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Journal

Proceedings of the National Academy of Sciences of the United States of America, 113(33)

ISSN

0027-8424

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Publication Date

2016-08-16

DOI

10.1073/pnas.1602960113

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FAR-RED ELONGATED HYPOCOTYL3 activates *SEPALLATA2* but inhibits *CLAVATA3* to regulate meristem determinacy and maintenance in *Arabidopsis*

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Edited by Xing Wang Deng, Peking University, Beijing, China, and approved June 27, 2016 (received for review February 22, 2016)

Plant meristems are responsible for the generation of all plant tissues and organs. Here we show that the transcription factor (TF) FAR-RED ELONGATED HYPOCOTYL3 (FHY3) plays an important role in both floral meristem (FM) determinacy and shoot apical meristem maintenance in *Arabidopsis*, in addition to its well-known multifaceted roles in plant growth and development during the vegetative stage. Through genetic analyses, we show that *WUSCHEL* (*WUS*) and *CLAVATA3* (*CLV3*), two central players in the establishment and maintenance of meristems, are epistatic to *FHY3*. Using genome-wide ChIP-seq and RNA-seq data, we identify hundreds of FHY3 target genes in flowers and find that FHY3 mainly acts as a transcriptional repressor in flower development, in contrast to its transcriptional activator role in seedlings. Binding motif-enrichment analyses indicate that FHY3 may coregulate flower development with three flower-specific MADS-domain TFs and four basic helix–loop–helix TFs that are involved in photomorphogenesis. We further demonstrate that *CLV3*, *SEPALLATA1* (*SEP1*), and *SEP2* are FHY3 target genes. In shoot apical meristem, FHY3 directly represses *CLV3*, which consequently regulates *WUS* to maintain the stem cell pool. Intriguingly, *CLV3* expression did not change significantly in *fhy3* and *phytochrome B* mutants before and after light treatment, indicating that FHY3 and phytochrome B are involved in light-regulated meristem activity. In FM, FHY3 directly represses *CLV3*, but activates *SEP2*, to ultimately promote FM determinacy. Taken together, our results reveal insights into the mechanisms of meristem maintenance and determinacy, and illustrate how the roles of a single TF may vary in different organs and developmental stages.

meristem maintenance | meristem determinacy | FHY3 | CLV3 | SEP2

Plant meristems are responsible for the generation of all plant tissues and organs. Unlike the shoot apical meristem (SAM), whose activity is maintained throughout the life of plants, the floral meristem (FM) is precisely programmed to terminate in a process known as FM determinacy (1). *WUSCHEL* (*WUS*) plays a central role in the establishment and maintenance of SAM, inflorescence meristem, and FM, as well as in FM determinacy (2–4). *WUS* is expressed in the organizing center located beneath the stem cells in the meristem to promote cell proliferation by maintaining stem cell potential (2). The *WUS*/*CLAVATA3* (*CLV3*) signaling pathway maintains the stabilization of meristem size and the stem cell pool (3, 5). Consistent with *WUS* overactivation, *clv3* mutants have an enlarged SAM and increased numbers of floral organs and whorls (5). In addition to the *WUS*/*CLV3* loop, several other pathways are known to regulate FM determinacy (4). *AGAMOUS* (*AG*) encodes a MADS-box transcription factor (TF) and is the lynchpin of the FM determinacy network (4, 6, 7). In the null *ag-1* mutant, FM determinacy is severely impaired, resulting in a flower-in-flower phenotype (6). AG inhibits *WUS* expression through both indirect and direct means (8, 9). A number of other genes have been shown to regulate FM determinacy through the *AG* pathway or in parallel pathways, and additional players in this critical developmental process await characterization (4).

Floral organs are produced by the FM based on the classic ABC model in *Arabidopsis* (10, 11). However, the ABC genes were found to be necessary but not sufficient for the determination of floral organ identity (12, 13), and the E class genes—*SEPALLATA1* (*SEP1*), *SEP2*, *SEP3*, and *SEP4*—were subsequently incorporated into the model (14). Although single or double *sep* mutants produce flowers indistinguishable from those of the wild-type, the *sep1sep2sep3sep4* quadruple mutant develops flowers with

Significance

The transcription factor *FAR-RED ELONGATED HYPOCOTYL3* (*FHY3*) is known to play multiple roles at the vegetative stage in *Arabidopsis*, but its functions in reproductive stage are unclear. We find that FHY3 is required for floral meristem determinacy and shoot apical meristem maintenance by mainly acting as a transcriptional repressor. *FHY3* mediates light-regulated *CLAVATA3* expression to regulate *WUSCHEL* expression in shoot apical meristem and directly represses *CLAVATA3*, but activates *SEPALLATA2*, to promote floral meristem determinacy. Furthermore, FHY3 may coregulate flower development with three flower-specific MADS-domain transcription factors and four basic helix–loop–helix transcription factors that are involved in photomorphogenesis, and thus may act as a bridge molecule in the cross-talk between external signals and endogenous cues to coordinate plant development.

Author contributions: R.L. and X.L. designed research; D.L., X.F., L.G., Z. Huang, Y. Li, Y. Liu, Z. He, X. Cao, X.M., and M.Z. performed research; G.Z. and L.X. contributed new reagents/analytic tools; X.F., L.G., H.W., X. Chen, R.L., and X.L. analyzed data; and H.W., X. Chen, R.L., and X.L. wrote the paper.

The authors declare no conflict of interest.

This article is a PNAS Direct Submission.

Data deposition: The data reported in this paper have been deposited in the Gene Expression Omnibus (GEO) database, www.ncbi.nlm.nih.gov/geo (accession nos. GSE69422, GSE69424, and GSE69425).

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This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1602960113/-/DCSupplemental.

leaf-like organs in all floral whorls and indeterminate FM activity, thereby demonstrating the functional redundancy of *SEP* genes and their fundamental roles in floral organ identity and FM determinacy (14, 15). On the other hand, the differing spatiotemporal expression patterns of individual *SEP* genes are suggestive of their distinct functions in flower development. Once the FM is produced, *SEP1* and *SEP2* are expressed in all four whorls. However, the mechanisms underlying the regulation of *SEP1* and *SEP2* expression remain unclear. Moreover, although the precise timing of the early events in floral organ production mediated by *SEP* genes has been well studied (16, 17), the molecular mechanisms mediated by *SEP* genes in the context of FM determinacy are largely unknown.

Light is one of the most important environmental signals for plant growth and development. Light could regulate stem cell activity through auxin and cytokinin (18). The detailed mechanisms underlying this process are still waiting dissection. The transposase-derived TF FAR-RED ELONGATED HYPOCOTYL3 (FHY3) was isolated as a key positive regulator of the phytochrome A (phyA) signaling pathway in *Arabidopsis* (19). FHY3 regulates the expression of target genes by directly binding to FHY3-binding sites (FBS, CACGCGC) (20, 21). ChIP-sequencing (ChIP-seq) analysis and genome-wide gene-expression profiling have identified hundreds of FHY3 target genes predicted to function in diverse environmental, hormonal, and developmental contexts. Under far-red (FR) conditions, FHY3 mainly acts as a transcriptional activator (21). In addition to phytochrome and circadian signaling, *FHY3* has been found to function in diverse plant developmental and physiological processes, including UV-B signaling, chloroplast biogenesis, chlorophyll biosynthesis, programmed cell death, ABA signaling, and branching (22). It is important to note that although the functions of *FHY3* during the plant vegetative stage are well studied, its roles in flower development remain poorly understood.

In this study, we isolated several *fhy3* mutations that dramatically enhanced the FM indeterminacy phenotype of *ag-10*, a weak *ag* allele. Through genetic analyses we show that *FHY3* is required for FM determinacy and SAM maintenance and that *wus* is epistatic to *fhy3* in FM determinacy. Through ChIP-seq and RNA-seq analyses, we identify hundreds of FHY3 binding sites and FHY3 target genes in floral organs and find that FHY3 mainly acts as a transcriptional repressor during flower development. Further analyses show that FHY3 functions in meristem determinacy and maintenance by directly binding the promoters of *CLV3*, *SEP1*, and *SEP2*, resulting in direct *CLV3* repression, direct *SEP2* activation, and downstream regulation of *WUS* expression.

Results

***FHY3* Is Required for FM Determinacy and SAM Maintenance.** To identify new players involved in FM determinacy, an ethyl methanesulfonate (EMS) mutagenesis screen was performed in the *ag-10* background, as previously reported (9). In contrast to *ag-1* null mutants (6), *ag-10* exhibits only a weak FM determinacy defect showing a few curved and bulged siliques with additional floral tissue inside (Fig. 1 *A* and *B* and Fig. S1*A*) (9). For screening, we focused on mutants with more bulged siliques throughout the entire plant as an indicator of prolonged FM activity.

Several such mutants were isolated with similar phenotype, showing very short and bulged siliques in whole plants with a mean carpel number of 4.3 ± 0.4 (n = 50) (Fig. 1*C*) and additional floral organs growing inside (Fig. S1 *A* and *B*). The mutants also produced very small petals and sterile anthers (Fig. 1*D*). Longitudinal and transversal silique sections revealed plenty of floral organs growing inside the pistils from the indeterminate FM (Fig. S1*B*), resulting in infertile siliques in the mutant in a Landsberg *erecta* (L*er*) background (Fig. S1*A*). Through genetic mapping, all mutation sites were found in *FHY3* and the mutations were named *fhy3-27*, *fhy3-39*, *fhy3-46*, and *fhy3-68* (Fig. S1*C*). *fhy3-68* was used for subsequent analysis. Introducing a *35S:FHY3-FLAG* transgene into *fhy3-68 ag-10* rescued the mutant phenotype, confirming that *fhy3-68* was responsible for the enhanced FM determinacy defects (Fig. 1*E*).

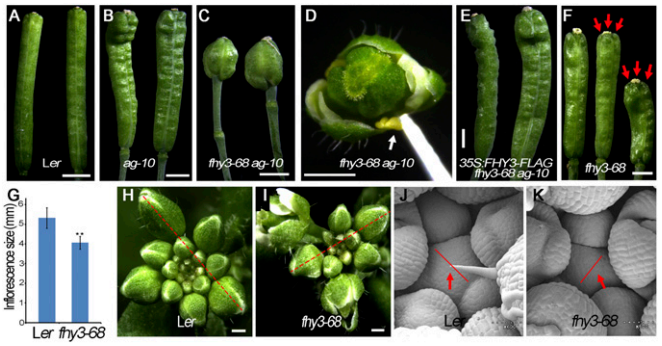

Fig. 1. *FHY3* is required for FM determinacy and SAM maintenance. (*A–C*, *E*, and *F*) Siliques of L*er* (*A*), *ag-10* (*B*), *fhy3-68 ag-10* (*C*), *35S:FHY3-FLAG fhy3-68 ag-10* (*E*), and *fhy3-68* (*F*). Carpels marked by red arrows in *F*. (*D*) Flowers of *fhy3-68 ag-10*. Sterile anthers are marked by a white arrow in *D*. (*G*) Quantification of inflorescence size (mm) of L*er* (n = 15) and *fhy3-68* (n = 15). $^{**}P < 0.01$. (*H* and *I*) Inflorescences of L*er* (*H*) and *fhy3-68* (*I*). Dashed lines mark the width used to measure inflorescence size in *G*. (*J* and *K*) SAM (marked by a red arrow) of L*er* (*J*) and *fhy3-68* (*K*). Red lines mark the width of SAM. (Scale bars: 1 mm in *A–C*, *E*, and *F*; 250 μm in *D*; 500 μm in *H* and *I*; 60 μm in *J* and *K*.)

Through outcrossing of *fhy3-68 ag-10* with L*er*, we obtained the *fhy3-68* single mutant. The number of carpels of *fhy3-68* was 2.61 ± 0.6 (n = 50), which was similar to that of *fhy3-4* plants in the No-0 background (3.2 ± 0.5, n = 50), indicated enhanced or prolonged FM activity of *fhy3* (Fig. 1*F* and Fig. S1*D*). Taken together, these findings show that *FHY3* is required for FM determinacy in *Arabidopsis*. In addition, we noticed that compared with the wild-type, the inflorescences of *fhy3-68* were smaller (Fig. 1 *G–I*) and the SAM size of *fhy3-68* was dramatically reduced (Fig. 1 *J* and *K*), indicating that *FHY3* is also required for proper SAM maintenance, consistent with the high expression level of FHY3 in SAM and FM (Fig. S1 *E* and *F*).

***wus* Is Epistatic to *fhy3* in FM Determinacy.** *WUS* plays a pivotal role in FM initiation, maintenance, and determinacy (2), and several genes have been characterized as FM determinacy factors through their regulation of *WUS* expression (4). To dissect the interaction between *FHY3* and *WUS* in FM determinacy, we performed in situ hybridization to assess the temporal-spatial expression pattern of *WUS*. In wild-type, *WUS* expression is shut off at stage 6 of flower development (23). In *ag-10* plants, most flowers have normal *WUS* expression patterns, whereas a few flowers exhibit *WUS* expression until stage 7 (Fig. S2 *A* and *B*) (9). For *fhy3-68*, the tested flowers (n = 9) showed slight *WUS* expression at the end of stage 6, indicating slightly prolonged *WUS* expression (Fig. S2*C*). All of the *fhy3-68 ag-10* stage 9 flowers examined (n = 9) had obviously prolonged *WUS* expression (Fig. S2*D*), showing that *FHY3* is required for the temporally precise repression of *WUS*. To determine the genetic relationship of *FHY3* and *WUS*, we crossed *fhy3-68 ag-10* with the *wus-1* loss-of-function mutant (2). *wus-1* flowers exhibited premature FM termination with normal sepals and petals and one or two stamens (Fig. S2*E*). The precocious termination of *fhy3-68 ag-10 wus-1* flowers resembled that of *wus-1* flowers (Fig. S2*F*), demonstrating that *wus-1* was epistatic to *fhy3-68 ag-10*.

To investigate whether FHY3 is a direct transcriptional regulator of *WUS*, we used an *FHY3:FHY3-GR fhy3-4* transgenic line (20). After 4- and 8-h treatment with dexamethasone (DEX) or dimethyl sulfoxide (DMSO, as a negative control), *WUS* expression was similar in both treatments (Fig. S2*G*). Additionally,

the ChIP-quantitative PCR (qPCR) data using the *35S:3FLAG-FHY3-3HA fhy3-4* transgenic line (21) revealed no significant enrichment of FHY3 binding activity at the *WUS* locus, indicating that *WUS* is not a direct target gene of FHY3 (Fig. S2*H*).

AG is the major FM terminator and acts via *WUS* repression (7). Thus, *AG* expression was analyzed to assess the relationship between *FHY3* and *AG* in FM determinacy. *AG* transcript levels were normal in *fhy3-68* and *fhy3-68 ag-10* compared with L*er* and *ag-10*, respectively (Fig. S2*I*). In addition, the expression of *FHY3* was not affected in *ag-1* compared with L*er* (Fig. S2*J*), indicating that *FHY3* and *AG* do not regulate each other. RT-qPCR assays using DEX- and DMSO-treated *FHY3:FHY3-GR fhy3-4* transgenic plants revealed no significant change in *AG* transcript levels after DEX treatment, further indicating that FHY3 does not regulate *AG* expression (Fig. S2*K*). These results suggest that *FHY3* may act independently of the *AG* pathway in FM determinacy.

Genome-Wide Identification of FHY3 Binding Sites. To investigate the molecular mechanisms underlying the *FHY3* functions in flower development, we performed ChIP-seq analysis using a *35S:3FLAG-FHY3-3HA fhy3-4* transgenic line (21) to identify the binding sites of FHY3 in floral organs. Inflorescences containing stage 8 and younger flowers were harvested for ChIP-seq analysis. We identified 1,885 FHY3 binding sites (FBSs, $P < 5e\text{-}3$) distributed across the five chromosomes (Fig. S3*A* and Dataset S1), of which 51% (960) were subsequently assigned to genic regions [from −2,000 bp of the transcription start site (TSS) to the 3′UTR] and grouped into 1,507 genes (Dataset S1), which were referred to as FHY3-associated genes. The remaining 49% (925) FBSs were localized in intergenic regions. Consistent with a previous report (21), 74% (688) of the intergenic FBSs resided in centromeric regions (Fig. 2*A*). *FHY1*, *CIRCADIAN CLOCK ASSOCIATED1* (*CCA1*), and *EARLY FLOWERING4* (*ELF4*) are well-characterized FHY3 target genes (21). Specific enrichment of FHY3 was detected in the promoter regions rather than the transcribed regions of these genes, confirming the reliability of our ChIP-seq data (Fig. S3*B*).

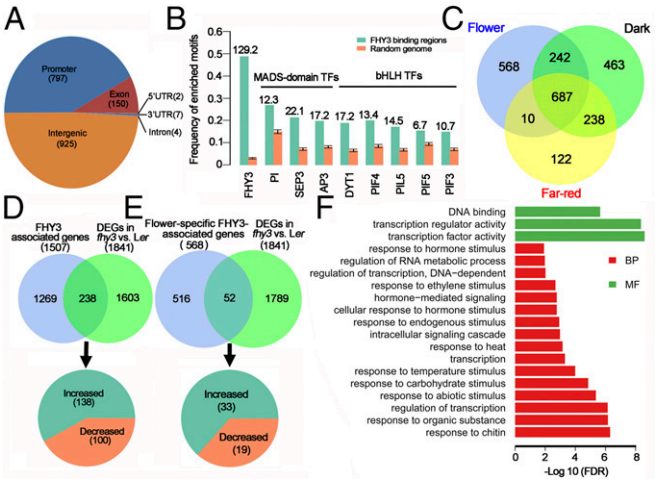

Fig. 2. Genome-wide identification of FHY3 binding sites and target genes. (*A*) Classification of FHY3 binding sites in the *Arabidopsis* genome. The numbers of binding sites are indicated in parentheses. (*B*) The binding motifs of several TFs were significantly enriched around the FHY3 binding peaks compared with randomly selected genomic regions. The numbers on the top of columns are *z*-scores computed from the permutation test. A *z*-score of 2 or above is considered statistically significant. (*C*) Venn diagram showing the number and overlap of FHY3-associated genes in flower and seedling under D and FR conditions. (*D* and *E*) The FHY3 ChIP-seq data and RNA-seq data were compared to identify FHY3 target genes (*D*) and flower-specific FHY3 target genes (*E*). (*F*) Enrichment of GO terms among flower-specific FHY3 target genes. BP, biological process; MF, molecular function.

Further analysis revealed that more than 42% of the FBSs occurred in promoter and TSS regions of annotated *Arabidopsis* genes, with the peak regions located at −2,000 to +200 bp from the TSS, confirming the role of FHY3 as a TF (Fig. S3*C*). We next searched for significantly enriched motifs in FBSs using the Multiple Em for Motif Elicitation (MEME) program, and the FBS motif (CACGCGC) (E-value = 7.4e-244) was identified (Fig. S3*D*). One or more FBS motifs were found in 47% of the identified FBSs, indicating that FHY3 may bind other motifs or coordinately regulate target genes with other factors (24). To find potential FHY3 cofactors, we investigated whether the binding motifs of other known plant TFs were present in FBSs. Interestingly, the binding motifs of three flower-specific MADS-domain TFs, PISTILLATA (PI), SEP3, and APETALA3 (AP3), were significantly enriched in FBSs (Fig. 2*B*). Moreover, the binding motifs of PHYTOCHROME INTERACTING FACTOR 3, -4, and -5 (PIF3, -4, and -5) and PHYTOCHROME INTERACTING FACTOR 3-LIKE 5 (PIL5), a group of basic helix–loop–helix (bHLH) TFs that mediate light signal in photomorphogenesis, were also enriched around the FHY3 binding peaks (Fig. 2*B*). ChIP-qPCR assays showed that the promoters of 10 randomly chosen target genes were indeed bound by FHY3 and one of the aforementioned TFs (Fig. S3*E*), confirming that these TFs are cofactors of FHY3. These results suggest that FHY3 functions synergistically with flower-specific MADS-domain TFs and bHLH TFs to regulate flower development. Using ChIP-seq data from a previous study (21), we identified 1,630 and 1,057 FHY3-associated genes in seedlings under dark (D) and FR conditions, respectively. A comparison of FHY3-associated genes under three tissues/conditions (D, FR, and flower) uncovered 687 genes that were commonly bound by FHY3 under three tissues/conditions and 568 genes (38% of total genes) that were bound by FHY3 specifically in floral organs (Fig. 2*C*).

Identification of FHY3 Target Genes in Floral Organs. To identify FHY3 target genes in floral organs, we compared the gene-expression profiles in the inflorescences containing stage 8 and younger flowers from L*er*, *ag-10*, *fhy3-68*, and *fhy3-68 ag-10* using RNA-seq. For each genotype, the expression profiles of two biological replicates were highly correlated with each other (Fig. S3*F*), indicating that our RNA-seq data were highly reproducible. Using edgeR, we identified differentially expressed genes (DEGs) for each pairwise comparison using false-discovery rate (FDR) < 0.05 as significance cut-off (Fig. S3*G* and Dataset S2). Compared with L*er*, a very small number of DEGs (29 up-regulated and 3 down-regulated genes) were found in the *ag-10* sample, consistent with its weak FM determinacy defects. Among the DEGs in *fhy3-68* vs. L*er*, 78% (1,442 of 1,841 DEGs) were up-regulated (Fig. S3*G* and Dataset S2), indicating that *FHY3* plays a repressive role in flower development.

Comparing the results of the ChIP-seq and RNA-seq analyses defined 238 FHY3 target genes that were bound directly and transcriptionally regulated by FHY3 in flower development (Fig. 2*D* and Dataset S3), of which 58% (138 genes) were up-regulated and 42% (100 genes) were down-regulated in *fhy3-68* (Fig. 2*D*). Based on the specific binding sites in flower (Fig. 2*C*), we identified 52 flower-specific FHY3 target genes, of which 63% (33 genes) were up-regulated in *fhy3-68*, once again suggesting a repressive role of *FHY3* in flower development (Fig. 2*E* and Dataset S3). Gene Ontology (GO) analysis of the DEGs between *fhy3-68* vs. L*er* revealed that GO terms related to cell cycle, DNA metabolism, and cell division were enriched in FHY3 up-regulated genes, and GO terms related to defense or immune response, cell death, and hormone signaling were enriched in FHY3 down-regulated genes (Fig. S3 *H* and *I*), indicating that FHY3 functions in cell proliferation and environmental and hormone response. Notably, among the flower-specific FHY3 target genes, genes assigned to the terms "transcription factor activity,"

"transcription regulator activity," and "DNA binding" in the "molecular function" category were highly enriched (Fig. 2*F*), suggesting that these genes may function as early target genes of FHY3 in flower development.

***SEP1* and *SEP2* Are FHY3 Target Genes.** *SEP* genes have fundamental roles in floral organ identity and FM determinacy. Our genome-wide analyses revealed that *SEP1* and *SEP2* were putative target genes of FHY3 (Dataset S3). Real-time RT-PCR analysis showed that *SEP* genes are differentially regulated by FHY3. *SEP1* and *SEP2* were down-regulated in *fhy3-68 ag-10* inflorescences and *SEP3* transcripts increased in *fhy3-68* (Fig. S4*A*). The Integrative Genomics Viewer (IGV) showed that FHY3-FLAG peaks were located at the promoter regions of *SEP1* and *SEP2*, respectively (Fig. 3*A*). We conducted ChIP-qPCR to confirm the occupancy of FHY3 at *SEP1* and *SEP2* in *35S:3FLAG-FHY3-3HA fhy3-4* inflorescences (21). Significant enrichment of FHY3 at the TSS regions but not the intragenic regions of *SEP1* and *SEP2* (Fig. 3*B*) demonstrated the direct and specific binding of FHY3 at the promoters of *SEP1* and *SEP2*. We also asked whether FHY3 directly regulates *SEP1* and *SEP2*. In *FHY3:FHY3-GR fhy3-4* transgenic plants treated with DEX or DMSO, *SEP1* and *SEP2* were induced after DEX or DEX/cycloheximide (CHX; a protein synthesis inhibitor) treatment but not DMSO or CHX (Fig. 3*C*), indicating that FHY3 induces *SEP1* and *SEP2* expression independent of new protein synthesis (Fig. 3*C* and Fig. S4*B*). Collectively, these findings show that *SEP1* and *SEP2* are *FHY3* direct target genes in floral organs.

***SEP2*-Mediated *FHY3* Functions in FM Determinacy.** To investigate whether the *FHY3* involvement in FM determinacy is mediated by *SEP1* and *SEP2*, *SEP1* and *SEP2* overexpression constructs under the CaMV35S promoter were generated as previously described (14) and then transformed into an *ag-10 fhy3-68*/+ population. Compared with *fhy3-68 ag-10* plants, all of the *35S:SEP1 fhy3-68 ag-10* and *35S:SEP2 fhy3-68 ag-10* transgenic plants exhibited an early flowering phenotype (Fig. S4 *C*–*E*). Real-time RT-PCR confirmed that *SEP1* and *SEP2* were overexpressed in the transgenic plants (Fig. S4*F*). Although *SEP1* overexpression in *35S:SEP1 fhy3-68 ag-10* plants (n = 12) failed to rescue the FM determinacy defects (Fig. S4*D*), the siliques of *35S:SEP2 fhy3-68 ag-10* transgenic plants (n = 8) were composed of two carpels with normal gynophores (Fig. 3*D* and Fig. S4*E*). Moreover, sliced open siliques showed no more layered carpeloid organs growing inside (compare the *Inset* in Fig. 3*D* to Fig. S1*B*), indicating that *SEP2* overexpression rescued the FM determinacy defects of *fhy3-68 ag-10*. However, it failed to rescue the small inflorescence, short petal, and sterile anther phenotypes of *fhy3-68 ag-10* (Fig. S4*E*). These findings indicate that *SEP2* only mediates the function of *FHY3* in FM determinacy.

To avoid the pleiotropic phenotypes resulted from constitutive overexpression of *SEP2*, we generated a *SEP3:SEP2 fhy3-68 ag-10* transgene because *SEP3* expression was flower-specific and unchanged in *fhy3-68 ag-10* (Fig. S4*A*). Although they showed normal development at the vegetative stage, the transgenic plants also produced short siliques because of the sterile anther (Fig. S4 *G* and *H*). The siliques were composed of 2.2 ± 0.3 carpels (n = 30) (Fig. S4*H*) and sliced-open siliques contained fewer additional organs growing inside than those of *fhy3-68 ag-10* (Fig. S4*I*), indicating that the *SEP3:SEP2* transgene could mainly rescue the FM indeterminacy of *fhy3-68 ag-10*. Real-time PCR analysis showed that the expression of *SEP2* reached normal level in the transgenic plants as in L*er* (Fig. S4*J*), indicating that other factors may also mediate the function of *FHY3* in FM determinacy besides *SEP2*.

To further confirm the role of *SEP2* in FM determinacy, two artificial miRNAs (amiRNAs) targeting *SEP2* (amiR-*sep2*A and amiR-*sep2*B) were generated and introduced into the *ag-10*. All amiR-*sep2*A *ag-10* plants (n = 15) and a small percentage of amiR-*sep2*B *ag-10* plants (8 of 52) produced more bulged siliques with additional organs growing inside, similar to the phenotype of *fhy3-68 ag-10* (Fig. 3*E*). Thus, reduced *SEP2* expression enhanced the FM indeterminacy of *ag-10*. qPCR analysis confirmed the reduced expression of *SEP2* but not *SEP1* in the amiR-*sep2 ag-10* lines (Fig. S4*K*). Taken together, these findings show that *SEP2* mediates the functions of *FHY3* in FM determinacy.

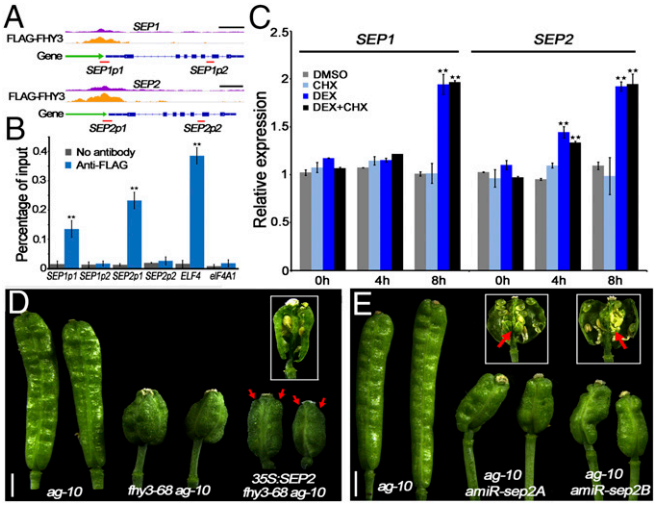

Fig. 3. *SEP2* mediates the function of FHY3 in FM determinacy. (*A*) The FHY3-FLAG ChIP-seq peaks (two biological replicates) at *SEP1* and *SEP2* revealed in IGV. FLAG-FHY3 peaks (purple and orange), gene structure, and the regions examined by ChIP are shown in the top, middle, and bottom rows, respectively. (Scale bars, 500 bp.) (*B*) ChIP to measure FHY3 occupancy at *SEP1* and *SEP2* in *35S:3FLAG-FHY3-3HA fhy3-4* inflorescences. The regions examined are shown in *A*. *eIF4A1* served as a negative control. Error bars represent SD from three biological replicates. $^{**}P < 0.01$ compared with no antibody (negative control). (*C*) The transcript levels of *SEP1* and *SEP2* in *FHY3:FHY3-GR fhy3-4* inflorescences measured by RT-qPCR. *Ubiquitin 5* (*UBQ5*) served as the internal control. Three biological replicates were performed. Error bars represent SD from three biological repeats. $^{**}P < 0.01$. (*D* and *E*) Siliques from plants of the indicated genotypes. Carpels were indicated by red arrows; Sliced open siliques were indicated by white arrows. (Scale bars, 1 mm.)

FHY3 Contributes to SAM and FM Regulation by Directly Repressing *CLV3*. Besides the FM determinacy defects, we found the SAM maintenance was impaired in the *fhy3-68* mutant (Fig. 1 *J* and *K*). It is well established that *CLV3* encodes a secreted peptide that restricts the domain of *WUS* expression and the size of the stem cell domain (5). We therefore examined the genetic relationship between *FHY3* and *CLV3*. Like *clv3-1*, *fhy3-68 clv3-1* produced a larger SAM than L*er* and *fhy3-68* (Fig. S5 *A*–*C*), indicating that *clv3* is epistatic to *fhy3* in SAM maintenance. Consistent with these observations, qPCR revealed increased *CLV3* transcript levels in *fhy3-68* and *fhy3-68 ag-10*, compared with L*er* and *ag-10*, respectively (Fig. S5*D*). In situ hybridization was then used to detect *CLV3* and *WUS* expression in L*er* and *fhy3-68*. Whereas *CLV3* expression increased and expanded in the SAM of *fhy3-68* (Fig. 4 *A* and *B*), *WUS* expression decreased in the SAM of *fhy3-68* compared with L*er* but remained unchanged in the early stage of FM (Fig. 4 *C* and *D*). These findings indicate that *CLV3* mediates *FHY3* function in the SAM by regulating *WUS* expression to balance the stem cell pool and SAM size.

We next examined the genetic relationship of *FHY3* and *CLV3* in FM determinacy. In the *clv3-1* mutant, the number of floral organs, particularly the stamen number (7.7 ± 0.5; n = 50) and carpel number (4.8 ± 0.6; n = 50), increased (Fig. 4*E*). The FM indeterminacy of *fhy3-68 clv3-1* was more severe than that of *clv3-1*, with increased stamen number (8.5 ± 0.5; n = 20) and unfused

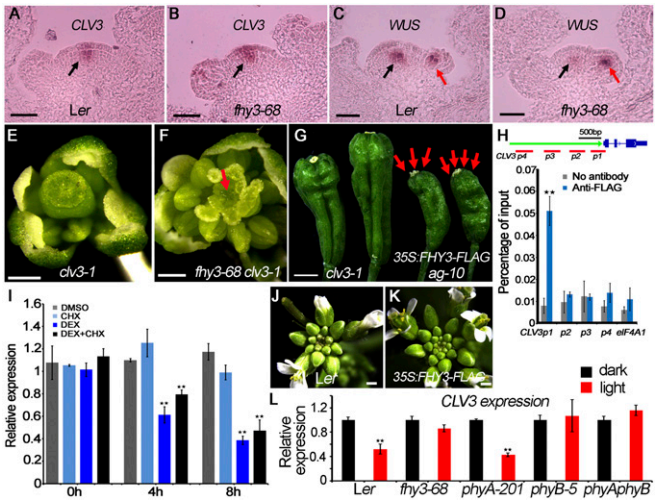

Fig. 4. *CLV3* mediates *FHY3* functions in regulating the stem cell pool in the SAM and FM meristem activity. (*A–D*) In situ hybridization to examine the expression of *CLV3* (*A* and *B*) and *WUS* (*C* and *D*) in L*er* (*A* and *C*) and *fhy3-68* (*B* and *D*). *CLV3* signals are marked by a black arrow in *A* and *B*. *WUS* signals are marked by a black arrow in SAM and a red arrow in FM in *C* and *D*. (*E* and *F*) Flowers of *clv3-1* (*E*) and *fhy3-68 clv3-1* (*F*). Dome-shaped meristem is marked by a red arrow. (*G*) Representative siliques of *clv3-1* (*Left*) and *35S:FHY3-FLAG ag-10* (*Right*) plants. Carpels are marked by red arrows. (*H*) ChIP to measure FHY3 occupancy at *CLV3* in *35S:3FLAG-FHY3-3HA fhy3-4* inflorescences. The regions examined are shown on the *Upper* panel. *CLV3* gene structure was shown. (Scale bar, 500 bp.) *eIF4A1* served as a negative control. Error bars represent SD from three biological replicates. $^{**}P < 0.01$ compared with no antibody (negative control). (*I*) The *CLV3* transcript levels in *FHY3:FHY3-GR fhy3-4* inflorescences measured by RT-qPCR. (*J* and *K*) Inflorescence of L*er* (*J*) and *35S:FHY3-FLAG* (*K*). *35S:FHY3-FLAG* developed a larger inflorescence containing more unopened buds than L*er*. (*L*) The *CLV3* transcript levels in seedlings of indicated plants after light treatment measured by RT-qPCR. In *I* and *L*, *UBQ5* served as the internal control. Three biological replicates were performed. Error bars represent SD from three biological repeats. $^{**}P < 0.01$. (Scale bars: 50 μm in *A–D* and 500 μm in *E–G*, *J*, and *K*.)

carpels with a dome-shaped meristem growing inside (Fig. 4*F*). The enhanced FM indeterminacy of *fhy3-68 clv3-1* was consistent with the finding that *FHY3* acts in *CLV3* pathway besides the *SEP2* pathway to regulate *WUS* expression in FM determinacy. Accordingly, we found that 16% ($n = 50$) siliques of *35S:FHY3-FLAG ag-10* plants were composed of three or more fused carpels, similar to those of weak *clv3* mutants (Fig. 4*G*). qPCR revealed decreased *CLV3* transcript levels in the inflorescence of *35S:FHY3-FLAG ag-10* compared with *ag-10* (Fig. S5*E*).

We performed additional ChIP-qPCR to investigate whether *CLV3* is an FHY3 direct target gene. The high occupancy of FHY3 in the upstream region of the *CLV3* TSS instead of other tested regions suggested that FHY3 binds specifically to the *CLV3* promoter (Fig. 4*H*). We then examined the *CLV3* expression in *FHY3:FHY3-GR fhy3-4* plants treated with DEX and DEX/CHX or DMSO and CHX (control). RT-qPCR revealed severely attenuated *CLV3* transcript levels in the DEX- or DEX/CHX-treated plants within 4 h of treatment (Fig. 4*I*), indicating that FHY3 directly represses *CLV3*. Correspondingly, the *35S:FHY3-FLAG* plants developed larger inflorescences that contained more unopened buds than L*er* (Fig. 4 *J* and *K* and Fig. S5*F*). Taken together, these results suggest that FHY3 functions in FM determinacy and SAM maintenance by directly repressing *CLV3*.

***FHY3* Mediates the Light-Repressed *CLV3* Expression.** A recent report showed that light regulates meristem activity by activating cytokinin signaling and repressing *CLV3* expression (18). To investigate whether *FHY3* is involved in light-regulated meristem activity, we grew seedlings 4 d in the dark after germination, followed by 12-h light treatment and examined the *CLV3* expression in diverse genotypes. As expected, *CLV3* expression was repressed by light in the wild-type plants (Fig. 4*L*). However, *CLV3* expression did not change significantly in *fhy3-68* before and after light treatment (Fig. 4*L*), indicating that *FHY3* is essential for light-regulated expression of *CLV3* in SAM. Unexpectedly and interestingly, light-repressed *CLV3* expression was observed in the *phyA* mutant, but not in *phyB* and *phyAphyB* mutants (Fig. 4*L*), indicating that phyB, but not phyA, may be involved in the light-regulated meristem activity. The interaction of *FHY3* and phyB in light-regulated meristem activity is an open and interesting question.

Discussion

Plant meristem maintenance and determinacy are critical for plant growth, life cycle, and crop yield. Most studies have been focused on the cross-talk between phytohormones, such as auxin and cytokinin, and gene expression in these processes (25, 26). Little is known whether, and if so how, external signals like light and temperature contribute to meristem regulation. *FHY3* is known to play pivotal roles in the phyA signaling and the circadian clock pathways, and other developmental and physiological processes at the vegetative stage (22), but its roles in the reproductive stage remain unclear. Our findings describe the previously unknown functions of *FHY3* in SAM maintenance, FM determinacy, as well as petal and stamen development during flower development (Figs. 1 and 3*F*). Previous studies have shown that most of FHY3 target genes (99% in FR or 78% in D) were activated, indicating that FHY3 mainly acts as a transcriptional activator in seedlings (21). In this study, we identified hundreds flower-specific FHY3-associated genes (Fig. 2*C*), suggesting that FHY3 has distinctive roles in flower development. Of the DEGs identified in *fhy3-68* vs. L*er*, 78% were up-regulated, indicating that FHY3 mainly functions as a transcriptional repressor in flower development (Fig. S3*G*). Notably, the expressions of *MYB77* in ethylene signaling, *BTB AND TAZ DOMAIN PROTEIN4* (*BT4*) in gibberellin signaling, and *MYBR1* in ABA, auxin, and ethylene signaling were activated in seedlings but repressed in flowers by FHY3 (Dataset S3) (21). The enrichment of genes related to cell cycle, DNA metabolic process, and DNA replication genes among the FHY3-activated genes (Fig. S3*H*), and the clustering of FHY3 intergenic binding sites in centromeric regions (Fig. S3*A*), raise the possibility that FHY3 may also function in cell division and epigenetic regulation. These findings underscore how the same TF may have vastly different roles in different organs and developmental stages.

Certain *cis*-elements, such as the G-box and GCC-box that are bound by bHLH TFs (like PIL5 and PIF3) and bZIP TFs, are enriched around the FHY3 binding sites in the vegetative stage (21). Here, we show that the binding motifs of bHLH TFs PIF3, -4, -5, and PIL5, which mediate light signal in photomorphogenesis, were significantly enriched around the FHY3 binding sites (Fig. 2*B* and Fig. S3*E*), indicating that FHY3- and PIFs-mediated light signaling may function in flower development. Furthermore, the binding motifs of AP3, PI, and SEP3 were also enriched in the FHY3 binding regions (Fig. 2*B* and Fig. S3*E*). Given that AP3 and PI, B-class MADS-domain TFs, form a protein complex with SEP3 to function in sepal and stamen development (27), our results suggest that FHY3 may coregulate sepal and stamen development with AP3, PI, and SEP3, consistent with the sepal and stamen developmental defects of *fhy3 ag-10* (Fig. 1*D*). Remarkably, we find that *FHY3* and phyB are involved in light-repressed *CLV3* expression (Fig. 4*L*). The relationship between FHY3 and pyhB in this process awaits further investigation (Fig. S6*A*). Combined with recent reports that light could regulate stem cell activity (18), and that two key meristem regulatory genes, *BASIC PENTACYSTEINE 3* (*BPC3*) and *AG* were uniquely bound by light signal transducer FHY1 and phyA, respectively (28), our results highlight

the possibility that FHY3 may act as a bridge molecule in the cross-talk between endogenous cues and external signals to coordinate plant development.

The *CLV3/WUS* feedback loop was well characterized in meristem maintenance (5). However, more detailed mechanisms in this process are unclear. It was recently reported that *HECATE1* (*HEC1*) is repressed by *WUS* and in turn represses *CLV3* to fine-tune the balance of stem cell proliferation (29). The present findings add FHY3 as a new player in this regulation system. We propose that *FHY3* indirectly regulates *WUS* expression and stem cell pool maintenance through a direct target gene, *CLV3* (Fig. S6*A*). How *FHY3* coordinates with *HEC1* in terms of stem cell pool maintenance is an open question. Meanwhile, we noticed that the SAM size of *fhy3-68* was small but the SAM size of *fhy3-68 clv3-1* was larger than that of *clv3-1* (Fig. S5 *A* and *B*). Therefore, the possibility that FHY3 directly promotes cell proliferation in the SAM besides the *CLV3* pathway cannot be ruled out because RNA-seq and GO analysis showed that FHY3-induced genes were enriched for "cell cycle" and "DNA replication" genes (Figs. S3*H* and S5*G*).

The small SAM size of *fhy3* as a result of the de-repressed *CLV3* expression and the reduced *WUS* expression was paradoxical to the severe FM indeterminacy of *fhy3 ag-10* with prolonged *WUS* expression, which indicates that *FHY3* may act through a genetic pathway parallel to the *CLV3* pathway in the FM determinacy because the temporal expression of *WUS* is more important than its relative expression level for FM activity regulation (30). Subsequently, this hypothesis was reinforced by the identification of *SEP1* and *SEP2* as FHY3 target genes. Overexpression of *SEP2* but not *SEP1* rescued the FM indeterminacy of *fhy3-68 ag-10*, and reduced *SEP2* expression in *ag-10* resulted in FM indeterminacy similar to those of *fhy3-68 ag-10* (Fig. 3*E* and Fig. S4*E*). It nevertheless remains possible that *SEP1* coordinates *SEP2* in promoting FM determinacy because the *SEP3:SEP2* transgene could only mainly rescue FM indeterminacy of *fhy3-68 ag-10*. Therefore, it is highly possible that other factors may mediate the function of *FHY3* in FM determinacy (Fig. S6*B*). Collectively, the present findings reveal the dual roles of *FHY3* in regulating meristem activity (Fig. S6), and therefore give insights into the mechanisms of SAM maintenance and FM determinacy.

Materials and Methods

All plants were grown in soil and maintained in a greenhouse at 23 °C under LD conditions (16 h of light/8 h of dark). Standard genetic and molecular biology techniques were used for crossing and for the construction of plasmids. Quantitative real-time PCR, in situ hybridization, and ChIP were performed as previously described (9). The primers used for genotyping and construction are listed in Table S1. Details are provided in *SI Materials and Methods*.

ACKNOWLEDGMENTS. We thank Rae Eden Yumul for valuable advice and language editing. This work was supported by National Science Foundation of China (NSFC) Project 31500985 (to M.Z.); National Basic Research Program of China Grant 2014CB138100; NSFC Project 31471168 (to X.L.); CAS Pioneer Hundred Talents Program (to X.L. and R.L.); and NSFC Grants 31401039 (to L.G.) and 91317312 (to L.X.).

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