810 | Putative retrotransposon protein | | | | 0.013 | 0.205 | 14.77 | | | | |
| 262679_at | At1g75830 | Probable antifungal protein | | | | 0.083 | 0.975 | 11.685 | | | | |
| 265211_at | At2g36640 | Late embryogenesis abundant protein (AtECP63) | | | | 0.049 | 0.453 | 9.217 | | | | |
| 251671_at | At3g57210 | Unknown protein | | | | 0.106 | 0.337 | 3.175 | | | | |
| 262883_at | At1g64780 | Putative ammonium transporter | | | | 0.421 | 1.026 | 2.435 | | | | |
| 257232_at | At3g16500 | Phytochrome-associated protein 1 (PAP1) -03.01 cell growth IAA7 like protein | | | | 0.695 | 1.598 | 2.296 | | | | |
| 251109_at | At5g01600 | Ferritin 1 precursor | | | | 0.824 | 1.764 | 2.14 | | | | |
| 260693_at | At1g32450 | Peptide transporter PTR2-B | | | | 0.783 | 1.654 | 2.112 | 0.384 | 1.427 | 3.713 | Stamen |
| 262033_at | At1g37140 | Homologue of terminal ear 1 (maize), RNA binding protein | | | | | | | 0.249 | 1.898 | 7.614 | |
| 259382_s_at | At3g16430 | Putative lectin | | | | | | | 0.295 | 1.57 | 5.313 | |
| 265153_at | *At1g30950* | UNUSUAL FLORAL ORGANS (UFO) | | | | | | | 0.334 | 1.382 | 4.129 | |
| 251653_at | At3g57130 | Homologue of NPR1, contains PFAM PF00023 (Ankyrin repeat) and PF00651 (BTB/POZ domain) | | | | | | | 0.472 | 1.724 | 3.647 | |
| 256243_at | At3g12500 | Chitinase homologue | | | | | | | 0.361 | 1.273 | 3.527 | |
| 246137_at | At5g28490 | Unknown protein | | | | | | | 0.424 | 1.485 | 3.502 | |
| 245136_at | At2g45210 | Putative auxin-regulated protein | | | | | | | 0.053 | 0.186 | 3.464 | |
| 253732_at | At4g29140 | Contains PFAM PF01554 MatE (multidrug transporter) | | | | | | | 0.105 | 0.316 | 3.005 | |
| 256319_at | At1g35910 | Putative trehalose-6-phosphate phosphatase | | | | | | | 0.524 | 1.453 | 2.77 | |
| 263775_at | At2g46410 | CAPRICE (CPC) | | | | | | | 0.599 | 1.564 | 2.607 | |
| 259632_at | At1g56430 | Nicotianamine synthase | | | | | | | 0.633 | 1.601 | 2.528 | |
| 246642_s_at | At5g34920 | Putative transposon protein | | | | | | | 0.361 | 0.893 | 2.469 | |
| 257670_at | At3g20340 | Unknown protein | | | | | | | 0.833 | 2.036 | 2.443 | |
| 264054_at | At2g22550 | Unknown protein | | | | | | | 0.733 | 1.759 | 2.399 | |

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 254397_at | At4g21690 | Gibberellin 3 beta-hydroxylase - like protein | | | | | | | 0.46 | 1.097 | 2.386 | |
| 261991_at | At1g33700 | Unknown protein | | | | | | | 0.333 | 0.796 | 2.383 | Stamen |
| 261292_at | At1g36940 | Unknown protein | | | | | | | 1.548 | 3.682 | 2.377 | |
| 265342_at | At2g18300 | Putative bHLH transcription factor | | | | | | | 0.024 | 0.057 | 2.356 | |
| 258492_at | At3g02390 | Unknown protein | | | | | | | 0.234 | 0.54 | 2.306 | |
| 264400_at | At1g61800 | Similar to glucose-6-phosphate/phosphate-translocator | | | | | | | 0.81 | 1.866 | 2.302 | |
| 249858_at | At5g23000 | AtMYB37 R2R3-MYB transcription factor. | | | | | | | 0.648 | 1.466 | 2.259 | |
| 260012_at | At1g67865 | Unknown protein | | | | | | | 0.632 | 1.423 | 2.25 | |
| 261266_at | At1g26770 | Expansin 10 | | | | | | | 1.064 | 2.365 | 2.222 | |
| 257186_at | At3g13130 | Unknown protein | | | | | | | 0.707 | 1.571 | 2.221 | |
| 258727_at | At3g11930 | Putative ethylene-responsive protein | | | | | | | 0.817 | 1.809 | 2.213 | Stamen |
| 246601_at | At1g31710 | Copper amine oxidase | | | | | | | 0.394 | 0.871 | 2.207 | |
| 263452_at | At2g22190 | Putative trehalose-6-phosphate phosphatase | | | | | | | 0.567 | 1.217 | 2.145 | |
| 254024_at | At4g25780 | Putative pathogenesis-related protein | | | | | | | 1.082 | 2.31 | 2.134 | Stamen |
| 248163_at | At5g54510 | Auxin-regulated protein GH3 homolog | | | | | | | 0.978 | 2.076 | 2.122 | |
| 266108_at | At2g37900 | Putative peptide/amino acid transporter | | | | | | | 0.534 | 1.13 | 2.116 | |
| 267262_at | At2g22990 | Putative serine carboxypeptidase | | | | | | | 0.88 | 1.854 | 2.106 | |
| 244992_s_at | rps7.1_chloroplast | | | | | | | | 0.191 | 0.397 | 2.075 | |
| 266139_at | At2g28085 | Contains PFAM PF02519 (auxin responsive protein) | | | | | | | 0.36 | 0.745 | 2.069 | |
| 256759_at | At3g25640 | Unknown protein | | | | | | | 2.436 | 5.039 | 2.068 | |
| 252327_at | At3g48740 | MTN3-like protein | | | | | | | 0.92 | 1.902 | 2.067 | |
| 258114_at | At3g14660 | Putative cytochrome P450 | | | | | | | 0.506 | 1.032 | 2.038 | |
| 245385_at | At4g14020 | Unknown protein | | | | | | | 0.711 | 1.449 | 2.036 | |
| 255521_at | At4g02280 | Putative sucrose synthetase | | | | | | | 0.518 | 1.054 | 2.032 | Stamen |
| 260783_at | At1g06160 | Contains AP2 DNA-binding domain | | | | | | | 1.027 | 2.08 | 2.025 | |
| 249817_at | At5g23820 | Contains PFAM PF02221 (MD-2-related lipid recognition domain) | | | | | | | 0.774 | 1.567 | 2.023 | |
| 248111_at | At5g55330 | Wax synthase-like protein | | | | | | | 0.894 | 1.803 | 2.016 | |
| 263995_at | At2g22540 | MADS-domain protein similar to tomato JOINTLESS | | | | | | | 0.896 | 1.799 | 2.007 | |

Numbers show average normalized intensity and fold decrease, and are only presented when the difference was statistically significant (details in the manuscript).

Genes previously shown to have a role in carpel or stamen development are italicized; genes reported to be expressed in carpels or stamens by Wellmer et al. (Wellmer et al., 2004) are underlined, with the corresponding organs indicated.

**Table S3. Sequences of oligonucleotides used for RT-PCR and EMSA**

**A.** RT-PCR

| Name | Sequence (3′ to 5′) |
|---|---|
| CRC for | ATGAACCTAGAAGAGAAACCAACC |
| CRC rev | TCACTTCTTCTCACCGAATCCC |
| AP3 for | AACACCACAACGAAGGAGATCGT |
| AP3 rev | AAGGTAATGATGTCAGAGGCAGA |
| At3g11000 for | CACTCGGAGCAGGTTCGAGAT |
| At3g11000 rev | AGCCGAAGTATATCCCTTCTTG |
| GA for | CCAACATCACCTCAACTACTG |
| GA rev | CTCTTTCCATGTCACCGATTG |
| ATH1 for | AGGCGGGTTTCGGATCTACAT |
| ATH1 rev | TTATTTATGCATTGCTTGGCT |
| At3g17010 for | ATGGGTAAGAGTAGTAACATAGTT |
| At3g17010 rev | TCAATCGATCATGCACTGATC |
| SEP3 for | ATGGGAAGAGGGAGAGTAGAA |
| SEP3 rev | TGGTGTCATAAGGTAACCAACC |
| At4g21590 for | ATGGGTTGGTCGTTGAGAATG |
| At4g21590 rev | TCATGCTCTAGCAAGCTTCCG |
| At1g02190 for | CGCCACCATGAGTCCATCAAC |
| At1g02190 rev | TCATAGAGGAGATGGTGGGAG |
| AG for | GCTCTCCAGTTAGTGTAA |
| AG rev | TCTATAATAAAATCATTAATAGAGCAGG |
| At1g47610 for | ATGCCTCGATCCGACCTAACC |
| At1g47610 rev | TTAATGACGTGGTACCCTCCA |
| At1g13400 for | GAGGGTTTAGAGAGAAGCAAAC |
| At1g13400 rev | TTATAGCCCATGATGTGGAGG |
| APT for | TCCCAGAATCGCTAAGATTGCC |
| APT rev | CCTTTCCCTTAAGCTCTG |

**B.** EMSA

| Probe number (Fig. 5A) | Probe name | Complementary oligonucleotide sequences (5′ to 3′) |
|---|---|---|
| m | AP3 mut | TAAGTGATAAATTTTAAATTTATGTAAACTG |
| | | TACAACAATCAGTTTACATAAATTTAAAAT |
| 1 | CRC | TTATTAGCTCTCTTCCCTTTTTTGGCAATC |
| | | GAGATGGGACGATTGCCAAAAAAGGGAAGA |
| 2 | AP3 | TAAGTGATAAATTTTCCATTTATGTAAACTG |
| | | TACAACAATCAGTTTACATAAATGGAAAAT |
| 3 | At3g11000 | AGTTTTCTTTGGTTTCTTAATTAGGAACTG |
| | | ACGTTAACAACAGTTCCTAATTAAGAAACC |
| 4 | GA4CArG1 | ATTAATTTGCAAATACCTTTTTTGTTAATT |
| | | AGTACCTACAAATTAACAAAAAAGGTATTT |
| 5 | GA4CArG2 | ATTGGATTCCAAACACCATATTAGATTGTA |
| | | CCTAGTTACCTACAATCTAATATGGTGTTT |
| 6 | ATH1 | CATCTCATCAATTCTCCTTAAATGGCTAAA |
| | | TGAGATCTTTTTTAGCCATTTAAGGAGAAT |
| 7 | At3g17010 | AATGAAAAGAAGCATACATATTTGGATACT |
| | | GGCAAAAGTGAGTATCCAAATATGTATGCT |
| 8 | SEP3 | AGTATAATATAGATGGCTAAATAGGTAAAC |
| | | ACGTAATTTTGTTTACCTATTTAGCCATCT |
| 9 | AG intron2 | GAAATTTAATTATATTCCAAATAAGGAAAGT |
| | | AACGTTCCATACTTTCCTTATTTGGAATAT |
| 10 | At1g47610 | AGCACTTCTTGCTTAACAAAATTGGTTATT |
| | | TGATTAAGTAAATAACCAATTTTGTTAAGC |
| 11 | At1g13400 | ATGTTTAGATATTTGCCTTTTTTGGTACAA |
| | | CCGCCTTCCTTTGTACCAAAAAAGGCAAAT |