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Transcriptional programs regulated by both LEAFY and APETALA1 at the time of flower formation

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Two key regulators of the switch to flower formation and of flower patterning in *Arabidopsis* are the plant-specific helix-turn-helix transcription factor LEAFY (LFY) and the MADS box transcription factor APETALA1 (AP1). The interactions between these two transcriptional regulators are complex. AP1 is both a direct target of LFY and can act in parallel with LFY. Available genetic and molecular evidence suggests that LFY and AP1 together orchestrate the switch to flower formation and early events during flower morphogenesis by altering transcriptional programs. However, very little is known about target genes regulated by both transcription factors. Here, we performed a meta-analysis of public datasets to identify genes that are likely to be regulated by both LFY and AP1. Our analyses uncovered known and novel direct LFY and AP1 targets with a role in the control of onset of flower formation. It also identified additional families of proteins and regulatory pathways that may be under transcriptional control by both transcription factors. In particular, several of these genes are linked to response to hormones, to transport and to development. Finally, we show that the gibberellin catabolism enzyme ELA1, which was recently shown to be important for the timing of the switch to flower formation, is positively feedback-regulated by AP1. Our study contributes to the elucidation of the regulatory network that leads to formation of a vital plant organ system, the flower.

Introduction

Optimal timing of the developmental phase transitions that culminate in the formation of flowers is critical for reproductive success, especially in monocarpic or annual plants. If monocarpic plants form flowers too late, they may not complete their lifecycle in the growing season (Roux et al. 2006, Anderson et al. 2011). If they form flowers too early, the accumulated resources cannot support formation of a large number of seeds, leading to poor competition in natural populations (Lunn et al. 2014). In many monocarpic plants including *Arabidopsis thaliana*, the switch to flower formation is biphasic (Hempel et al. 1997, 1998, Prusinkiewicz et al. 2007, Liu et al. 2013, Yamaguchi et al. 2014a). This is

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Abbreviations – APETALA1, AP1; *ARF*, *AUXIN RESPONSE FACTOR*; *ARR9*, cytokinin-induced type-A authentic response regulator 9; ELA1, EUI-LIKE P450 A1; *ENP*, *ENHANCER OF PINOID*; FT, FLOWERING LOCUS T; GA, gibberellin; GO, gene ontology; JA, jasmonic acid; LEAFY, LFY; *LMI*, *LATE MERISTEM IDENTITY 1*; *LSH*, *LIGHT-DEPENDENT SHORT HYPOCOTYLS*; *OPT*, oligopeptide transporter; *PHOT*, *PHOTOTROPIN1*; qRT-PCR, quantitative reverse transcriptase polymerase chain reaction; *SEP3*, *SEPALLATA3*; SPL, SQUAMOSA PROMOTER BINDING-LIKE; UFO, UNUSUAL FLORAL ORGAN; *ZFP3*, *ZINC FINGER PROTEIN3*.

due to the fact that the first few new lateral primordia formed on the inflorescence after cessation of vegetative development are not competent to adopt a floral fate (Hempel et al. 1997). They instead give rise to branches subtended by cauline leaves. The degree of branching is important for optimal pollination and yield (Evers et al. 2011, Iwata et al. 2012). Primordia that form subsequent to the branch-producing prefloral inflorescence phase give rise to flowers. Once floral competence is acquired, there is no reversion to earlier primordial identities in *Arabidopsis* (Tooke et al. 2005, Wang et al. 2007, Melzer et al. 2008, Wagner and Meyerowitz 2011, Muller-Xing et al. 2014).

Many extrinsic and intrinsic cues direct cessation of vegetative development in *Arabidopsis*, which is also referred to as bolting. These cues include prolonged cold/vernalization, ambient temperature and photoperiod, age sensing, the autonomous pathway, carbohydrate signaling and hormonal cues such as gibberellin (GA) (Fornara et al. 2010, Pose et al. 2012, Song et al. 2013a, Wahl et al. 2013, Romera-Branchat et al. 2014, Verhage et al. 2014). Sensing of the stimuli occurs both in leaves and at the shoot apex. Their perception ultimately leads to accumulation of factors that promote the transition from vegetative development to formation of an inflorescence; these include the transcriptional co-regulator FLOWERING LOCUS T (FT) and the SQUAMOSA PROMOTER BINDING-LIKE (SPL) family of transcription factors (Cardon et al. 1997, Kardailsky et al. 1999, Corbesier et al. 2007, Jaeger and Wigge 2007, Schwarz et al. 2008, Wang et al. 2009, Bergonzi et al. 2013, Poethig 2013). The subsequent switch to flower formation is in large part controlled by the plant-specific transcription factor LEAFY (LFY) in *Arabidopsis* (Schultz and Haughn 1991, Weigel et al. 1992, Wagner et al. 1999). Recently, it was shown that the SPL transcription factor SPL9 and GA-sensitive DELLA proteins promote onset of flower formation synergistically with LFY (Yamaguchi et al. 2014a). Because GA levels increase prior to flower formation (Eriksson et al. 2006), the accumulation of DELLA proteins depends on GA catabolism. The reduction in GA levels is achieved at least in part by LFY directly inducing expression of the GA catabolism enzyme EUI-LIKE P450 A1 (ELA1) (Zhang et al. 2011, Yamaguchi et al. 2014a). The combined activity of LFY, DELLA and SPL proteins leads to strong upregulation of the MADS box transcription factor APETALA1 (AP1).

AP1 is a direct target of LFY (Parcy et al. 1998, Wagner et al. 1999). Unlike LFY, which is expressed prior to formation of the first flower in cauline leaf primordia that form during the prefloral inflorescence phase, *AP1* expression is restricted to primordia that have committed to a floral fate (Mandel et al. 1992, Weigel et al. 1992). Consistent with this, photoinduction experiments revealed a significant temporal delay between *LFY* and *AP1* upregulation (Hempel et al. 1997). The regulatory interactions that lead from LFY to *AP1* induction are complex but increasingly well understood (Wagner et al. 1999, Saddic et al. 2006, Pastore et al. 2011, Yamaguchi et al. 2014a). Loss of LFY activity or AP1 activity prolongs the prefloral inflorescence phase (more cauline leaves and branches form), with more severe phenotypic defects observed in the *lfy* than *ap1* null mutants (Weigel et al. 1992, Bowman et al. 1993). In addition, *lfy ap1* double mutant plants display a very dramatic delay in the onset of flower formation, far exceeding that observed in *lfy* null mutants (Huala and Sussex 1992, Weigel et al. 1992). This finding has been attributed to the fact that *AP1* can be induced independent of LFY, albeit later in development. The transcriptional co-activator FT plays a role in this process (Ruiz-Garcia et al. 1997, Abe et al. 2005, Wigge et al. 2005). Thus, AP1 acts not only downstream of LFY, but also in parallel with LFY. LFY and AP1 are co-expressed in young flower primordia from stage 1 onward (Mandel et al. 1992, Weigel et al. 1992). In addition to promoting onset of flower formation, LFY and AP1 also jointly regulate early events during flower morphogenesis including flower patterning (Bowman et al. 1993, Weigel and Meyerowitz 1993, Liu et al. 2009, Wu et al. 2012). However, thus far, only a handful of common LFY and AP1 target genes has been identified.

Here, we systematically identified shared LFY and AP1 targets with the aim to better understand the joint activities of the two transcription factors during early flower development. We identified several gene families and new pathways as likely directly regulated by both LFY and AP1. In addition, we show that the GA catabolism enzyme gene *ELA1* is positively feedback-regulated by AP1.

Materials and methods

Identification of common target genes of LFY and AP1

Four datasets were used to identify genes bound by both LFY and AP1 (Kaufmann et al. 2010, Moyroud et al. 2011, Winter et al. 2011, Pajoro et al. 2014). The specific genotypes, developmental stages, number of peaks and number of genes identified in each of these are described in Table S1. Peak locations and assigned genes for the four datasets can be found in Tables S2 and S3.

LFY-bound genes were identified from the Moyroud et al. (2011) and Winter et al. (2011) datasets. ChIP-chip data (seedlings and inflorescences) from

Winter et al. (2011) were remapped to the TAIR9/10 genome assembly by reanalyzing the CEL files (NCBI GSE28063) in CisGenome (version 2) (Ji et al. 2008) using the TAIR9 bpmap file. This resulted in 1725 seedling peaks and 1039 inflorescence peaks (False Discovery Rate (FDR) < 0.05). These were assigned to 1405 and 864 genes, respectively, using a custom Python script (Winter et al. 2011), modified to use TAIR10 annotations (as described in the TAIR10_GFF_genes.gff file, ftp://ftp.arabidopsis.org/). This script was used to assign all peaks from all LFY and AP1 datasets. The 1564 published LFY peak locations (FDR < 0.01) from Moyroud et al. (2011) were assigned to 1311 genes. The union of all LFY datasets resulted in 2460 LFY-bound genes.

AP1-bound genes were identified from the Kaufmann et al. (2010) and Pajoro et al. (2014) datasets. AP1 peak locations from Kaufmann et al. (2010) remapped to the TAIR9/10 genome assembly were downloaded from the PRI-CAT website ('AP1_Replicate1_admin_BindingSites.csv' and 'AP1_Replicate2_admin_BindingSites.csv'; http://www.ab.wur.nl/pricat/quickload/A_thaliana_Jun_2009/ChIP-seq/) (Muino et al. 2011). The 3536 (FDR < 0.01) AP1 peaks from replicate 1 and the 6392 (FDR < 0.01) peaks from replicate 2 were assigned to 2411 and 4437 genes, respectively (Table S3). A gene was classified as bound by AP1 in the Kaufmann et al. (2010) dataset if it was bound by AP1 in both replicates. The 498, 956 and 1075 (FDR < 0.001) published peak locations from the 2-, 4- and 8-day induction experiments from Pajoro et al. (2014) (see Table S1) were assigned to 338, 647 and 714 genes, respectively. The union of the genes identified in all AP1 datasets resulted in 2389 AP1-bound genes.

Common LFY and AP1 targets (769 genes) were taken as the intersection of the two lists of LFY- and AP1-bound genes.

Identification of high-confidence LFY and AP1 target genes

Expression data from five datasets were used to identify high-confidence LFY and AP1 target genes (Schmid et al. 2003, 2005, Kaufmann et al. 2010, Moyroud et al. 2011, Winter et al. 2011, Pajoro et al. 2014). Genotypes, numbers of differentially expressed genes and data sources are described in detail in Table S4.

Published lists of differentially expressed genes were used for 35S:LFY-GR (Winter et al. 2011), 35S:AP1-GR (Kaufmann et al. 2010) and AP1:AP1-GR (Pajoro et al. 2014) (see Table S4). For the Kaufmann data, we filtered for the most significant probe and platform (Operon 4200, Operon 4000, or Agilent, Waltham, Massachusetts, USA) for each time point for display in Table S5. Schmid et al. (2003, 2005) raw data were analyzed using Bioconductor in R. Data were gcrma normalized (Wu et al. 2004). Non-specific filtering was performed by including only those genes that were classified as 'Present', using the mas5.0 algorithm, in at least one of the arrays for a given comparison. Differentially expressed genes were identified using limma (Smyth 2004). The following comparisons were tested: d7 vs d14, d14 vs d21 and d7 vs d21 (Schmid et al. 2005), and d0 vs d3, d3 vs d5, d5 vs d7 and d0 vs d7 (Schmid et al. 2003, 2005).

The 769 LFY- and AP1-bound genes were successively filtered using these expression data to identify high-confidence LFY and AP1 target genes. (Tables S5 and S6)

GO term analysis

Enriched gene ontology (GO) terms were identified using ChipEnrich software (Orlando et al. 2009) (http://www.arexdb.org/software.jsp) using gene annotations downloaded from TAIR on August 28, 2014. The genes AT5G24910, AT4G23060 and AT3G55560 were manually added to the GO term 'response to GA stimulus'. *P*-values were adjusted for multiple testing using the Benjamini–Hochberg method (Benjamini and Hochberg 1995) implemented in R.

Plant growth and treatment

All plants were grown on soil at 23°C in a 16 h light/8 h dark cycle. The following plant lines were previously described: *lfy-1* null mutants (Weigel et al. 1992), *ap1-10* (Schultz and Haughn 1993), *ela1-1* (Yamaguchi et al. 2014a), *ap1 cal* 35S:LFY-GR (Winter et al. 2011) and *ap1 cal* 35S:AP1-GR (Wellmer et al. 2006). All mutants were in the Columbia background, while all inducible lines were in the Landsberg *erecta* background.

To test *ELA1* induction upon dexamethasone activation of 35S:LFY-GR or 35S:AP1-GR, the synthetic steroid was dissolved in ethanol, and stored at −20°C. For mock treatment, 0.01% ethanol with 0.01% silwet L-77 was used. For steroid treatment, 0.01% ethanol with 0.01% silwet L-77 and 10 μ*M* dexamethasone was used. Soil-grown *ap1 cal* 35S:LFY-GR or *ap1 cal* 35S:AP1-GR inflorescences were treated just after bolting by spraying.

Expression analyses

Quantitative reverse transcriptase polymerase chain reaction (qRT-PCR) was performed as previously described (Yamaguchi et al. 2009). Briefly, RNA was isolated from plants using TRIzol reagent (Invitrogen, Waltham, Massachusetts, USA) and purified

using RNeasy Mini kits (Qiagen, Fitchburg, Wisconsin, USA). cDNA was synthesized using a Superscript III kit (Invitrogen).The resulting cDNA was quantified by Power SYBR Green PCR Master Mix and 7100 Real-time PCR System (Applied Biosystems, Waltham, Massachusetts, USA). Mean and SE of at least three technical replicates is shown. *EIF4* was used as a loading control. Primers used were as follows: *ELA1* primers: TCCGCGATGAAGTCTTTCTT and TTGTGTCCTCAAGGGCTTCT; *CAL* primers: CATTTCAACACCCCCATCTT and GCCGTTTGGTCTT CTTCTTG; *LATE MERISTEM IDENTITY 2* (*LMI2*) primers: TTCAGGAATCTCGCTCCATT and CGCCACA GTAACCTCTTTCC; *EIF4* primers: AAACTCAAT-GAAGTACTTGAGGGACA and TCTCAAAACCATAAG-CATAAATACCC.

In situ hybridization was performed as previously described (Wu and Wagner 2012). The *ELA1* probe consisted of base pairs 112–1512 (Transcription start site (TSS) = 1). The probe was cloned into the pGEM-T Easy vector (Promega, Fitchburg, Wisconsin, USA). Antisense *ELA1* probe was digested with *Nco*I and transcribed with the T7 polymerase using the Riboprobe Combination System (Promega) and DIG RNA labeling mix (Roche, Penzberg, Upper Bavaria, Germany).

Results

To better understand how LFY and AP1 together regulate early events in flower development, we computationally identified common direct targets of both transcription factors after reanalysis of published transcriptome and ChIP-seq or ChIP-chip datasets (Kaufmann et al. 2010, Winter et al. 2011, Moyroud et al. 2011, Pajoro et al. 2014) (Tables S1–S6, Fig. S1). We first identified genes whose regulatory regions were bound by both LFY and AP1. This analysis identified 769 genes as bound by both transcription factors, a highly significant enrichment (P-value $< 10^{-15}$, χ^2 test; Figs 1A and S1). In total, 31% of all LFY targets and 32% of all AP1 targets were bound by the other transcription factor (Fig. 1A). Moreover, the genomic regions occupied by LFY and AP1 were in close proximity to one another in their common target genes (Fig. 1B). More than 37% the AP1-binding sites were <200 bp away from an LFY-binding site. To discern among the 769 LFY- and AP1-bound genes those that are likely to be regulated by LFY or AP1, we next filtered for significant differential expression (FDR < 0.05) after LFY-GR or AP1-GR activation using the available public transcriptome datasets (Kaufmann et al. 2010, Winter et al. 2011, Pajoro et al. 2014) (see section Materials and methods for details). A total of 33% (249 of the 769 LFY- and AP1-bound genes) were differentially

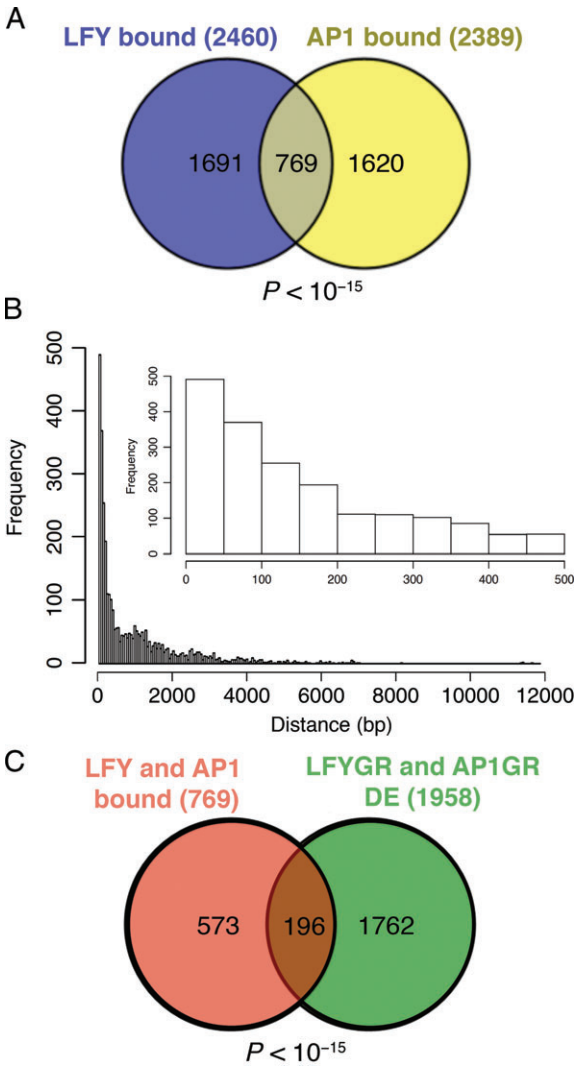

Fig. 1. Identification of direct LFY and AP1 target genes. (A) Genes directly bound by either LFY (Moyroud et al. 2011, Winter et al. 2011) or AP1 (Kaufmann et al. 2010, Pajoro et al. 2014) or by both transcription factors. While LFY and AP1 have unique target genes, there is a significant subset of genes (769 genes; P-value $< 10^{-15}$, χ^2 test) whose regulatory regions are bound by both LFY and AP1. (B) Distance between AP1 peak summits and the nearest LFY-binding peak summit in the genes bound by both transcription factors. Inset: Close-up showing AP1-binding sites 500 bp or less away from LFY-binding sites. (C) Of the 769 genes bound by LFY and AP1, a significant subset (196 genes; P-value $< 10^{-15}$, χ^2 test) was differentially expressed (FDR < 0.01) in both LFY-GR and AP1-GR plants on the basis of public transcriptome data (Kaufmann et al. 2010, Winter et al. 2011, Pajoro et al. 2014).

expressed upon LFY-GR activation (one developmental stage, 4 h activation; see Table S4). A total of 65% of the LFY- and AP1-bound genes (497 of the 769) were differentially expressed in at least one of the AP1-GR datasets (one developmental stage assayed, different treatment durations) (2 h to 8 days; see Table S4).

Of the 769 genes bound by LFY and AP1, a total of 196 (26%) were differentially expressed after steroid activation of LFY-GR and of AP1-GR (P-value $< 10^{-15}$, χ^2 test; Fig. 1C, Table S5). To examine the likely functions of the 196 putative LFY and AP1 target genes, we tested for GO term enrichment among them using ChipEnrich (Orlando et al. 2009). Significantly enriched (FDR < 0.001) GO terms included 'regulation of transcription', 'flower development' and 'stamen development', as expected (Fig. 2). In addition, the GO terms 'response to GA', 'response to auxin', 'response to jasmonic acid (JA)' and 'response to ethylene' were enriched. 'Auxin transport' and 'oligopeptide transport' were also among the enriched GO terms, as was 'phosphorylation' and 'polarity specification'. Auxin and GA have been implicated in the switch to flower formation in *Arabidopsis* (Yamaguchi et al. 2013, 2014a). Besides the previously described GA catabolism gene *EUI-LIKE P450 A1* (*ELA1*) (Magome et al. 2013, Zhang et al. 2011, Yamaguchi et al. 2014a), another GA catabolism enzyme, *GA2OX2* (Sun 2008), was linked to the 'response to GA' GO term. The genes linked to 'response to auxin stimulus' included regulators of the transcriptional response to auxin such as *AUXIN RESPONSE FACTOR6* (*ARF6*) and *IAA18* (Chapman and Estelle 2009), while *ENHANCER OF PINOID* (*ENP*) (Cheng et al. 2008, Furutani et al. 2014) and the *ABCB1* and *ABCB19* transporters (Titapiwatanakun et al. 2009, Cecchetti et al. 2015) were among the genes linked to the 'auxin transport' GO term. Ethylene and JA have been implicated in floral organ development and abscission (Kim 2014). Among the genes assigned to these GO terms were the transcriptional regulator *JASMONATE ZIM-DOMAIN2* (*JAZ2*) (Chini et al. 2007) and *ALLENE OXIDE CYCLASE3* (*AOC3*), which encodes an enzyme in the JA biosynthesis pathway (Gfeller et al. 2010). The GO term 'regulation of polarity' contained *PINHEAD/ZWILLE* (*PNH*) and *ASYMMETRIC LEAVES1* (*AS1*), while *TEOSINTE-LIKE1*, *CYCLOIDEA* and *PROLIFERATING CELL FACTOR1* transcription factors (*TCP5*, *TCP10*) and signaling components *CORYNE* (*CRN*) and *ERECTA* (*ER*) were linked to the 'leaf development' GO term.

We next asked how many of the 196 candidate meristem identity/flower development genes were also significantly differentially expressed (FDR < 0.05) during the switch from vegetative to reproductive development using a publicly available shoot apex expression dataset that measured changes in gene expression between day 7, day 14 and day 21 of development (Schmid et al. 2005). Of the 196 genes, 168 genes were differentially expressed in at least one of the conditions tested (d7 vs d14, d14 vs d21 and d7 vs d21). Flower formation can also be triggered in *Arabidopsis* by shifting plants from non-inductive to inductive (long-day) photoperiods (Hempel et al. 1997, Schmid et al. 2003). To narrow down the list to high-confidence direct LFY and AP1 target genes, we next asked which of the 168 direct LFY and AP1 targets were also regulated by switch from non-inductive to inductive photoperiod (FDR < 0.05) using a public expression dataset (Schmid et al. 2003). Of the 168 genes, 104 genes were differentially expressed in at least one of the conditions tested (d0 vs d3, d3 vs d5, d5 vs d7 and d0 vs d7). Figure 3 shows a clustering heatmap of the expression changes observed for these 104 genes in all datasets. Two general trends are apparent. First, genes differentially expressed after steroid activation of LFY-GR co-cluster with those that change during development and during photoperiod. Second, the different datasets that probe gene expression changes upon AP1-GR activation clustered together. In addition, several groups of genes can be

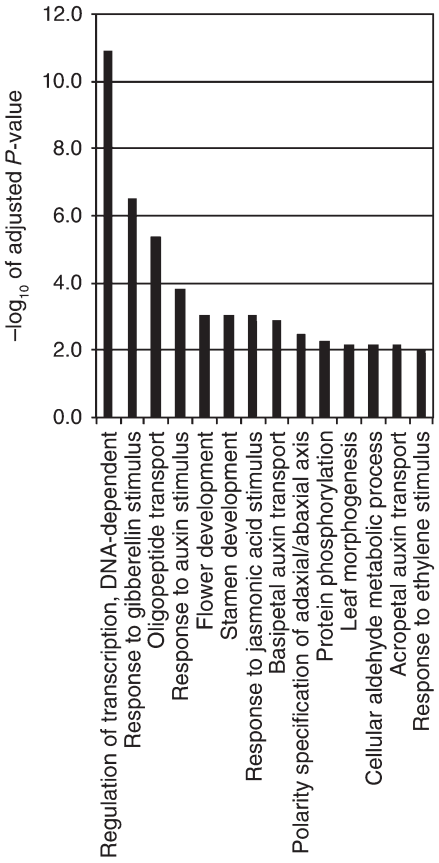

Fig. 2. GO term enrichment in direct AP1 and LFY targets with a likely role in the meristem identity transition GO terms enriched among the 196 candidate LFY and AP1 target genes identified in Fig. 1C. For a list of these genes, see Table S5. An FDR correction was performed and FDR cutoff of less than 0.001% was implemented. $-\log_{10}$ P-values of all significant GO terms are shown.

identified that show a coordinate regulation of gene expression (i.e. their expression increased in most conditions or it decreases in most conditions) (Fig. 3).

Consistent with this analysis, about one third of the 104 genes (34) changed in expression coordinately (FDR < 0.05) over all four conditions, with 21 genes upregulated and 13 genes downregulated significantly (FDR < 0.05) in at least one condition during development (d7 vs d14, d14 vs d21 or d7 vs d21), during photoperiod switch (d0 vs d3, d3 vs d5, d5 vs d7 or d0 vs d7) after activation of AP1-GR, and after activation of LFY-GR (see Table S5). To narrow the list of likely coordinately LFY- and AP1-regulated direct targets down further, we asked which of the 34 genes show an expression fold change of 1.5 or greater in all conditions. This identified a total of 11 genes as AP1- and LFY-regulated targets (Fig. 3 arrows, see Table S5). Five of these were coordinately upregulated by LFY and AP1. Among the upregulated genes are the known direct LFY and AP1 targets *SEPALLATA3* (*SEP3*) and *LFY*, as expected (Kaufmann et al. 2010, Winter et al. 2011, Wu et al. 2012). SEP3 acts together with LFY during flower patterning (Liu et al. 2009, Kaufmann et al. 2010, Winter et al. 2011, Wu et al. 2012). *LFY* autoregulates and is positively feedback-regulated by AP1 (Liljegren et al. 1999, Kaufmann et al. 2010, Winter et al. 2011). In addition, a gene encoding a cytochrome P450 enzyme *CYP714A1/EUI-LIKE P450 A1* (*ELA1*), a GA 2 oxidase (*GA2OX2*) and an F-box protein were among the upregulated LFY and AP1 targets. Both *GA2OX2* and *ELA1* have a role in GA catabolism (Zhu et al. 2006, Zhang et al. 2011, Nomura et al. 2013). The biological function of the F-box protein we identified (At4G33160) has not yet been elucidated. The UNUSUAL FLORAL ORGANS (UFO) F-box protein acts as an LFY co-factor for the activation of the class B floral homeotic genes (Levin and Meyerowitz 1995, Lee et al. 1997, Chae et al. 2008).

We next examined the expression of the five LFY and AP1 upregulated genes during development and photoinduction (Schmid et al. 2003, 2005) more closely. *SEP3*, *ELA1* and *LFY* were strongly upregulated during the onset of reproductive development, while the F-box protein shows weaker upregulation and *GA2OX2* appears to first decrease in levels (d7–d14) before it increases (d14–21) (Fig. 4A). In response to inductive photoperiod, all five genes are upregulated, with *LFY* and *ELA1* accumulation already increasing at day 5, while all other genes did not increase until day 7 (Fig. 4B). In the *lfy* mutant, neither LFY activity nor *AP1* RNA is present by day 7 (Fig. S2). This dataset hence provides an opportunity to assess whether upregulation by photoinduction requires these transcription factors. *SEP3*, *ELA1* and the F-box protein were not induced by photoperiod in the *lfy* mutant, suggesting that LFY and AP1 are important for their upregulation in response to inductive photoperiod (Figs 4B and S2). *LFY* expression is slightly upregulated in the *lfy* mutant, as expected. The SOC1 and AGL24 transcription factors are thought to mediate photoperiod induction of *LFY* (Lee et al. 2008, Liu et al. 2008). *GA2OX2* induction by long-day photoperiod was not at all impaired in the *lfy* mutant (Fig. 4B). Hence, other factors mediate photoperiod induction of *GA2OX2*. Finally, we examined LFY and AP1 binding to these five loci based on public ChIP-chip or ChIP-seq datasets (Kaufmann et al. 2010, Moyroud et al. 2011, Winter et al. 2011, Pajoro et al. 2014). LFY and AP1 bound to similar regions at these genes, in the 5′ intergenic region of the *SEP3*, *ELA1* and the F-box protein loci and to the second intron of the *LFY* and *GA2OX2* loci (Fig. 4C). In addition, sites uniquely bound by AP1 were present in *LFY* and *GA2OX2* and a unique LFY-bound site was present at the *LFY* locus (Fig. 4C).

Among the six genes coordinately downregulated by LFY and AP1 was a gene encoding a putative oligopeptide transporter (*OPT*), the cytokinin-induced type-A authentic response regulator *ARR9*, the class II TCP transcription factor *TCP5*, the blue light receptor *PHOTOTROPIN1* (*PHOT1*), a zinc finger transcription factor, *ZINC FINGER PROTEIN3* (*ZFP3*) and a protein of unknown function (At1g67050) (Joseph et al. 2014). The *OPT* gene identified here has been assigned the name *NFP4.3* for *NITRATE TRANSPORTER PEPTIDE TRANSPORTER FAMILY*, but the cargo of this transporter is unknown (Leran et al. 2014). *ARR9* and *TCP5* have both been linked to negative regulation of transcriptional response to cytokinin (Efroni et al. 2013, Schaller et al. 2015). *PHOT1* has recently been linked to inflorescence phototropism (Kagawa et al. 2009). *ZFP3* contains a single zinc finger protein and is expressed in flowers (Joseph et al. 2014). *ZFP3* has recently been shown to be a negative regulator of ABA and a positive regulator of red light response (Joseph et al. 2014).

During development *OPT*, *ARR9* and *TCP5* are strongly downregulated (Fig. 5A). *PHOT1* is slightly less dramatically downregulated, while repression of *ZFP3* is only seen at the last time point (d21), and that of At1g67050 does not change further after d14 (Fig. 5A). In response to inductive photoperiod, *OPT*, *ARR9*, *TCP5* and *PHOT1* expression decreased (Fig. 5B). By contrast, *ZFP3* expression first increased and then decreased, while that of At1g67050 decreased only late in the time course, between day 5 and day 7 (Fig. 5B). When we examined response to photoperiod in the *lfy* mutant, four genes showed elevated expression compared with the wild-type in at least one time point assayed; these were *OPT*, *ARR9*, *TCP5* and *ZFP3* (Fig. 5B).

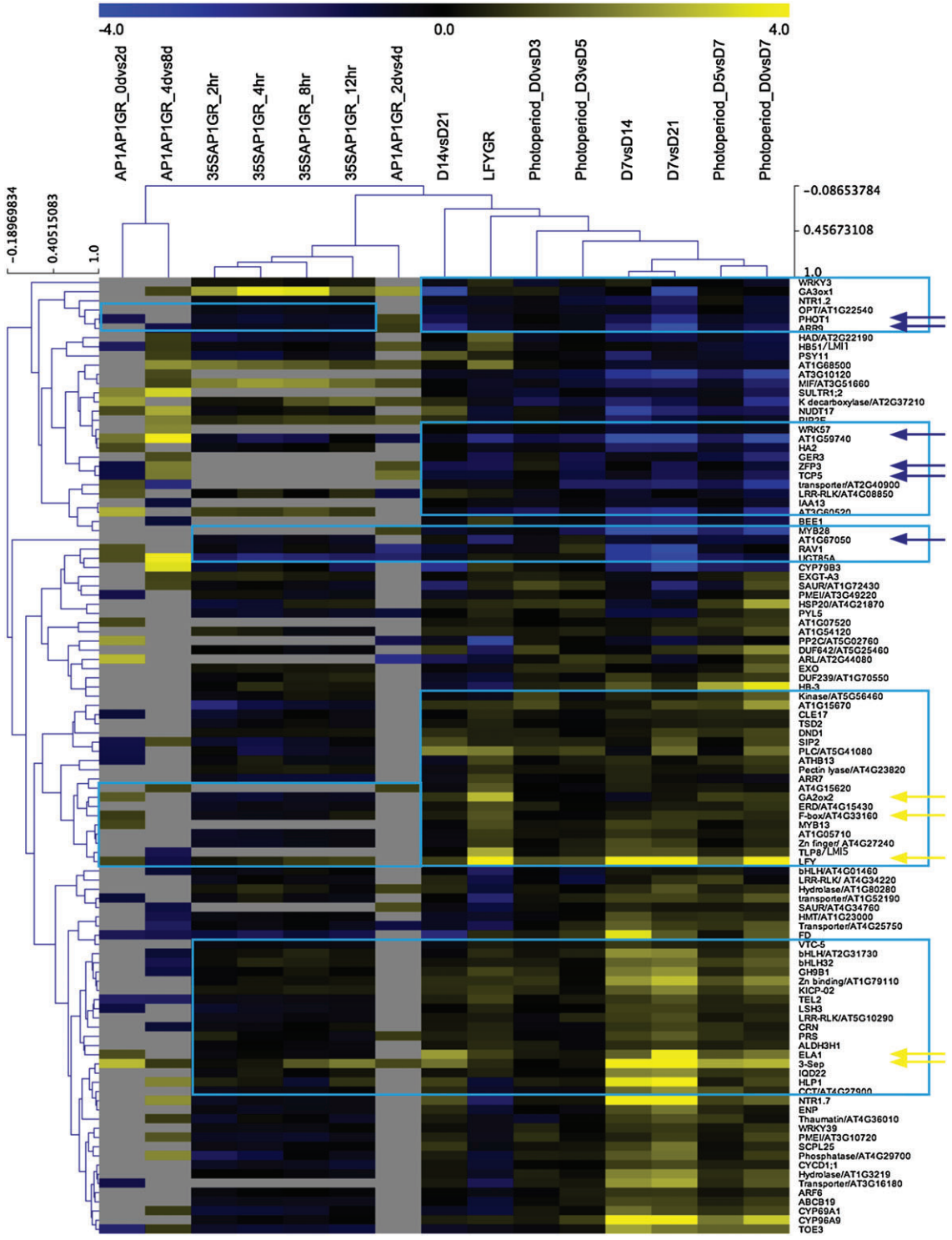

Fig. 3. Clustering of direct AP1 and LFY targets with a likely role in the meristem identity transition. (A) Hierarchical clustering of direct LFY- and AP1-regulated genes. Heatmap displays log2 fold changes of the 104 direct LFY and AP1 targets differentially expressed in LFY-GR, AP1-GR, in response to photoperiod induction and during the switch to flower formation (day 7, day 14 and day 21) based on public transcriptome datasets (Schmid et al. 2003, 2005, Kaufmann et al. 2010, Winter et al. 2011, Pajoro et al. 2014). See Table S4 for a detailed description of data sources. Blue boxes highlight genes coordinately regulated over multiple datasets. Arrows highlight high-confidence direct LFY- and AP1-regulated genes (see Figs 4 and 5 below). Blue: downregulation, Yellow: upregulation. Clustering was performed using TMEV freeware (http://www.tm4.org/) using default values.

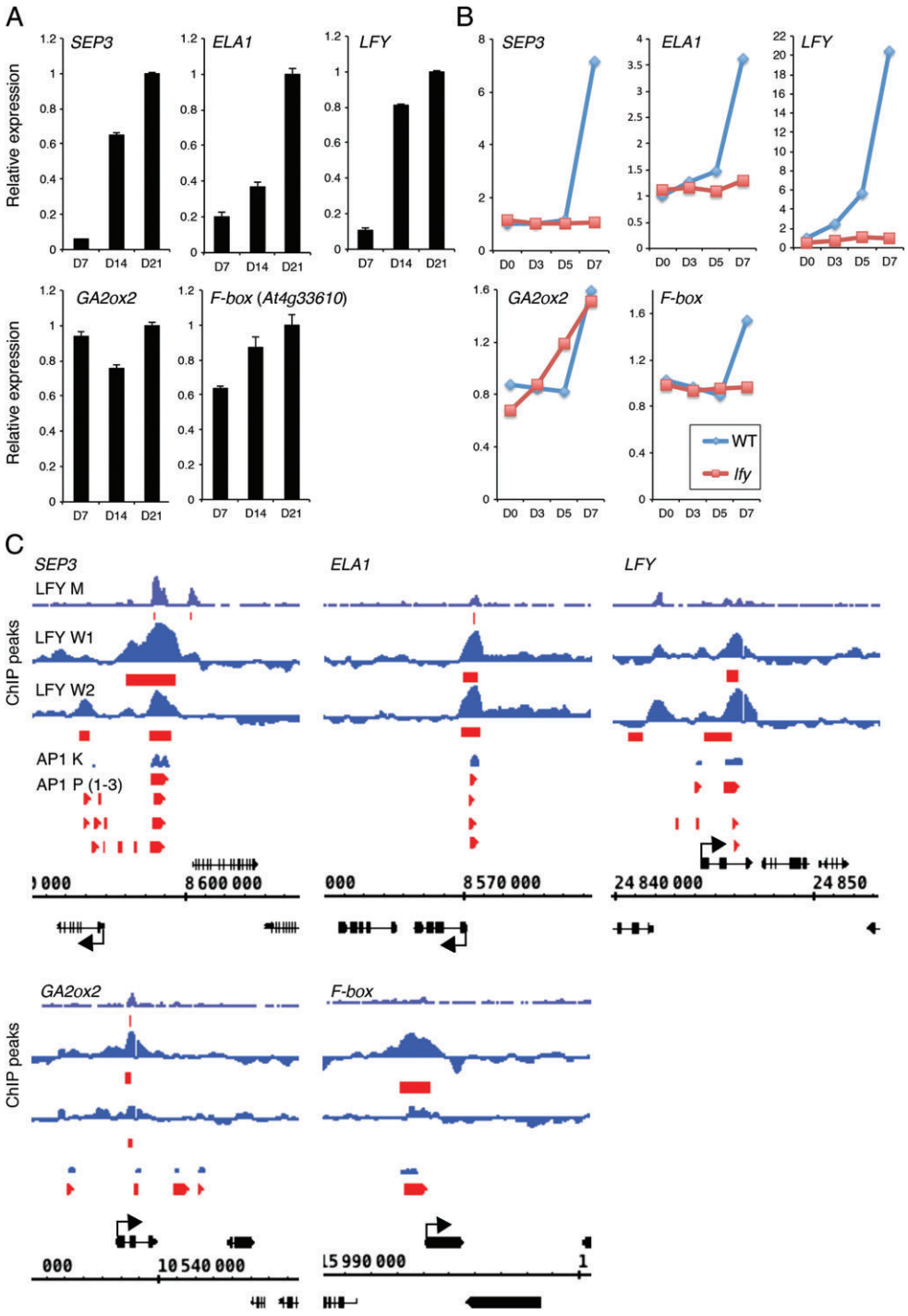

Fig. 4. High-confidence direct LFY and AP1 upregulated targets during the switch to flower formation. (A) Expression of high-confidence direct LFY and AP1 target genes at different times of development and in different genetic backgrounds based on a public transcriptome dataset (Schmid et al. 2005). For criteria used to identify these genes, see Table S6. (B) Induction of high-confidence direct LFY and AP1 target genes by long-day photoperiod in wild-type or *lfy* mutant inflorescences based on a public transcriptome dataset (Schmid et al. 2003). (C) LFY and AP1 binding peaks to the regulatory regions of high-confidence direct LFY and AP1 target genes on the basis of chromatin immunoprecipitation. Screenshots were taken from the Integrated Genome Browser (IGB; http://bioviz.org/igb/) using available data for LFY [LFY M (Moyroud et al. 2011); LFY W1, W2 (Winter et al. 2011)]. Data for AP1 [AP1 K (Kaufmann et al. 2010)] were obtained through IGB (Nicol et al. 2009) using the PRI-CAT data source (http://www.ab.wur.nl/pricat/tutorial.html). AP1 K: replicate 1 data are displayed. AP1 P (1–3) (Pajoro et al. 2014): only significantly bound regions are displayed.

The remaining two genes did not require LFY and AP1 activity for response to photoperiod (Fig. 5B). Finally, we examined the LFY- and AP1-bound sites at all six loci. Common LFY- and AP1-bound regions upstream of *ARR9*, *TCP5*, *PHOT1*, *ZFP3* and At1g67050 were identified (Fig. 5C). LFY bound to the 5′ intergenic region of *OPT*, while AP1 bound in the second intron and the 3′ intergenic region of this locus (Fig. 5C). Unique AP1- or LFY-bound sites were also present at four loci (*ARR9*, *TCP5*, *PHOT1* and *ZFP3*) (Fig. 5C).

We recently reported that LFY directly activates expression of the gene encoding the GA catabolism enzyme ELA1 in inflorescences, causing a reduction in the level of bioactive GA in this tissue (Yamaguchi et al. 2014a). The reduced GA levels in turn allow a stronger upregulation of the direct LFY target *AP1* and a more rapid switch to flower formation (Yamaguchi et al. 2014a). The current data suggest that AP1 also regulates *ELA1* expression, perhaps as part of a positive feedback mechanism. To further investigate a role for AP1 in this pathway, we performed *ELA1* in situ hybridization in wild-type and *ap1* mutant inflorescences. *ELA1* expression was much reduced in *ap1* mutant relative to wild-type inflorescences (Fig. 6A). qRT-PCR confirmed the reduction in *ELA1* expression in *ap1* mutant relative to wild-type inflorescences at the time of flower formation and revealed the defect in *ELA1* upregulation to be similar to that observed in *lfy-1* null mutants (Fig. 6B). In addition, when we activated 35S:AP1-GR with dexamethasone, we saw an increase in *ELA1* expression (Fig. 6C). The transcriptional upregulation of *ELA1* upon AP1-GR activation was slower that observed after LFY-GR activation (Fig. 6C). This finding as well as prior observations that place ELA1 upstream of *AP1* (Yamaguchi et al. 2014a) suggest that AP1 regulates *ELA1* via positive feedback (see section Discussion for more details).

That *ELA1* is a direct target of both LFY and AP1 further highlights the central role of this GA catabolism enzyme in the control of the onset of flower formation. We therefore hypothesized that reduced GA accumulation in the inflorescences may not only affect the accumulation of *AP1* but also perhaps affect the expression of additional regulators of the switch to flower formation. To test this hypothesis, we examined whether the *ela1-1* null mutant (Yamaguchi et al. 2014a) could enhance the delay in flower formation of a very strong/null *ap1* mutant, *ap1-10* (Schultz and Haughn 1993). It has previously been reported that neither loss of ELA1 function nor loss of AP1 function affect the cessation of vegetative development, consistent with the finding that neither gene is expressed during the transition from vegetative to prefloral inflorescence development (Mandel et al. 1992, Bowman et al. 1993, Yamaguchi et al. 2014a). Both AP1 and ELA1 promote cessation of the prefloral inflorescences phase (Mandel et al. 1992, Yamaguchi et al. 2014a) (Table 1). The duration of the prefloral, branch-forming, inflorescence phase can be estimated by counting the number of cauline leaves and secondary inflorescence branches formed. When we compared the number of cauline leaves and secondary inflorescences formed in *ela1-1 ap1-10* double mutants with those in the parental lines or in the wild-type, we found that *ela1-1 ap1-10* formed significantly more (P-value $< 3 \times 10^{-34}$, Student's t-test) secondary inflorescences (Table 1). The number of cauline leaves was not significantly increased (P-value $= 0.205$, Student's t-test). The data suggest that additional genes besides *AP1* depend on low GA levels for proper regulation of expression at this important juncture in the plant life cycle.

Discussion

Here, we identify a large cohort of genes (196) as likely directly regulated by LFY and AP1 during flower development. The filtering criteria we employed for identification of these direct LFY- and AP1-regulated targets uncovered several known LFY targets including *LMI1*, *LMI5*/*TLP8*, *RAX1*, *SEP3* and *ELA1* (William et al. 2004, Saddic et al. 2006, Chahtane et al. 2013, Yamaguchi et al. 2014a), thus validating our approach. Our findings suggest that LFY and AP1 jointly regulate many processes in early flower development.

To identify these LFY and AP1 targets, we did not analyze gene expression changes in constitutive *lfy* and *ap1* mutant compared with wild-type inflorescences (Schmid et al. 2005). The reason for this is that both *ap1* and *lfy* mutant plants have dramatically different tissue composition from the wild-type (Schultz and Haughn 1991, Weigel et al. 1992, Bowman et al. 1993). These differences in tissue composition can affect gene expression more than the loss of transcription factor activity. A case in point, *ap1* mutants form a large number of flower primordia relative to the wild-type and genes expressed in flower primordia, such as *LFY*, appear to exhibit increased expression when entire *ap1* inflorescences are assayed by transcriptomic or qRT-PCR analyses. It is well known that AP1 positively feedback-regulates *LFY* expression (Bowman et al. 1993, Schultz and Haughn 1993, Liljegren et al. 1999). We focus here on changes in gene expression triggered by dexamethasone activation of fusion proteins of LFY or AP1 to the glucocorticoid receptor (Wagner et al. 1999, Wellmer et al. 2006, Pajoro et al. 2014) to identify LFY- and AP1-regulated genes. For a discussion of this approach, see also Yamaguchi et al. (2015). The strength of this approach is

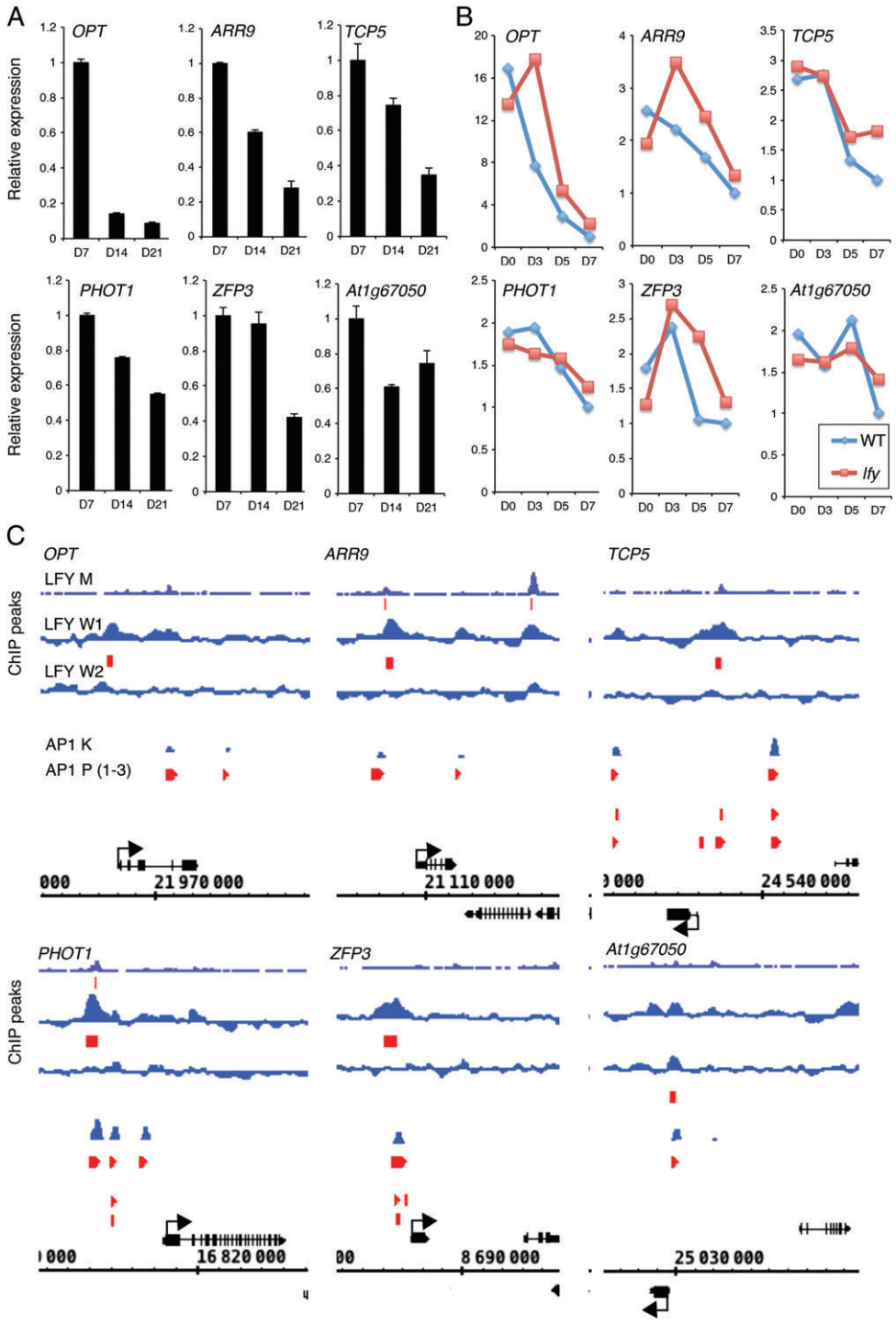

Fig. 5. High-confidence direct LFY and AP1 downregulated targets during the switch to flower formation. (A) Expression of high-confidence direct LFY and AP1 target genes at different times of development and in different genetic backgrounds based on a public transcriptome dataset (Schmid et al. 2005). For criteria used to identify these genes, see Table S6. (B) Induction of high-confidence direct LFY and AP1 target genes by long-day photoperiod in wild-type or *lfy* mutant inflorescences based on a public transcriptome dataset (Schmid et al. 2003). (C) LFY and AP1 binding peaks to the regulatory regions of high-confidence direct LFY and AP1 target genes on the basis of chromatin immunoprecipitation. Screenshots were taken from the Integrated Genome Browser (IGB) using available data for LFY [LFY M (Moyroud et al. 2011); LFY W1, W2 (Winter et al. 2011)]. Data for AP1 [AP1 K (Kaufmann et al. 2010)] were obtained through IGB (Nicol et al. 2009) using the PRI-CAT data source . AP1 K: replicate 1 data are displayed. AP1 P (1–3) (Pajoro et al. 2014): only significantly bound regions are displayed.

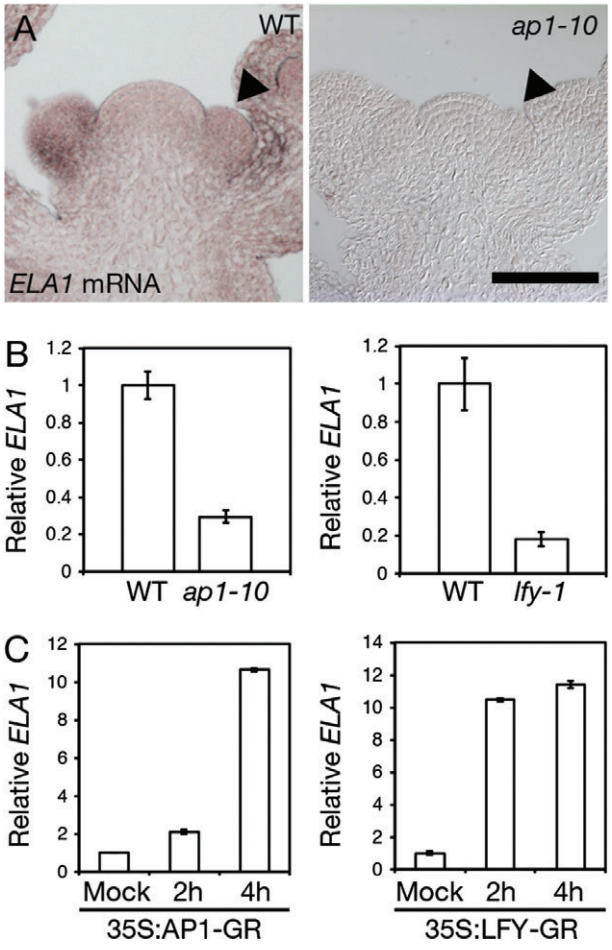

Fig. 6. *ELA1* expression is regulated by AP1. (A) In situ hybridization of *ELA1* in 30-day-old wild-type and *ap1-10* null mutant inflorescences. Arrows point to young flower primordia. (B) qRT-PCR-based expression of *ELA1* in wild-type (WT) compared with *lfy-1* null mutant or *ap1-10* null mutant inflorescences shortly after the switch to flower formation (day 15). (C) qRT-PCR-based expression of *ELA1* in 25-day-old *ap1 cal* 35S:LFY-GR or *ap1 cal* 35S:AP1-GR inflorescences 2 or 4 h after application of 10 μ*M* dexamethasone relative to mock-treated inflorescences.

(1) that it compares gene expression in morphologically identical plants and (2) that rapid changes in gene expression can be observed (Kaufmann et al. 2010, Winter et al. 2011). Its weakness lies in the different experimental set-ups used by different investigators. To address this issue and the fact that both LFY and AP1 might be required to activate some common targets effectively (Wagner and Meyerowitz 2011), future investigations should focus on simultaneous activation of both fusion proteins in the same genetic background.

The GO term enrichment analysis suggests a prominent role for both LFY and AP1 in hormone response, in particular response to GA, auxin and JA. GA plays multiple roles in the developmental phase transitions to flower formation. In *Arabidopsis*, it promotes cessation of vegetative development to allow onset of the prefloral inflorescence phase (Galvao et al. 2012, Osnato et al. 2012, Yu et al. 2012, Andres et al. 2014, Yamaguchi et al. 2014a). By contrast, GA represses the switch from the prefloral to the floral inflorescence phase (Yamaguchi et al. 2014a). Subsequent to this step, during flower development, GA levels increase again; this is required for the elaboration of several types of floral organs (Yu et al. 2004). The class C floral homeotic gene AGAMOUS may contribute to this increase in GA levels (Gomez-Mena et al. 2005). LFY and AP1 have both been directly linked to GA homeostasis (Kaufmann et al. 2010, Winter et al. 2011, Yamaguchi et al. 2014a). Among the 196 likely AP1- and LFY-regulated genes were six genes linked to GA biosynthesis (*GA2OX1*, *GA2OX2*, *GA2OX4*, *GA20OX1*, *GA3OX1* and *ELA1*), one to GA sensing (*GID1B*) and one to GA response (*IQD22*) (Sun 2008, Zou and Sun 2011, Daviere and Achard 2013, Magome et al. 2013, Yamaguchi et al. 2014a). LFY and AP1 directly upregulate *ELA1*; this causes a reduction in GA levels that enables the switch to flower formation [this study, Yamaguchi et al. (2014a)]. Future studies will reveal whether AP1 and LFY also control GA homeostasis in developing flower primordia.

A link from LFY and AP1 to auxin response and auxin transport is not surprising given the previously described roles for auxin in the switch to flower formation and in floral organ initiation (Okada et al. 1991, Bennett et al. 1995, Cheng et al. 2008, Krizek 2011, Yamaguchi et al. 2013, 2014b). In addition, recent investigations have linked LFY to regulation of polar auxin transport (Li et al. 2013, Yamaguchi et al. 2014b). Auxin and auxin response have also been implicated in the development of both male and female reproductive floral organs and of the ovules (Wu et al. 2006, Shi and Yang 2011, Reeves et al. 2012, Galbiati et al. 2013, Larsson et al. 2013, Song et al. 2013b, Cardarelli and Cecchetti 2014, Hawkins and Liu 2014). Finally, auxin promotes flower maturation, a role it executes at least in part together with JA (Nagpal et al. 2005, Tabata et al. 2010, Rubio-Somoza

Table 1. Meristem identity phenotypes of *ela1* mutants. Average number of cauline leaves and secondary inflorescences formed in the genotypes indicated. Mean ± SEM for one representative experiment is shown. All phenotypic experiments were performed multiple times and two-sided Student's *t*-tests were performed for each experiment. ***P-value $< 3 \times 10^{-34}$.

Genotype	Cauline leaves	Secondary inflorescences
Wild-type (Col)	2.8 ± 0.4	2.8 ± 0.4
ela1-1	4.6 ± 0.1	4.6 ± 0.1
ap1-10	3.4 ± 0.1	4.3 ± 0.1
ap1-10 ela1-1	4.9 ± 0.1	11.3 ± 0.2***

and Weigel 2013, Wang et al. 2013). AP1 has not as yet been directly linked to auxin response or transport. Three genes with roles in auxin transport (*ENP*, *ABCB1* and *ABCB19*) and seven genes with roles in auxin response (*ARF6*, *ARF11*, *IAA13*, *IAA17*, *ARGOS-LIKE*, *SAUR/AT1G72430* and *SAUR/AT4G34760*) were among the direct LFY- and AP1-regulated genes. It will be interesting to investigate whether additional auxin-dependent pathways are controlled by LFY and AP1 in young flower primordia. Such a study would be enabled by generating conditional *lfy ap1* double mutants at defined stages of flower development [see Wu et al. (2012) for an example of this approach]. Most of the roles described for JA occur late in *Arabidopsis* flower development, although earlier roles have been described in other plant species [see Ito et al. (2007), Acosta et al. (2009) and Cai et al. (2014) for examples].

Other new families of genes and pathways could be identified among the 196 genes possibly regulated by both LFY and AP1. For example, among the genes linked to onset of flower formation and flower development were known flowering repressors and activators (*TEMPRANILLO1*, *FLOWERING LOCUS D* and *NF-YB2*) (Abe et al. 2005, Wigge et al. 2005, Castillejo and Pelaz 2008, Cao et al. 2014). Likewise, three related genes, ALOG family transcription factors [*LIGHT-DEPENDENT SHORT HYPOCOTYLS* (*LSH*) *1*, *2* and *3*] were among the direct LFY and AP1 targets. The rice LSH-like protein TAWAWA1 (TAW1) and the tomato LSH-like protein TERMINATING FLOWER (TMF) shape the inflorescence architecture in each species, a role similar to that executed by LFY and AP1 in *Arabidopsis* (Liu et al. 2013, Yamaguchi et al. 2014a). In agreement with this idea, the regulator of axillary meristem initiation *RAX1* (Muller et al. 2006) was among the direct LFY and AP1 targets we identified.

Several of the new high-confidence direct LFY and AP1 targets we identified warrant further investigation, primarily those whose induction or repression by long-day photoperiod is altered when LFY and AP1 activity are absent. These include two upregulated genes: the F-box protein and *ELA1* (see below). The F-box protein is of interest, because LFY requires the F-box UFO as a co-factor during flower patterning in *Arabidopsis* (Levin and Meyerowitz 1995, Lee et al. 1997, Chae et al. 2008). In other plant species, in particular in the Solanaceae (tomato and petunia), LFY activity generally depends on F-Box proteins during additional stages and in additional pathways such as the switch to flower formation (Lippman et al. 2008, Souer et al. 2008). Besides the high-confidence target gene F-box (AT4G33160), the 196 LFY/AP1 targets contained another F-box protein (AT1G78100). It is possible that UFO is not required for all LFY activity in *Arabidopsis* because of the presence of other F-box proteins with overlapping function.

Among the downregulated LFY and AP1 direct high-confidence targets, three showed abnormal expression upon the shift to inductive photoperiod. OPT (At1g59740) is very strongly downregulated by photoperiod and is dependent on LFY and AP1 for this response. Three other transporters of this family were among the 196 LFY/AP1 targets: AT1g22540, AT1g52190 and At3g16180. The latter two have recently been implicated in nitrate transport (Hsu and Tsay 2013). It will be of interest to determine what peptides and other molecules are differentially transported in flowers or inflorescences. A second high-confidence direct LFY and AP1 downregulated gene that displays different response to photoperiod in the absence of LFY and AP1 activity is a negative regulator of cytokinin response, *ARR9* (Schaller et al. 2015). The significance of this is not understood. Recent data have suggested extensive crosstalk between cytokinin and auxin during initiation of flower primordia (Besnard et al. 2014a, 2014b, Schaller et al. 2015) and in gynoecium development (Reyes-Olalde et al. 2013, Zuniga-Mayo et al. 2014). Cytokinin may be required for AP1 function (Han et al. 2014). Finally, ZFP3 is a Zn finger nuclear factor with a role in ABA signaling (Joseph et al. 2014). Interestingly, several other genes with a link to this pathway were among the 196 direct LFY- and AP1-regulated genes, including components of the ABA receptor (PYL5, PYL6 and PP2C/At5G41080) (Cutler et al. 2010, Raghavendra et al. 2010). Whether there is a link from this stress hormone to flower initiation or flower patterning by LFY and AP1 remains to be elucidated.

Common LFY- and AP1-regulated targets can theoretically be grouped into distinct categories. A first category of shared LFY and AP1 targets are those that act downstream of both LFY and AP1 and are transcriptionally regulated by both. There is precedent for this type of interaction. For example, the gene encoding the MADS box transcription factor *SEP3* is directly induced by both LFY and AP1 (Liu et al. 2009, Kaufmann et al. 2010, Winter et al. 2011). This type of regulatory interaction, where one transcription factor activates another, and together they regulate a third gene, constitutes a feed-forward loop network motif (Alon 2007). Feed-forward loops are very common in developmental switches and help to buffer against noisy inputs (Alon 2007). Because LFY acts upstream of *AP1* (Weigel and Nilsson 1995, Hempel et al. 1997, Liljegren et al. 1999, Wagner et al. 1999), a second theoretical category of shared LFY and AP1 targets is comprised of genes induced by LFY that are feedback-regulated

by AP1, i.e. genes that act downstream of LFY, but upstream of AP1. Known examples of genes regulated in this manner are *LMI1* and *LMI2*. Both *LMI1* and *LMI2* are activated by LFY, act upstream of AP1 and are positively feedback-regulated by AP1 (William et al. 2004, Saddic et al. 2006, Kaufmann et al. 2010). Positive feedback loops are also common network motifs in developmental switches and are known to drive the network equilibrium toward the next step; in this case, commitment to floral fate. A third category of direct LFY and AP1 are those that act upstream of both LFY and AP1 and are feedback-regulated by both genes. Candidate direct LFY and AP1 targets in this category are *FLOWERING LOCUS D* (this study) (Kaufmann et al. 2010, Winter et al. 2011, Jaeger et al. 2013) and *TFL1*. *TFL1* is a direct target of LFY and of AP1 (Kaufmann et al. 2010, Moyroud et al. 2011, Winter et al. 2011), but was not identified here because of the stringent peak to gene assignment criteria used. Of note, we have focused here on genes coordinately regulated by both LFY and AP1 and 67% of the 196 direct LFY- and AP1-regulated targets behave in this manner (Table S6). In some cases (33% of the 196 targets), genes are regulated in the opposite manner by the two transcription factors (Table S6). For example, the opposite regulation could be due to genes activated by LFY that are subsequently or in different tissues repressed by AP1.

We do not have conclusive evidence for the newly identified LFY and AP1 targets acting downstream of LFY and AP1 or upstream and being feedback regulated. One indicator that can be used for a preliminary classification is the temporal upregulation of the identified genes relative to that of *LFY* or *AP1* upon photoperiod shift (Schmid et al. 2003) (see Figs 4 and 5). Of the high-confidence target genes that are dependent on LFY and AP1 for correct photoperiod response, four (*ELA1*, *OPT*, *ARR9* and *ZFP3*) respond prior to *AP1* during photoperiod shift, suggesting that these genes might be feedback-regulated by AP1. In addition, all of these genes except *ELA1* may also be feedback-regulated by LFY based on these criteria. By contrast, the F-box protein-encoding gene is not induced before *AP1* and hence (like *SEP3*) is likely a downstream target of both LFY and AP1.

In the case of the GA catabolism enzyme ELA1, prior and current data suggest that it is indeed feedback-regulated by AP1. ELA1 is an important direct target of LFY in the control of onset of flower formation (Yamaguchi et al. 2014a). Several pieces of evidence suggest that ELA1 acts upstream of *AP1*: in *ela1* mutants *AP1* upregulation is delayed, *AP1* expression is more strongly induced by LFY when GA levels are reduced and induction of *ELA1* precedes that of *AP1* not only upon photoperiod shift but also during development (Schmid et al. 2003, Yamaguchi et al. 2014a). In this study, we saw a more rapid upregulation of *ELA1* upon LFY-GR than upon AP1-GR activation. This finding suggests that AP1 may first need to upregulate a co-factor such as LFY before it can induce *ELA1* expression. Thus, both LFY and AP1 are required for *ELA1* upregulation with LFY acting upstream and AP1 downstream of ELA1. The positive feedback from AP1 to *ELA1* likely contributes to a more robust reduction in GA levels at the time when the first flower is formed. Together with the previously described positive feedback from AP1 to transcriptional regulators that act upstream of AP1 such as *LFY*, *LMI2* and *LMI1* (Saddic et al. 2006, Kaufmann et al. 2010, Pastore et al. 2011, Winter et al. 2011), this feedback would help tip the balance toward the next step in development (Ferrell 2012), here commitment to floral fate. Our data also fit well with the prior observation that AP1 is important for the plant's ability to respond to LFY, when LFY activity or other signals that promote formation of flowers are limiting (Liljegren et al. 1999, Wagner and Meyerowitz 2011).

Finally, we provide genetic evidence that the reduced GA level triggered by ELA1 accumulation is important for *AP1* accumulation. *ela1 ap1* double mutants displayed a more significant delay in the onset of flower formation than either of the parental lines. One possible explanation for this finding is that additional regulators of the switch to flower formation besides AP1 are dependent on reduced GA levels for proper expression in incipient primordia. Future studies are needed to uncover additional roles for low GA during the switch to flower formation.

Author contributions

C. M. W., N. Y. and D. W. conceived of this study; C. M. W. performed the computational analyses; N. Y. performed the phenotypic and qRT-PCR analyses; M.-F. W. performed the in situ hybridization and D. W. wrote the manuscript with input from C. M. W. and N. Y.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Identification of LFY- and AP1-bound genes. Individual LFY and AP1 ChIP-seq and ChIP-chip datasets

and sources, and the number of genes identified in each are listed below. Unless noted, published peak locations were assigned to genes using a custom PYTHON script (Winter et al. 2011), rewritten to use TAIR10 annotations. The total number of LFY-bound genes was taken as the union of the genes identified in the three LFY ChIP datasets. See Fig. S1A for a detailed overlap analysis of the three LFY datasets. The total number of AP1-bound genes was taken as the union of the four AP1 ChIP datasets. See Fig. S1B for a detailed overlap analysis of the four AP1 ChIP datasets. The 769 LFY- and AP1-bound genes are the intersects of the total LFY-bound genes and total AP1-bound genes (see Fig. 1A).

Table S2. LFY peaks and gene assignments.

Table S3. AP1 peaks and gene assignments.

Table S4. Summary of LFY-GR, AP1-GR, development and photoperiod transcriptome datasets used for the identification of high-confidence LFY- and AP1-bound genes.

Table S5. One hundred ninety six genes bound by LFY and AP1 and differentially expressed in LFY-GR and AP1-GR.

Table S6. Filtering criteria used to identify high-confidence LFY and AP1 targets. The steps below were applied to successively filter the list of 769 LFY- and AP1-bound genes (see Table S1) to obtain a list of 11 high-confidence LFY and AP1 targets. See also Table S5 for genes and expression data. See Table S4 for more information regarding the transcriptome datasets employed.

Fig. S1. Identification of LFY- and AP1-bound genes.

Fig. S2. Expression of *AP1* in *lfy* mutants during photoperiod induction.

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