Plants monitor seasonal cues to optimize reproductive success by tuning onset of reproduction and inflorescence architecture. TERMINAL FLOWER 1 (TFL1) and FLOWERING LOCUS T (FT) and their orthologs antagonistically regulate these life history traits, yet their mechanism of action, antagonism and targets remain poorly understood. Here, we show that TFL1 is recruited to thousands of loci by the bZIP transcription factor FD. We identify the master regulator of floral fate, LEAFY (LFY) as a target under dual opposite regulation by TFL1 and FT and uncover a pivotal role of FT in promoting flower fate via LFY upregulation. We provide evidence that the antagonism between FT and TFL1 relies on competition for chromatin-bound FD at shared target loci. Direct TFL1-FD regulated target genes identify this complex as a hub for repressing both master regulators of reproductive development and endogenous signalling pathways. Our data provide mechanistic insight into how TFL1-FD sculpt inflorescence architecture, a trait important for reproductive success, plant architecture and yield.
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differentiation and of the transition from branch to
ifloral fate in the inflorescence in response to seasonal cues3–5. For example, in plants that flower only once, like Arabidopsis and most crops, an early switch to flower formation allows rapid completion of the life-cycle and is beneficial in a short growing season3–5. At the same time early onset of flower formation reduces seed set and yield since flowers form in lieu of branches, which support production of more flowers per plant3–5. By contrast, delaying flower formation increases branching and total flower number, but prolongs time to seed set3–5.

Key regulators of seasonal control of onset of reproductive development and of the switch from branch to floral fate in primordia of the inflorescence are members of the phosphatidylethanolamine-binding protein (PEBP) family of proteins5,6,9,10. Among these, FT promotes onset of the reproductive phase and flower formation (determinacy), while TFL1 promotes vegetative development and branch fate (indeterminacy)9,11–13. Arabidopsis flowers in the spring and FT accumulates when the daylength exceeds a critical threshold, while TFL1 is present in both short-day and long-day conditions2,3,14.

FT and TFL1 are small mobile proteins, which have been implicated in transcriptional regulation but do not have DNA-binding domains14–18. Biochemical and genetic studies showed that FT physically interacts with the bZIP transcription factor FD via 14–3–3 proteins and similar interactions have recently been described for TFL119–22. Indeed, despite their antagonistic roles, TFL1 and FT are distinguished by only a small number of non-conservative amino acid changes11,12,25,24. FT can be converted into TFL1 and vice versa by a single amino acid substitution and such mutations have been selected for during crop domestication23–26. Accumulating evidence suggests that FT acts as a transcriptional co-activator, while TFL1 may either prevent FT activity or act as a co-repressor23,27. However, non-nuclear roles have also been described for both TFL1 and FT28,29.

A key unanswered question is how the florigen modulates plant form—what are the downstream processes they set in motion and what is molecular basis for their antagonism? Here we show that TFL1 is recruited to target loci by the bZIP transcription factor FD. We identify the master regulator of floral fate, LEAFY, as a target under dual opposite regulation by TFL1 and FT and uncover a prominent role for FT in LFY upregulation. We find that the antagonism between TFL1 and FT relies on competition for access to chromatin bound FD at the LFY locus and other shared targets. Finally, we identify hundreds of TFL1–FD regulated genes linking this complex not only to repression of master regulators of floral fate, but also to diverse endogenous signalling pathways. The combined data reveals how TFL1 and FT tune transcriptional programs that direct primordium fate in the inflorescence.

**Result**

TFL1 is recruited to thousands of loci by the bZIP transcription factor FD. Mechanistic insight into TFL1 activity has been hampered by low protein abundance. To overcome this limitation and to test the role of TFL1 in the nucleus, we first generated a biologically active, genomic GFP-tagged version of TFL1 (gTFL1-GFP tfl1-1) (Supplementary Fig. 1a–c) and identified a developmental stage and tissue where TFL1 accumulates. TFL1 protein strongly accumulated in branch meristems in the axils of cauline leaves in 42-day-old short-day grown plants just prior to the switch to flower formation (Fig. 1a). To conduct TFL1 chromatin immunoprecipitation followed by sequencing (ChIP-seq), we next isolated shoot apices at this stage for anti-GFP immunoprecipitation. Because TFL1 is present in very few cells and binds chromatin indirectly, we combined eight individual ChIP-seq reactions per replicate to enhance detection. We conducted FD ChIP-seq in analogous fashion using a published, biologically active, genomic fusion protein (gFD-GUS tfl1-1)20 (Supplementary Fig. 1d). This approach yielded high-quality ChIP-seq data in both cases (Supplementary Figs. 2 and 3a).

In total, we identified 3308 and 4422 significant TFL1 and FD peaks (MACS2 summit qval ≤ 10−10), respectively (Fig. 1b). The TFL1 peaks significantly overlapped with the FD peaks (72% overlap, p val < 10−300, hypergeometric test; Fig. 1b–d). De novo motif analysis of ChIP peak summits identified the G-box cis motif, a known FD-binding site30, as most significantly enriched (p val < 10−470) and frequently present (>84%) under TFL1 bound and TFL1/FD co-bound peaks (Fig. 1e and Supplementary Fig. 2). To test whether TFL1 chromatin occupancy is dependent on the presence of FD, we also performed TFL1 ChIP-seq in the fd-1 null mutant. TFL1 chromatin occupancy was strongly reduced in fd-1 (Fig. 1c, d). Our data point to a prominent nuclear role for TFL1 and show that FD recruits TFL1 to the chromatin of target loci.

Annotating FD and TFL1 peaks to genes identified 2699 joint TFL1 and FD targets. Gene Ontology (GO) term enrichment analysis implicates these targets in abiotic and endogenous stimulus response and reproductive development (Supplementary Table 1). TFL1 and FD peaks were present at loci that promote onset of the reproductive phase in response to inductive photoperiod2,3,31 like GIGANTEA (GI), CONSTANS (CO), and SUPPRESSOR OF CONSTANS 1 (SOC1) and at loci that promote floral fate31,32 such as LFY, APETALA1 (API), and FRUITFULL (FUL) (Fig. 1f). Identification of these TFL1 and FD co-bound targets fits with the known biological role of TFL1 as a suppressor of onset of reproduction and of flower fate and the proposed molecular function of TFL1 in opposing gene activation11–13,27.

**Leafy** is selected under dual opposite regulation by TFL1/FD and FT/FD. We selected the LEAFY (LFY) gene, which encodes a master regulator of flower fate33,34, to further probe the molecular mechanism of action of TFL1. While TFL1 promotes branch fate, LFY promotes flower fate in primordia (Supplementary Fig. 4a–f)33,33–35. Using independent biological replicates, we confirmed FD-mediated TFL1 binding to LFY by ChIP-qPCR (Supplementary Fig. 4g, h). To test whether LFY expression is rapidly repressed by the TFL1–FD complex, we generated transgenic plants expressing a steroid inducible version of TFL1 (TFL1ER; Supplementary Fig. 5). A single steroid treatment reduced LFY levels by 50% after 4 h (Supplementary Fig. 4i). The combined data suggest that the TFL1–FD complex directly represses LFY.

To better understand TFL1 recruitment to the LFY locus, we identified the genomic region sufficient and the cis motifs necessary for TFL1 association with the LFY locus. TFL1 and FD peak summits located to the second exon of LFY (Fig. 1f and Supplementary Fig. 4g, h) and LFY reporters that lacked the second exon were not repressed in response to TFL1 overexpression (Supplementary Fig. 6a, b). Exonic transcription factor-binding sites, although rare, are found in both animals and plants, and frequently link to developmental regulation36,37. To test whether LFY exon 2 (e2) alone is sufficient to recruit TFL1–FD, we transformed gTFL1-GFP tfl1-1 plants with a T-DNA containing only LFY e2. We detected strong TFL1 recruitment to the introduced copy of e2, using primer sets that specifically amplify the transgene borne exon (Fig. 2a). Next we identified three putative bZIP-binding sites in the second exon of LFY; These include an evolutionarily conserved G-box and two
motifs identified by de novo motif analysis under TFL1 or FD peak summits. Most significant motifs include the G-box (as for the narrowPeak file format in ENCODE). See also Supplementary Figs. 1-3 and Supplementary Data 1.

Having identified the cis motifs necessary for TFL1 recruitment to LFY, we next probed their contribution to spatiotemporal LFY accumulation. LFY reporters that contain e2 (pLFY2-GUS, Supplementary Fig. 6a) and a genomic LFY reporter (gLFY-GUS, Fig. 2b) recapitulated endogenous LFY expression (Fig. 2c, Supplementary Fig. 6d). This is the precise region where TFL1 protein accumulates during reproductive development (Fig. 2c)\(^{14}\). Indeed, LFY is known to be ectopically expressed in the inflorescence shoot apex of tfl1 mutants during reproductive development\(^{15}\). Thus, TFL1–FD binding to the bZIP motifs of e2 is required to prevent ectopic LFY accumulation in the centre of the shoot apex.

Surprisingly, the bZIP-binding site mutations in the second exon of LFY are instead required for TFL1 recruitment. The three bZIP-binding sites in the second exon of LFY are partially conserved C-boxes (Fig. 2b). When we transformed gTFL1-GFP tfl1-1 with a version of LFY e2 in which the three bZIP binding were mutated (e2m3), we were unable to detect TFL1 binding to the introduced copy of e2m3 (Fig. 2a). The three bZIP-binding sites in the second exon of LFY are almost completely conserved in TFL1 binding to the introduced copy of e2m3 (Fig. 2a). The three bZIP-binding sites in the second exon of LFY are required for TFL1 recruitment to e2 and e2m3 via FD in yeast (Supplementary Fig. 6c).

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FT (35S:FT-HAER) (Supplementary Fig. 9a–c). A single steroid treatment triggered significant LFY induction after 4 h (Supplementary Fig. 9d). After crossing FT-HAER to LFY reporters containing (pLFYi2:GUS) or lacking (pLFYi2m3:GUS) the bZIP-binding sites in e2, we tested reporter activity in response to steroid activation. GUS upregulation was similar to that of endogenous LFY when the bZIP-binding sites were present (Supplementary Fig. 9e). By contrast, GUS expression was not upregulated in the pLFYi2m3:GUS FT-HAER plants after steroid induction (Supplementary Fig. 9e). The combined loss-of-function, photoinduction and gain-of-function data indicate that FT-FD directly activates LFY expression via bZIP-binding sites in the second exon.

These findings prompted us to assess the biological importance of the LFY bZIP-binding sites for inflorescence architecture.

Fig. 2 Exonic bZIP cis motifs mediate TFL1 recruitment to LFY. a TFL1 recruitment to LFY exon 2 (e2) or a bZIP-binding site mutated version thereof (e2m3) in 42-day-old short-day-grown plants. Top: LFY exon 2 (e2, black rectangle) and T-DNA vector (grey line). Centre: location of amplicons P1 and P2, each consisting of one exon 2-specific and one vector-specific primer. Bottom: gTFL1-GFP ChIP-qPCR. Progeny pools of >50 random gTFL1-GFP T1 plants were analyzed. Shown are mean ± SEM of three independent biological experiments (black dots). P values unpaired one-tailed t-test: **P1 = 0.0018, **P2 = 0.0078; n.s. TA3 = 0.35. b Top: genomic LFY construct with GFP (gGLFY) or beta-glucuronidase (gLFY-GUS). Putative bZIP-binding motifs in LFY exon 2 are colour-coded based on conservation. For the pLFYi2:GUS reporter diagram see Supplementary Fig. 6a. Centre: mutation of the three bZIP-binding motifs without changing the primary amino acid sequence. Bottom: evolutionary conservation of the G-box (3rd bZIP). c Top: expression domain of LFY and TFL1 proteins in inflorescence apices with flower primordia. Centre and Bottom: accumulation of beta-glucuronidase in a wild-type or bZIP-binding site mutated (m3) reporter (pLFYi2:GUS) or genomic construct (gLFY-GUS). Plants were grown in long day. Staining was conducted under identical conditions. Arrowheads: flower primordia; asterisk: inflorescence shoot apex; Scale bars: 2 mm. See also Supplementary Figs. 4–6.
Indeed, it has been shown that the terminal members FT and the closely related TWIN SISTER OF FT (TSF). locus prevent access of both TFL1 and of activating PEBP family members FT and the closely related TWIN SISTER OF FT (TSF). This is expected since the bZIP mutations at the FT in LFY (Fig. 3c, d and Supplementary Fig. 10). In the gGLFYm3 only partial rescue (in 15 out of 25 independent transgenic lines) a construct which preserves LFY protein sequence but has mutated bZIP-binding sites (gGLFYm3) yielded transgenic lines), a construct which preserves LFY protein. While a genomic GFP-tagged LFY construct (gGLFY) fully rescued the lfy-1 null mutant (in 24 out of 25 independent transgenic lines), a construct which preserves LFY protein expression to promote floral fate. a Expression of FT and LFY in above-ground tissues of long-day-grown wild-type (WT) and pFT4kb:amiRF7 plants prior to onset of (day 7 and day 10) or during (day 16) reproductive development. b Effect of a single far-red enriched photoperiod (FRP) on LFY (left) and reporter (GUS, right) accumulation in 42-day-old short day (SD) grown plants. a, b Expression was normalized over UBIQ10. Shown are mean ± SEM of three independent biological experiments (black dots). Unpaired one-tailed t-test; p values: n.s. FT day 7 = 0.407; n.s. FT day 10 = 0.052, ***FT day 16 = 6E-05; n.s. LFY day 7 = 0.258, n.s. LFY day 10 = 0.07, **LFY day 16 = 0.0045 (a) ***LFY WT ± FRP = 0.0004, **gLFY-GUS ± FRP = 0.0001, n.s. gLFY-GUSm3 ± FRP = 0.217 (b). c Rescue of lfy-1 null mutants by genomic GFP-tagged LFY (gGLFY) or a bZIP-binding site mutated version thereof (gGLFYm3) in long-day-grown plants. Representative inflorescence images (top and side view). Arrowheads indicate branches formed on the main stem. For m3 mutations see Fig. 2a. d Phenotype quantification of 15 independent transgenic lines for gGLFY lfy and gGLFYm3 lfy. RL rosette leaves, CL cauline leaves, Br branches. Box plot-median (red line), upper and lower quartiles (box edges), and minima and maxima (whiskers). Letters: significantly different groups p value < 0.05 based on Kruskal-Wallis test with Dunn’s post hoc test. Scale bars, 1 cm. See also Supplementary Figs. 6–10.

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FT competes TFL1 from FD bound at shared target loci. Having identified LFY as a target under dual opposite regulation by TFL1 and FT, we next investigated the mechanism underlying the TFL1–FT antagonism at this locus. To test for possible competition between FT and TFL1 at the chromatin, we conducted anti-HA ChIP-qPCR in 42-day-old short-day grown FT-HA/ER gTFL1-GFP plants four hours after mock or steroid application. Estradiol induction led to rapid recruitment of FT-HA to the second exon of LFY, the region occupied by FD and TFL1 (compare Fig. 4a, b to Supplementary Fig. 4b). Anti-GFP ChIP-qPCR performed on the same sample uncovered a concomitant reduction in TFL1 occupancy (Fig. 4a, b). We next asked whether upregulation of endogenous FT also triggers reduced TFL1 occupancy at the LFY locus. Towards this end, we treated plants with a single FRP to upregulate FT (Supplementary Fig. 8b). The single FRP likewise significantly reduced TFL1 occupancy at the LFY chromatin (Fig. 4c). By contrast, photoinduction of FT did not alter FD occupancy (Fig. 4d). To probe whether FT is recruited to the second exon of LFY via the bZIP-binding sites, we transformed FT-HA/ER with either a wild-type version of e2 or a bZIP-binding site mutated version thereof (e2m3). After steroid induction, we used transgene-specific
Fig. 4 FT competes TFL1 from the chromatin. a LFY locus and primers used. b Top: FT-HAER occupancy at the LFY locus after 4-h mock (M) or steroid (T) treatment. Bottom: gTFL1 occupancy in the same sample. c, d Effect of FT upregulation by photoperiod (FRP, 24 h) on TFL1 (c) or FD (d) occupancy at the LFY locus. e FT-HAER recruitment to LFY exon 2 (e2) or a bZIP-binding site mutated version thereof (e2m3) after 4-hour steroid treatment. Top: LFY exon 2 (e2, black rectangle) and T-DNA vector (grey line). Centre: location of amplicons P1 and P2, each consisting of one exon 2-specific and one vector-specific primer. Below: FT-HAER ChIP-qPCR. Progeny pools of >50 random FT-HAER T1 plants transformed with e2 or e2m3 were analyzed (see also ref. 108). f Effect of photoperiod (FRP) on TFL1 occupancy at TFL1–FD target loci identified in Fig. 1f and LMI267 g FT-HAER occupancy with or without 4-h estradiol treatment at TFL1–FD bound regions of target loci shown in f. b–g ChIP was performed in 42-day-old short-day-grown plants. Shown are mean ± SEM of three independent biological experiments (black dots). P values (unpaired one-tailed t-test): b anti-HA ChIP ** region 4 = 0.009, region 5 = 0.003; anti-GFP ChIP *** region 4 = 6E−05, region 5 = 0.006; c * region 4 = 0.039, **; region 5 = 0.002; d n.s. LFY = 0.18, TA3 = 0.29. e **P1 = 0.004, P2 = 0.002, n.s. TA3 = 0.12; f * GI = 0.016, ***CO = 0.0007, **SOC1 = 0.0015, ***FUL = 0.0003, ***API = 0.0007, LMI2 = 0.0011, n.s. TA3 = 0.34. g **GI = 0.009, *CO = 0.011, **SOC1 = 0.003, *FUL = 0.015, ***API = 0.0001, **LMI2 = 0.008, n.s TA3 = 0.17. h Model for antagonistic roles of TFL1 (purple circles) and FT (red circles) in promoting branch fate or floral fate, respectively. Increased FT accumulation leads to competition of TFL1 from bZIP transcription factor FD bound to chromatin and to onset of flower formation. FD dimers (orange ovals), 14-3-3 proteins (black disks).
primers to monitor FT binding to the two versions of *LFY* e2 by ChiP-qPCR. As described above for TFL1 (Fig. 2a), FT was recruited to *LFY* e2 alone (Fig. 4e). In addition, FT recruitment to the introduced copy of *LFY* e2 was abolished when the three bZIP-binding sites were mutated (Fig. 4e).

Our combined data suggest that FT competes TFL1 from FD bound at exonic bZIP motifs at the *LFY* locus. The loss of TFL1 from the *LFY* locus via competition by FT is further supported by the finding that neither steroid nor FRP induction of FT reduced TFL1 mRNA accumulation (Supplementary Figs. 8c and 9d). Competition of TFL1 from FD by FT is not limited to the *LFY* locus. We tested whether FT induction by FRP competes TFL1 from the other direct TFL1–FD target loci we identified (Fig. 1f). FRP treatment reduced TFL1 occupancy at all loci tested (Fig. 4f). To confirm that FT indeed occupies the TFL1–FD bound sites at these loci, we also conducted ChiP-qPCR in FT-HA ER after steroid induction. In the estradiol treated samples, we saw significant FT recruitment to the TFL1–FD bound regions at all loci tested (Fig. 4g). We conclude that the antagonism between FT and TFL1 relies on competition for FD bound at the chromatin of shared target loci (Fig. 4h).

**Direct TFL1–FD repressed genes promote onset of flower formation and endogenous signalling.** Our findings place florigens directly upstream of *LFY*, yet prior genetic data suggest that florigens act both upstream of and in parallel with *LFY*39,40. To gain insight into additional gene expression programs repressed by the TFL1–FD complex, we next conducted RNA-seq with and without FRP treatment. We isolated inflorescences with associated primordia from 42-day-old short-day-grown *ft* mutant, wild-type and *ftfl* mutant plants and identified the significant gene expression changes in each genotype relative to untreated siblings. On the basis of Principle Component Analysis (PCA) and replicate analysis, RNA-seq quality was high (Supplementary Fig. 11). We next defined genes directly repressed by TFL1–FD. Towards this end, we focussed on TFL1–FD complex bound loci that exhibit FT-dependent de-repression upon photoinduction. Six-hundred four TFL1–FD bound genes were significantly (DESeq2 adjusted \( p < 0.005 \)) de-repressed upon FRP treatment in the wild-type or in *ftfl* mutants but not in *ft* mutants (Fig. 5a). GO term enrichment linked the TFL1–FD repressed genes to reproductive development and to response to endogenous and abiotic signals (Fig. 5b).

K-means clustering of the 604 genes identified three main patterns of gene expression. Genes encoding promoters of floral fate ( *LFY*, *AP1*, *FUL* and *LM12*) clustered together (cluster III in Fig. 5c) and displayed stronger upregulation in *ftfl* mutants than in the wild type. This pattern of de-repression was confirmed for all four loci using independent biological samples and qRT-PCR (Supplementary Fig. 12a). *SOC1* clusters with these genes, but was not included in further analyses because it was weakly, but significantly, de-repressed in *ft* mutants (Fig. 5c, Supplementary Data 1 and Supplementary Fig. 12b). By contrast, *CO* and *GI*, which promote cessation of vegetative development24, were more strongly upregulated in the wild-type than in *ftfl* mutants (cluster I in Fig. 5c), perhaps because these genes are already partially de-repressed in *ftfl* mutants in the absence of FRP treatment. Indeed, like RNA-seq, qRT-PCR of independent biological replicates revealed higher accumulation of *GI* and *CO* in untreated *ftfl* mutant compared to wild-type plants (Fig. 5c, Supplementary Data 1 and Supplementary Fig. 12b). Cluster II genes are only upregulated in the wild type and may represent genes that are transiently de-repressed. Our data identify the TFL1–FD complex as a hub for repression of key regulators of the onset of reproductive development and of the switch to flower fate (Fig. 5c).

Consistent with the GO-term enrichment analysis (Fig. 5b), combined ChiP-seq and RNA-seq analysis additionally identified components of endogenous stimulus response. We identified genes linked to sugar signalling (trehalose-6-phosphate) and hormonal signalling and response (abscisic acid, cytokinin, brassinosteroid, auxin and strigolactone) as direct TFL1–FD complex repressed targets (Fig. 5c). Using qRT-PCR and independent biological samples, we confirmed FT-dependent de-repression of members of these pathways by FRP photoinduction (Fig. 5c). Several of the identified pathways link to repression of branching or to promotion of onset of flower formation in the inflorescence. For example, we identified four trehalose-6-phosphate phosphatases (TPPH, TPP, TPPG and TPPE) as direct TFL1–FD complex repressed targets; TPPs were recently shown to repress branching in the maize inflorescence51. In addition, auxin and the auxin-activated transcription factor *MONOPTEROS* (MP) were direct TFL1–FD complex repressed targets (Fig. 5c). MP promotes the switch to floral fate in *Arabidopsis*52 and the tomato ortholog of TFL1 executes its role in inflorescence architecture at least in part by modulating auxin flux and response33. Finally, we identified key components of the brassinosteroid pathway including the *BRI1* receptor and the bHLH transcription factor *BMI1*54,55 as direct TFL1–FD complex repressed targets. Brassinosteroid signalling represses inflorescence branching in *Setaria*56. The combined data implicate TFL1–FD in direct repression of genes that promote floral fate or repress branch fate, consistent with the role of TFL1 in promoting branch formation.

We also identified the cytokinin activating enzyme *LOG5*57, abscisic acid biosynthesis (*ABA1*) and response regulators (*ABI5*, *ABF4*, *APETALA2*38,59, and components of strigolactone signalling, *SMXL6* and *SMXL8*)60,61, as direct TFL1–FD complex repressed targets.

To gain further insight into the role of the TFL1–FD complex in hormone signalling, we next assessed indirect, downstream, gene expression changes triggered by FRP treatment. In particular, we identified genes not bound by TFL1 or *FT* that were significantly differentially expressed (DESeq2 adjusted \( p < 0.005 \)) in the wild type and in *ftfl*, but not in *ft*. The identified indirect targets provide a ‘molecular phenotype’ that is consistent with de-repression of the auxin, brassinosteroid and cytokinin hormone pathways upon FRP treatment in the wild-type and in *ftfl* mutants (Fig. 5c). By contrast, the abscisic acid signalling pathway signature was more complex (Fig. 5c). Our combined data uncover a prominent role for the TFL1–FD complex in regulation of endogenous signalling.

It is conceivable that components of some of the identified TFL1–FD dependant pathways (strigolactone, cytokinin, auxin, abscisic acid as well as sugar signalling) may modulate additional aspects of the inflorescence architecture, such as branch outgrowth62–64. Support for this hypothesis comes from our phenotypic analyses. We examined the effect of a single FRP on inflorescence architecture in the *ft* mutant, the wild-type and the *ftfl* mutant. Photoperiod induction triggered a reduction in the number of branches, but not cauline leaves, formed in the wild type and more strongly, in *ftfl* (Supplementary Fig. 13a–j). This suggests that branch meristems adopt floral fate upon stimulus perception65. *ftfl* mutants also formed fewer branches than the wild type in the absence of photoperiod. These phenotypes are consistent with the observed gene expression changes (Fig. 5c). In addition, FRP triggered a significant increase in inflorescence branch outgrowth in both wild-type and *ftfl* plants (Supplementary Fig. 13k). FRP had no phenotypic effect in *ft* mutants. Our combined data suggest that florigens tune plant form to the environment by controlling expression of master developmental regulators and endogenous signalling pathway components. These developmental changes likely require large-scale transcriptional reprogramming in the context of chromatin. Consistently, we identified transcriptional co-
regulators and chromatin regulators among the direct TFL1–FD repressed targets (Supplementary Fig. 14a, b).

Discussion

Here we identify LFY, a master regulator of flower fate\textsuperscript{33,34}, as a target under dual opposite transcriptional regulation by TFL1 and FD and demonstrate that FT activation of LFY expression is critical to promote floral fate. We provide a molecular framework for the antagonistic roles\textsuperscript{27} of FT and TFL1 that relies on competition for bZIP transcription factor mediated access to binding sites at regulatory regions of shared target loci. Additional support for this mechanism comes from recent in vitro studies\textsuperscript{21}.

Our data suggest that TFL1 may not simply prevent access of the FT co-activator to the chromatin\textsuperscript{23} but may be an active repressor, as mutating bZIP-binding sites results in LFY de-repression specifically in the TFL1 expression domain. The identity of the transcription factors that activate LFY in the centre of the inflorescence shoot apex in the absence of PEBP/FD binding to LFY is not known. Our identification of FT recruiting motifs in the second exon of LFY fits with prior data demonstrating that the 2.3 kb upstream intergenic ‘LFY promoter’ is unresponsive to FT\textsuperscript{38}. This upstream regulatory region drives reporter expression in similar domains as endogenous LFY\textsuperscript{66}. The requirement of the bZIP motifs for LFY upregulation in the context of the genomic construct, which contains the 2.3 kb ‘LFY
promoter’, suggests the presence of repressive regulatory elements in the genomic region of LFY. We identify hundreds of TFI1–FD repressed genes many of which, based on our computational analyses of recently published FD and TFI1 ChiP-seq datasets, are also immediate early genes, which, based on our computational analyses of recently published and previously described datasets, are also immediate early genes. One example is DIA2, which is repressed by TFI1 in at least one of the long-day ChIP-seq datasets (Supplementary Fig. 3b). The 604 direct TFI1–FD repressed genes include key regulators of onset of the reproductive phase and of floral fate. Of note, TFI1 opposes not only LFY, but also LFY targets, such as LMI2 and API76–80. This is consistent with prior genetic investigations that place TFI1 both upstream of LFY and as a modulator of plant response to LFY81. Finally, we link the TFI1–FD complex to repression of divergent endogenous signalling pathways including sugar and hormonal signals. Several of these pathways have been shown to impact the switch from branch or flower fate in other plant species81–85. The combined data point to an important role of the hormonal environment for the switch from branch to flower fate in primordia of the inflorescence. Our findings also set the stage for elucidating co-activities as well as differences between flavirugene regulated cell fate reprogramming during flower initiation and other developmental pathways under seasonal control by florigen such as tuberization, bulbing formation and seed dormancy59–72.

Changes in the relative balance of activating and repressive PEBP family members occurred during domestication of diverse crop species to give rise to desirable traits like everbearing and compact growth habits58,69,73. Thus, mechanistic insight into the antagonism and identification of the targets of PEBPs will benefit traditional or genome editing-based crop improvement. It should further facilitate elucidation that how PEBP protein acts as co-activators or co-repressors in the nucleus.

Methods

Plant materials. Arabidopsis ecotype Columbia plants were grown in soil at 22 °C in long-day photoperiod (LD, 16 h light/8 h dark, 100 µmol/m² s) or short-day photoperiod (SD, 8 h light/16 h dark, 120 µmol/m² s). gGUS88, fd1 null mutants, fl1–14 homozygous mutants77, ft–11 mutant75,76,79,87,90,91,92,93–95. For qRT-PCR analysis, total RNA was extracted from leaves or shoot apices using TRIzol (Thermo Fisher Scientific, 15599-026) and purified using TRIzol (Thermo Fisher Scientific, 15599-026). Primer sequences are listed in Supplementary Table 3.

For yeast one-hybrid analysis, true total RNA was extracted from leaves or shoot apices using TRIzol (Thermo Fisher Scientific, 15599-026) and purified using TRIzol (Thermo Fisher Scientific, 15599-026). Primer sequences are listed in Supplementary Table 3.

Yeast one-hybrid assay. The previously described 3994-bp truncated FT promoter87 was PCR amplified from genomic DNA as the published amirRTF88 from pBs800 (ref. 88). The amirRTF fragment was introduced into EcolI-HF (NEB, R3101S) digested pENTR3C Thermofisher Scientific vector by Gibson Assembly. Next, LBHA was transformed into pENTR3C by thermal Fisher scientific strain (NEB, E5510S) and shuffled into binary vector pMCsG81U81 using LR reaction, which resulted in pMcsAamiRTF. The previously described 3994-bp FT promoter was inserted to XhoI (NEB, R0146S) digested pMcsAamiRTF by Gibson Assembly. GUS promoter and LFY-genic region up to and including the second intron were sequenced long-read sequencing (HiSeq Mini Kit (Illumina, 704054); cDNA was synthesized using SuperScript III First-Strand Synthesis (Invitrogen, 18080051) from 1 µg of RNA. Real time PCR was performed using a CDNA standard curve. Normalized expression levels were calculated using the 2−(ΔΔCT) method with the housekeeping gene UBQ10 (AT1G05520) as the control. Expression of multiple different genes was compared. Normalized gene expression is shown relative to the control treatment. Primer sequences are listed in Supplementary Table 2.

Chil-qPCR, Chil-seq and data analysis. Forty-two-day-old short-day grown plants were trimmed and 1.6 g of non-bolted inflorescences were harvested from 36 plants. Chromatim immunoprecipitation was conducted following a published protocol89 for Chil-qPCR. For Chil-seq, each biological replicate consisted of 200 mg of total RNA obtained from one MinElute Plant RNA purification column. For Chil-seq and Chil-qPCR, anti-GFP antibody (Thermo Fisher Scientific, A-11122; 1:200 dilution) and anti-GUS antibody (Abcam, ab50148: 1:200 dilution) were used. The antibodies were validated by the manufacturers. Chil-qPCR was performed using Platinum Taq DNA Polymerase (Invitrogen, 10966634) and EvaGreen dye (Biotochrom, 31000). For Chil-qPCR, the value of the Chil samples was normalized over that of input DNA as previously described90. Non-transgenic wild-type plants were used as the negative genetic
control for anti-GFP and anti-GUS antibody ChIP. The Ta3 retrotransposon (AT1G37110) was used as the negative control region for ChIP-qPCR. Primer sequences for all primer sets are listed in Supplementary Table S2.

Anti-GFP ChIP-seq was performed for gTFL1-GFP (A), gTFL1-GFP (B), wild-type and TFL1-Matus as for RNA-seq, followed by further growth in short-day conditions. To assess onset of reproductive development, the number of rosette leaves formed were counted at bolting. To analyze the inflorescence architecture, the number of sessile buds, outgrowing branches, flower branches, and single flowers subtended by a cauline leaf were counted weekly after bolting until the first normal flower (not subtended by a cauline leaf) formed.

Statistical analyses. The Kolmogorov–Smirnov (K–S) test was used to assess whether the data were normally distributed. All ChIP and qRT-PCR data were normally distributed. An unpaired one-tailed t-test was used to test for changes in one direction. Error bars represent the standard error of the mean (SEM). Two to three independent biological replicates were analyzed. For multiple-group comparisons (the non-parametric Kruskal–Wallis test) followed by the Dunn’s post hoc test were employed. Box and whisker plots display minima and maxima (whiskers), lower and upper quartile (box) and median (red vertical line).

Reporting summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability

The authors declare that the data supporting the findings of this study are available within this paper, its Supplementary information files and public data repositories. Source data are provided with this paper. The ChIP-seq and RNA-seq datasets were deposited to the GEO database (GSE141894). Individual replicates and P values for all figures are provided as a source data file. Source data are provided with this paper.

Code availability

Speeches for plots to peak annotation can be found at https://github.com/sklasfeld/ChIP_Annotation.

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Author contributions

D.W. and Y.Z. conceived of the study and Y.Z. conducted the majority of the experiments. S.K. conducted the bioinformatic analyses. N.Y. and C.W.J. identified the optimal stage to study primordium fate regulation by TFL1, conducted initial TFL1 ChIP analyses and mapped the FD-binding sites in LFY. R.J. and Y.Z. constructed and sequenced ChIP-seq libraries. K.G. generated the biologically active genomic GEP-TFL1 construct. D.W. wrote the manuscript with the help of Y.Z. and input from all other authors.

Competing interests

The authors declare no competing interests.

Additional information

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