LEAFY is a pioneer transcription factor and licenses cell reprogramming to floral fate

Run Jin1, Samantha Klasfeld1, Yang Zhu1, Meilin Fernandez Garcia2,3, Jun Xiao1,4,5, Soon-Ki Han1,6, Adam Konkol1 & Doris Wagner1

Master transcription factors reprogram cell fate in multicellular eukaryotes. Pioneer transcription factors have prominent roles in this process because of their ability to contact their cognate binding motifs in closed chromatin. Reprogramming is pervasive in plants, whose development is plastic and tuned by the environment, yet little is known about pioneer transcription factors in this kingdom. Here, we show that the master transcription factor LEAFY (LFY), which promotes floral fate through upregulation of the floral commitment factor APETALA1 (AP1), is a pioneer transcription factor. In vitro, LFY binds to the endogenous AP1 target locus DNA assembled into a nucleosome. In vivo, LFY associates with nucleosome occupied binding sites at the majority of its target loci, including AP1. Upon binding, LFY ‘unlocks’ chromatin locally by displacing the H1 linker histone and by recruiting SWI/SNF chromatin remodelers, but broad changes in chromatin accessibility occur later. Our study provides a mechanistic framework for patterning of inflorescence architecture and uncovers striking similarities between LFY and animal pioneer transcription factor.
Chromatin prevents expression of inappropriate or detrimental gene expression programs, allowing the formation of distinct cell types from the same genome. The basic unit of chromatin is the nucleosome comprised of 147 base-pairs of DNA wrapped nearly two turns around the histone octamer. Chromatin is further compacted by nucleosome/nucleosome interactions and by the linker histone H1, which associates with the dyad (midpoint) of the nucleosome and the linker DNA near the nucleosome entry and exit. During cell fate reprogramming in eukaryotes, master transcription factors silence and activate new gene expression programs in the context of chromatin. While it is easy to imagine how master transcription factors bind to active genes in open chromatin to trigger silencing, it is difficult to envision how these sequence-specific binding proteins can access their cognate motifs in silent chromatin to activate gene expression. This is because nucleosomes are refractory for most transcription factor binding. However, a special class of transcription factors, termed pioneer transcription factors, can access their cognate binding motifs in the nucleosome.

These pioneer transcription factors play important roles in cell fate reprogramming. For example, the mammalian pioneer transcription factor FoxA reprograms fibroblast to hepatocytes, and Sox2 pioneer transcription factors reprogram fibroblasts to induced pluripotent stem cells. Defining criteria for pioneer transcription factors are (1) ability to bind cis motifs in the context of a nucleosome both in vitro and in vivo, (2) facilitating access of additional, non-pioneer, transcription factors to target loci via local chromatin opening and (3) cell fate reprogramming.

Plant development occurs after embryogenesis and is tuned by the environment, a likely adaptation to the sessile lifestyle. Not surprisingly, many master transcription factors that reprogram cell fate have been identified in plants. For example, the bHLH transcription factors MUTE and FAMA reprogram leaf epidermal cells to guard cell fate, while AP2 family transcription factors WOUND INDUCIBLE1 and BABY BOOM cause de-differentiation upon wounding and somatic embryo formation on seedlings, respectively. The NF-Y complex transcription factor LEAFY COTYLEDON1 (LEC1) also promotes embryo fate on seedlings, respectively. Both DNA fragments formed stable nucleosomes, which we further purified using glycerol gradients. The NF-Y complex transcription factor LEAFY (LFY) is necessary and sufficient to trigger flower formation on inflorescences and reprograms cells in roots of growing seedlings to flower fate, when ectopically expressed together with the pluriptency factor WUSCHEL. In root explants, inducible activation of LFY is sufficient to trigger synchronous, abundant flower formation bypassing elaboration of a shoot. Finally, MADS box transcription factors of the SEPALLATA (SEP) family can reprogram cauline leaves into floral organs.

Pioneering activity has been proposed for several of these plant transcription factors, including LEC1. The NF-YB homolog LEC1 has not been tested for pioneer activity in plants. In animals, NF-YB is recruited as a component of the histone fold NF-Y complex, which binds closed chromatin by displacing histones and reprograms cells in roots of growing seedlings to flower fate, when ectopically expressed together with the pluriptency factor WUSCHEL. In root explants, inducible activation of LFY is sufficient to trigger synchronous, abundant flower formation bypassing elaboration of a shoot. Finally, MADS box transcription factors of the SEPALLATA (SEP) family can reprogram cauline leaves into floral organs.

We have previously shown that LFY activates API together with a MYB transcription factor termed LATE MERISTEM IDENTITITY 2 (LMII) in a coherent 'and' logic feed-forward loops. The LFY binding site at the API locus is critical for locus activation and LFY association with this site has been structurally characterized.

Here we use complimentary biochemical, genomic and structural approaches to test whether LFY is a bona fide pioneer transcription factor. We find that in vitro LFY binds with high affinity and specificity to a native regulatory fragment from the API locus in the context of a nucleosome. In vivo, the majority of the LFY bound sites, including that at API, are nucleosome occupied and isolated LFY-associated DNA fragments are co-bound by histones. LFY displaces linker histone H1 and recruits SWI/SNF chromatin remodelers at the API locus, this triggers subsequent changes in chromatin accessibility. Our findings identify LFY as a pioneer transcription factor, link pioneer activity of LFY to competency for cell fate reprogramming and pave the way for understanding the molecular basis for the developmental plasticity of plants.
and with high affinity (Supplementary Fig. 1c, d; Fig. 1c). We next tested LMI2 association with its binding motif in the context of a nucleosome. Unlike LFY, LMI2 was unable to bind its target sequence in a nucleosome even at high molar excess (Fig. 1e). Thus, although LMI2 in vitro shows higher affinity for the naked AP1 locus DNA fragment than LFY, its association with the DNA is blocked by a nucleosome.

**LFY binds nucleosomes in vivo.** We wished to use root explants to test whether LFY can associate with nucleosomes in vivo. This system uses inducible (dexamethasone steroid triggered) nuclear entry of a glucocorticoid receptor hormone binding domain fusion of LFY (35S:LFY-GR) 48. In root explants, unlike in inflorescences, 35S:LFY-GR activation triggers synchronous, abundant, flower formation34 (Supplementary Fig. 2a, b). We characterized the root explant reprogramming system by examining the kinetics of LFY binding and gene activation, focusing on the AP1 locus. LFY binding to AP1 was rapid, with strong occupancy observed already 20 min after dexamethasone application (Supplementary Fig. 2b, c). Robust AP1 induction was first observed 24 h after LFY activation with further increases in AP1 message accumulation until day five (Supplementary Fig. 2d). To probe whether the LFY binding site at the AP1 locus is nucleosome occupied, we conducted micrococcal nuclease (MNase) digestion followed by tiled oligo qPCR. This uncovered a nucleosome centered over the LFY binding site at the AP1 locus at a similar position as in the published dataset (Supplementary Fig. 2e and Fig. 1a).

To assess nucleosome occupancy at LFY bound sites genome-wide, we next conducted chromatin immunoprecipitation (ChIP) and MNase digestion in root explants one hour after steroid or mock treatment (Fig. 2a) followed by sequencing. ChIP-seq with LFY-specific antibodies57 identified 1177 significant (MACS2 summit qvalue \( \leq 10^{-100} \)) LFY binding peaks in the steroid treated tissue (Supplementary Data 1). The quality of the ChIP-seq data was high based on replicate concordance (Supplementary Fig. 3a–c). Moreover, the majority of the LFY binding peaks contained the known LFY consensus motif56,57 under the peak summit (Supplementary Fig. 3d, e). Lastly, a significant fraction of the LFY binding peaks identified in root explants overlapped with those previously identified.

**Fig. 1 The helix-turn-helix transcription factor LFY binds specifically and with high affinity to nucleosomal DNA in vitro.** a Top: Nucleosome at the AP1 regulatory region positioned over a functionally important LFY binding site53, 55. Vertical line: nucleosome mid-point (dyad). Browser screenshot: MNase-seq reads. Below: significant nucleosome (DANPOS q value \( \leq 10^{-100} \)) with color saturation proportional to the signal strength. Short horizontal line: LFY binding motif (consensus core = CCANTGG)53, 54, 57. Bottom: AP1 locus regulatory region with binding site for LFY and for the MYB transcription factor LMI251, 60 and their distance from the transcription start site. b EMSA of LFY binding to native AP1 regulatory DNA assembled into nucleosomes containing a wild-type (left) or a mutated (right) binding motif at the nucleosome dyad. Arrows and drawings on the left indicate nucleosome alone (bottom) and transcription factor nucleosome complex (top). The supershift observed at high molar excess is typical of nucleosomal EMSAs14, 15. The experiment was repeated three times with similar results. c Apparent dissociation constants (KD in nM) of LFY and LMI2 binding based on the decrement of nucleosome (total binding) or the first bound nucleosome (specific binding) as described in Ref. 14. ND: not detectable. d LFY and LMI2 feed-forward loop for transcriptional activation of AP151. e EMSA of LMI2 binding to native AP1 regulatory DNA containing the wild-type binding motif assembled into a nucleosome. The experiment was repeated three times with similar results. See also Supplementary Fig. 1.
during the switch to floral fate\textsuperscript{57} (Supplementary Fig. 3e). To probe nucleosome occupancy, we employed MNase-seq after using both low digestion, which is customarily used to capture ‘fragile’ nucleosomes when investigating pioneer transcription binding to nucleosomes in vivo\textsuperscript{16}, or standard (high) MNase digestion. Nucleosome occupancy was assessed immediately prior to (one hour mock treatment) or after (one hour steroid treatment) LFY nuclear entry. All MNase-seq datasets were of high quality on the basis of characteristic phased nucleosome occupancy (Supplementary Fig. 4a-c).

To identify LFY binding events at nucleosome occupied sites, we called significant nucleosomes using DANPOS (occupancy qval ≤10\textsuperscript{−50}) in the MNase-seq datasets. We next compared LFY binding and nucleosome occupancy in a 2 kb region centered on the significant LFY peaks and ranked on the signal strength of the nucleosomes present under the peak summit region (Fig. 2b, c, Supplementary Fig. 4d). Immediately prior to LFY nuclear entry, most LFY binding sites were nucleosome occupied (68%, Fig. 2b). Similar results were obtained with high MNase digest or when we probed nucleosome occupancy immediately after LFY binding (Fig. 2c, Supplementary Fig. 4d, Supplementary Data 1). The extent of the overlap between LFY binding and nucleosome occupancy (Fig. 2b, c, Supplementary Fig. 4d) is similar to that described for Sox2\textsuperscript{14} and FoxA2\textsuperscript{18}, supporting the notion that LFY can bind to nucleosomal DNA in vivo.

Genes at which the LFY binding site was nucleosome occupied include AP1, as expected (Fig. 2d, Supplementary Fig. 4d). At another known LFY target, REGULATOR OF AXILLARY MERISTEMS 1 (RAX1)\textsuperscript{62}, LFY bound naked DNA (Fig. 2d, Supplementary Fig. 4d). RAX1 promotes meristem growth prior to...
to onset of flower formation. The housekeeping gene ACTIN7 was neither bound by LFY nor had nucleosomes positioned over regulatory regions and was chosen as a negative control locus (Fig. 2d, Supplementary Fig. 4e).

To further test the ability of LFY to associate with nucleosome occupied regions in vivo, we conducted sequential ChIP in root explants using chromatin sonicated to nucleosome sized, 150 base-pair, DNA fragments. After initial immunoprecipitation for LFY, we dissociated the antigen/chromatin complex from the antibodies and subjected the chromatin to a second round of ChIP using a commercial anti-H3 histone antibody. Sequential ChIP uncovered significant enrichment of H3 at AP1, but not at RAX1 or the ACT7 control locus (Fig. 3a). We probed additional loci where the LFY bound site was not (LSH2) or partially (ACR7) nucleosome occupied on the basis of our ChIP-seq and MNase-seq analyses (Supplementary Fig. 4e). Neither LSH2 nor ACR7 displayed H3 enrichment by sequential ChIP (Fig. 3a). The combined data confirm that LFY binds to its target site at the AP1 locus in the context of a nucleosome in vivo.

Because the LMI2 binding motif is very close to that of LFY at the AP1 locus chromatin only after LFY activation (Fig. 4c). Thus, LFY binding to chromatin enriched DNA promotes floral fate in root explants. Pioneer transcription factors enable gene expression changes in the context of closed chromatin by allowing binding of additional non-pioneer transcription factors and by directly or indirectly opening the chromatin at target loci. Analysis of histone modifications in root explants revealed that in the absence of LFY, both the AP1 and the RAX1 loci are marked by the repressive histone modification H3K27me3, while no H3K27me3 was present at the ACT7 locus (Fig. 5a). Conversely, ACT7 was significantly associated with the active H3K4me3 histone modification, which was absent from the AP1 and RAX1 loci (Fig. 5a). To monitor gene expression changes triggered by LFY, we next conducted a time-course RNA-seq analysis 1, 6, or 24 h after dexamethasone or mock treatment in root explants (Supplementary Fig. 7). Transcriptomes of dexamethasone and mock treated root explants began to differ from each other 24 h after LFY activation.
RAX1 was upregulated significantly 6 h (weakly) or 24 h (strongly) after steroid application, while AP1 was significantly and strongly induced only 24 h after dexamethasone application (Fig. 5b, Supplementary Fig. 7b). In total we identified 5, 33, and 302 LFY bound and differentially expressed genes 1, 6, or 24 h after LFY activation, respectively (DESeq2 q-value ≤ 0.01 Supplementary Fig. 7c). The direct LFY regulated targets in root explants overlapped significantly with known direct LFY targets (Supplementary Fig. 7c)57. We next divided these direct LFY regulated targets into those where the LFY binding site was nucleosome occupied and those where the LFY binding site was nucleosome free. The two groups of genes showed similar expression fold changes in response to dexamethasone treatment at all timepoints assayed (Supplementary Fig. 7d). Despite their similar behavior, on the basis of gene ontology term enrichment analysis (AgriGO2 GOslim) only LFY regulated targets where LFY bound to a nucleosome occupied binding sites were linked to flower development (Fig. 5c, Supplementary data 2). Thus, LFY binding in the context of nucleosome-enriched chromatin activates flower related genes in root explants.

LFY triggers local chromatin opening. The helix-turn-helix DNA binding domain of LFY has structural similarity with linker histone H1 (Fig. 6a) and like LFY, the linker histones contacts the nucleosome near the dyad (Fig. 1a)3. Linker histones compact chromatin and H1 loss triggers precocious flowering in Arabidopsis3,70. To probe for chromatin opening by LFY, we therefore next determined occupancy of the H1 linker histone in the absence and presence of LFY. Anti H1 ChIP-qPCR 24 h after LFY activation compared to mock treatment revealed a strong reduction of linker histone occupancy at the LFY binding site of the AP1 locus, but not at RAX1 or ACT7 loci. Thus, LFY binding leads to loss of H1 linker histone at AP1. In developing flowers, LFY directly recruits the BRAHMA and the SPLAYED SWI/SNF chromatin remodeling ATPases to overcome Polycomb repression for flower patterning71. It is not known whether LFY recruits SWI/SNF complexes to activate floral fate. After introducing a tagged version of SWI3B—a core component of both the BRAHMA and the SPLAYED SWI/SNF complexes72—into LFY-GR plants, we examined SWI3B occupancy 24 h after mock or dexamethasone treatment. LFY activation lead to significant SWI3B recruitment to the AP1 locus,
but not to RAX1 or ACT7 (Fig. 6c, Supplementary Fig. 5c). Thus, LFY initiates local chromatin changes upon associating with its nucleosome bound target sites at the API locus. Finally, we tested for broad changes in chromatin accessibility at known DNase hypersensitive sites. We conducted formaldehyde assisted Identification of regulatory elements (FAIRE) followed by quantitative PCR at the API, RAX1 and ACT7 loci. We did not observe increased accessibility at any of the loci tested 24 h after LFY activation (Fig. 6d, Supplementary Fig. 5d). However, 5 days after LFY activation, we observed a significant increase in chromatin accessibility at the API locus, but not at RAX1 or ACT7 (Fig. 6e, Supplementary Fig. 5e). The delayed chromatin opening is consistent with the continued increase in API expression until day five after LFY upregulation (Supplementary Fig. 2d).

LFY DNA contact helix and nucleosomal binding sites are characteristic of pioneer transcription factors. Studies of animal pioneer transcription factors have highlighted structural properties of the DNA recognition moieties of transcription factors critical for pioneer activity. In particular, pioneer transcription factors have short DNA recognition helices, which make contacts on one face of the DNA, leaving more than 50% of the DNA surface free to interact with histones in a nucleosome. Indeed, such short anchoring alpha helices are frequently found in strong nucleosome binders like FoxA. Since ETT/ARF3 preferentially bind naked DNA (Supplementary Fig. 6a), we wished to examine the structure of its DNA contact domain bound to DNA. Structural data is available only for DNA contact domains of AUXIN RESPONSIVE FACTOR1 (ARF1), which is closely related to ARF3/ETT. The ARF1 monomer or homodimer DNA binding domain is comprised of beta sheets and disordered loops that interact with one face of the DNA (Supplementary Fig. 6b). Compared to LFY, the ARF1 DNA contact domains extend further into the DNA, especially in the ARF1 dimer (Supplementary Fig. 6b). Whether these ARF1 contacts would preclude simultaneous histone interactions is not clear. We conclude that LFY associates with cognate motifs in the context of nucleosomes and that this may be enabled by structural properties of its DNA contact helix.

Most pioneer transcription factors bind target sites in the context of nucleosomes as well in free DNA in vivo. However, the types of binding motifs they associate with in each case often differ. Binding sites in free DNA tend to be closer in sequence to the consensus motif, while sites bound in nucleosomes generally deviate more from the consensus. LFY binds a palindromic sequence, which at its core has the sequence CCANTGG. As described for Oct4 and Sox2, the top LFY motif identified by de novo motif analysis from the naked DNA more closely resembles the consensus than does the top motif identified from the nucleosome occupied binding sites.
**Fig. 6** LFY displaces histone H1 and recruits chromatin remodelers.  

**a** Comparison of the structure of linker histone H1 (PDB: 5NL0) and of the LFY DNA binding domain of LFY. Red: All base contacting residues, including the LFY anchoring helix. Warm pink and red: helix-turn-helix DNA binding domain of LFY. Gray: remainder of the LFY C-terminal domain. Turquoise: H1 linker histone.  

**b** Histone H1 ChIP-qPCR at the LFY bound sites of AP1, RAX1 and ACT7 24 h after dexamethasone (Dex) or control (Mock) treatment. Black dots: means from n = 2 independent biological experiments.  

**c** ChIP-qPCR of the SYD and BRM SWI/SNF complex subunit SWI3B at the LFY bound sites of AP1, RAX1 and ACT7 24 h after dexamethasone (Dex) or control (Mock) treatment.  

**d, e** FAIRE qPCR at known DNase hypersensitive sites of the loci indicated 24 h after LFY activation (d) or 5-days after LFY activation (e) relative to the control (Mock treated plants). Black dots: means from n = 2 independent biological experiments. See also Supplementary Fig. 5.

**Fig. 7** LFY DNA contact helix and motif preference.  

**a** Structure of the known pioneer transcription factor FoxA (PDB: 5X07 [https://www.rcsb.org/structure/5X07]), and LFY monomer (top right) or dimer (bottom) (PDB: 2VY1) bound to DNA. Red: All base contacting residues, including the DNA anchoring helix. Arrows delineate the DNA region contacted.  

**b** Position weight matrices of top motifs identified by de novo motif analysis (HOMER) in naked DNA (LFY motif (free); p value = 1E-43) or in nucleosomes (LFY motif (nucl.); p value = 1E-48). The nucleosomal LFY motif diverges more from the known CCANTGG56, 57 core consensus motif. Asterisk: Center of the palindrome. See also Supplementary Fig. 6.
In enhanced chromatin accessibility, strong transcriptional unlocking sets the stage for the chromatin to initiate a series of events, including binding of additional, non-pioneer, transcription factors and further recruitment of chromatin regulators, that collectively result in enhanced chromatin accessibility, strong transcriptional activation and altered cell fate (Fig. 8). Pioneer activity may be especially important for target genes with restricted spatio-temporal expression that commit cells to new fates.

LFY is a master regulator of onset of flower formation and can reduce time to formation of the first flower in trees from decades to months. Although LFY alone is sufficient to activate AP1, accumulation of this floral commitment factor is delayed relative LFY activity. Under floral inductive conditions in inflorescences, AP1 upregulation occurs 2-3 days after that of LFY. The delay in AP1 upregulation is of biological significance as it enables formation of branches prior to the irreversible switch to flower fate. The duration of the delay in flower formation tunes the inflorescence architecture to environmental cues to enhance reproductive success. Molecularly, the delayed AP1 upregulation is attributable—at least in part—to a requirement for co-factors activated by LFY in ‘and’ logic feed-forward loops (FFLs). Such FFLs not only make biological processes more robust to noisy stimuli such as environmental cues, but they also represent temporal delay elements. In one FFL, LFY activates LMI2, which upregulates AP1 together with LFY. In another FFL, LFY directly triggers reduced accumulation of bioactive gibberellin hormone; this stabilizes the DELLA/SQUAMOSA BINDING PROTEIN9 (SPL9) transcriptional complex, which activates AP1 in parallel with LFY.

Combining these and additional prior findings with those of our current study, we propose that the pioneer transcription factor LFY establishes competency for cell fate reprogramming to floral fate by associating with the nucleosome occupied binding site at the AP1 locus where it opens chromatin locally, concomitant with initial (low level) AP1 upregulation (Fig. 8, Supplementary Fig. 2d). This local chromatin opening may be the result of direct changes in DNA-histone contacts caused by LFY, as described for other pioneer transcription factors. Alternatively, local chromatin opening may be caused by changes in nucleosome-linker or nucleosome–nucleosome interaction due to linker histone H1 displacement by LFY (Fig. 5c) or by SWI/SNF mediated chromatin remodeling (Fig. 5d). That LFY ‘unlocks’ the chromatin at the AP1 locus in this manner is supported by the critical role of the LFY binding site for AP1 locus activation. As a result, LFY pioneer activity enables binding of additional (likely non-pioneer) transcription factors; these include not only LMI2, but also SPL9/DELLA and theflorigen activator complex, which all bind near the LFY binding site at the AP1 locus to activate gene expression. We propose that the activity of these LFY co-factors, in a manner not yet understood, contributes to further opening of the AP1 locus chromatin, enables strong AP1 upregulation and reprogramming to floral fate (Fig. 8).

In summary, the delay between LFY pioneer factor binding and local chromatin opening at AP1 on one hand, and broad locus accessibility and strong AP1 upregulation on the other, is likely attributable to the timed accumulation (FFLs) and hierarchical recruitment of the LFY co-factors LMI2, SPL9/DELLA and theflorigen activator complex to the AP1 locus. This ensures correct timing of the irreversible switch to flower formation, which is critical for reproductive success.

Animal pioneer transcription factors play important roles in developmental reprogramming in vivo, generation of iPS cells and trans-differentiation, enabled by their unique ability to bind cis motifs even when buried deep in the nucleosome. While first ‘rules’ or characteristics of pioneering activity of transcription factors are emerging, it is far from clear what their unique or defining set of properties is. For example, some pioneering factors act cooperatively with other transcription factors, but it is not known how widespread such interactions are. The LFY pioneer transcription factor shares many properties with animal pioneer factors in addition to...
the ability to bind nucleosomes in vitro and in vivo. Like the pioneer transcription factors Oct4, Ebf1, and Rap178,92, LFY recruits SWI/SNF chromatin remodeling complexes to key target loci. Like Foxa3,106, LFY displaces linker histone H1 in vivo. Like the pioneer transcription factors Pax7,43, PHA-497, and Rap178, LFY rapidly associates with nucleosome-occupied binding sites at target loci, but chromatin opening is delayed relative to binding. Finally, the LFY DNA contact helix shares structural properties with strong nucleosome binders95 and LFY binds weaker motifs in nucleosomes, as described for Oct4 and Sox294. Unique properties of the LFY pioneer transcription factor include its ability to bind both fragile and stable nucleosomes (this study) and to contact a consensus motif half site as a monomer in vitro and in vivo4,46,57. Combined with the fact that the LFY binding site is palindromic54,56,57, this enables LFY to bind its cognate motif in a nucleosome even if the DNA is rotated 180° to face the histone octamers (altered rotational nucleosome positioning)96,97. Moreover, LFY bound sites cluster around the transcription start site57, which enhances nucleosome positioning96,97. These combined characteristics make the LFY pioneer transcription factor a uniquely well-suited candidate to license key developmental transitions. Plants evolved multicellularity independently from animals and can change their final body plan in response to environmental cues98, suggesting that pioneer transcription factors may be more prevalent in this kingdom. Our study sets the stage for future engineering for enhanced cell fate reprogramming.

Methods

**Plant materials.** 35S::LFY-GR is in the Arabidopsis thaliana wild-type accession Landsberg erecta88. LMI2Δ in binary vector pMDC99 and SWI3B-3xHA in binary vector pGWB1100 were transformed into 35 S::LFY-GR plants. For experiments in inflorescence, plants were grown in soil at 22 °C short-day photoperiod (SD, 8 h light/16 h dark, 120 µmol/m2s). For all other experiments, plants were grown on 1/2 MS plates (half strength Murashige and Skoog medium supplemented with 0.5 g/L of MES monohydrate, pH = 5.7, 0.8% phytoagar) at 22 °C in long-day photoperiod (LD, 16 h light/8 h dark, 100 µmol/m2s). Arabidopsis seeds were stratified at 4 °C for 3 days. To obtain root explants, roots from 3-week-old seedlings were harvested and placed on callus inducing medium (CIM) (3.08 g/L Gamborgs B5 salts, 20 g/L sucrose, 0.5 g/L MES, pH = 5.7, 1.5 % BSA, 0.05 mg/L vitamin A, 0.04 mg/L B1, 0.1 mg/L C5, 0.05 mg/L C4, and 0.1 mg/L C3) and a further incubation for 6 h. For each sample, 2 grams of tissue was harvested at the end of day 5 on CIM. LMI2 ChIP-qPCR in inflorescence, for

each sample, 2 grams of shoot apices from 25-day-old, short-day grown LMI2Δ/ΔSS5: LFY-GR plants were harvested from 4 trays of plants. After trimming, shoot apices were frozen in 5 µL dexamethasone or mock solution for 18 h followed by addition of 5 µM beta-estradiol and a further incubation for 6 h. For LFY ChIP-qPCR and ChIP-seq, 35 S::LFY-GR root explants were treated with 5 µM dexamethasone for 1 hour prior to the end of day 5 on CIM. For each sample, 1.2 g of root explant tissue was harvested. For H1 and SWI3B-3xHA ChIP-qPCR, root explants were treated with 5 µM dexamethasone for 24 h. For each sample, 0.6 and 1.2 g of tissue was harvest for H1 and SWI3B ChIP-seq, respectively. At least two biological replicates were generated for each experimental condition.

ChIP was conducted using our protocol106 and the following antibodies: rabbit polyclonal anti-LFY antibody (Abcam, 65294), anti-LFY antibody (ROCHE, mouse anti-HA antibody (Abcam, 21175), Mouse IgG2b Anti-HA antibody (Abcam, 45640), rabbit anti-histone H1 antibody (Abcam, 61177), rabbit anti-histone H3 antibody (Abcam, 18521), and rabbit anti mouse IgG (Abcam, 46450). ChIP-qPCR was performed using Platinum Taq DNA Polymerase (Invitrogen, 10966034) and EvaGreen dye (Biotium, 31000). Inputs from each sample were used to generate the standard curve to compute sample enrichments106. Primer sequences for ChIP-qPCR are listed in Supplementary Table 1.

For sequential-ChIP, four ChIP reactions from 0.5 g root explant tissue each were combined into one biological replicate. Three such biological replicates were analyzed in 24 h steroid application (Fig. 3a). LFY bound sites were used to obtain chromatin fragments of ~150 bp to probe LFY and histone occupancy. The first (LFY) overnight immunoprecipitation and the subsequent wash steps were performed as in a published protocol106. The eluted chromatin was next immunoprecipitated for histone H3 or IgG following an established protocol85 using commercial antibodies (above). The anti-H3 or IgG chromatin bound to 5 µL Tris- HCl pH 8.0, 5 mM EDTA, 20 mM DTT, and 1% SDS at 37 °C for 30 min.

**ChIP-seq and data analysis.** For LFY ChIP-seq, each biological replicate was generated by pooling four individual ChIP reactions (each consisting of 0.6 g of root explant tissue) prior to DNA purification on MinElute PCR columns (Qiagen, 28040). Three biological replicates were sequenced for each condition. Dual index libraries were prepared for the six ChIP samples (three mock and three treatment) and three input samples using the SMARTer TruPlex DNA-Seq Kit (Takara Bio, R04046). After quantifying libraries using NEBNext Library Quant Kit for Illumina (NEB, E7330), libraries were pooled based on desired read depth. Single-end sequencing was conducted using NextSeq 550/500 High Output Kit v2 (Illumina, TruSeq v3) on the NextSeq platform (Illumina).

After trimming adapter sequences and low-quality reads by Trimomatic (v0.32)107, FastQC (v0.11.5) was performed on trimmed reads to confirm high-quality sequences108. Sequencing reads were then mapped to release 10 of the Arabidopsis Genome (TAIR10)109 by Bowtie2 (−phred33 -q -v 2.3.11)110. Next, uniquely mapped reads (quality score MAPQ ≤ 10) (Samtools v1.3.11)111 were filtered for low quality reads and mapping were done as described for LFY ChIP in root explants (above). For inflorescence ChIP-seq peaks, peaks were called using MACS2 (v2.1.2)112,113 (−keep-dup auto −峰 height 10−10) as described for LFY ChIP in root explants. A total of 30 cycles of sonication (summit q-value ≤ 10−10) were identified in the pooled dexamethasone-treated samples using the pooled mock-treated samples as controls in MACS2 (v2.1.2)112,113 (−keep-dup auto —nomodel —extsize 138 −call-signals - g 101724395). This yielded 1177 significant LFY peaks. For quality control, Spearman correlation coefficients of the reads in the LFY treatments of each biological replicate with the pooled control and the HF16 (summit phred33 -q -v 2.3.11)111 de novo motifs analysis was conducted using HOMER v4.114,115 for MACS2 q-value ≤ 10−10 peak signals (±150 base pairs) compared to genome matched background (unbound regions from similar genomic locations as the peak summits), for this example see Refs. 73,117. LFY ChIP-seq data (15-day-old 35S::LFY)146 was retrieved from GEO dataset GSE64245. Two replicates were analyzed. Inflorescence ETChIP-seq data was obtained from EBI-ENA database accession number PRIXEB9862. Three biological replicates and one negative control file were analyzed. Trimming, FastQC, filtering (low quality reads) and mapping were done as described for LFY ChIP in root explants (above). For inflorescence ChIP-seq, peaks were called using MACS2 (v2.1.2)112,113 using the same parameters as for the ChIP-seq analysis in root explants. Since no control files were available, we used more stringent criteria for peak calling (MACS2 summit q-value ≤ 10−46). This identified 1092 significant LFY binding peaks. For ETChIP ChIP-seq, peaks were called using (summit q-value0.01)119 as described for LFY ChIP in root explants. A total of 670 significant peaks were identified.

Histone modification ChIP-seq data from root explants cultured for 14 days on CIM106 were retrieved from DDBI Sequence Read Archive (DRA) under the accession number DRA000110, and was analyzed in the Columbia ecotype. Trimming, FastQC, filtering for low quality reads and mapping were done as described for LFY ChIP-seq (above). For all histone modification ChIP-seq, peaks were called using MACS2112,113 callpeak command -f BAM —call-summits —keep-dup auto —nomodel —extsize 138 -g 101724395, using the histone H3 file as the control file and the respective histone modification file as the treatment file. For H3K4me3 and H3K27me3 peaks, q-value ≤ 10−10 was used for significance calling and a total of 6032 and 1359 peaks were identified, respectively.

**FAIRE-qPCR.** For FAIRE- ChIP-seq, 35S::LFY-GK root explants were treated with 5 µM dexamethasone or mock solution for five days or for the last 24 h on CIM. For treatment

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Protein purification, nucleosome assembly and EMSA. pE-SUMOpro-LFY and pE-SUMOpro-LFY-GR were expressed in RosettaTM DE3 (Novagen, 71397) and purified using Ni-NTA affinity chromatography (GE healthcare, 17115301). Protein concentrations were determined by SDS-PAGE, using a BSA standard curve run on the same gel. Nucleosomes were reconstituted in 10 mM Tris-HCl pH 8, 50 mM NaCl, 1.0 mM MgCl2, 50 μM dithiothreitol, 0.5 μg/ml BSA, HEPES, and H4, which share 80–95% amino acid identity with their Arabidopsis counterparts, were expressed and purified.128 A 152 bp fragment region from the API regulatory region (TAIR10 Chr1:25986457–25986608) with the LFY binding site in the center and containing the LMI2 binding site served as wild type nucleosomal template. The LFY binding site was chemically changed from AA (GAGGAGACAGGTCGCGTA) to GAGGACAAAATGTAACCGCA, while the LMI2 binding site mutated fragment was then assembled around core histones by stepwise dialysis with decreasing concentration of salt and urea as previously described.14,15 Initially, assembled nucleosomes were run on a native gel to see whether multiple nucleosome bands formed—an additional 2 h at 42°C heat shift was performed if multiple nucleosome conformations were present as previously described.14,15 Glycerol gradients of the dialyzed assembled nucleosomes were employed to separate free DNA from nucleosomes and the fractions collected were then dialyzed in 10 mM Tris–HCl pH 7.5 for 2 h. DNA was purified using the QIAquick gel extraction kit (Qiagen, 28115). Puriﬁed DNA was diluted 50 times for qPCR analyses.

DNA and nucleosome binding reactions were performed using established procedures.14,15 Briefly, Cy-5 labeled DNA fragments and nucleosomes were diluted to 10 nm concentration in EMSA buffer (10 mM Tris–HCl (pH 7.5), 1 mM MgCl2, 10 μM ZnCl2, 1 mM DTT, 10 mM KCl, 0.5 mg/ml BSA). Serial dilutions of RNAse A were added to EMSA buffer to achieve the desired concentrations (ranging from 1 nm to 500 nm depending on the reaction). To test for RNAse A, 10 pl of diluted proteins of various concentrations were added to 10 μl of Cy-5 labeled DNA or nucleosomes. Reactions were then incubated at room temperature for 30 min in the dark followed by analysis in 5% nondenaturing polyacrylamide gels at 100 V for 75 min.

Dissociation constants. Apparent KdS were calculated from two separate EMSA binding curves per sample, each representing one independent experiment. Image analysis was conducted using image J.127 The experimental data was analyzed using the ‘non-linear regression’ function with ‘One Site – Total’ in GraphPad Prism (v8.0) software.14,15 Bmax less than 1 and R2 values between 0.8 and 0.99 were met to ensure actual fit of data.14,15,128 Kd was either computed from the reduction in DNA or nucleosome bound fractions (designated total KD) or based on the first appearance of a DNA or nucleosome bound complex (designated specific KD) as in Ref.14,15.

RNA-seq and data analysis. RNA-seq, two biological replicates were generated for each treatment (mock or steroid) and time point (1, 6, or 24 h treatment). Replicates were treated with either 0.5 μM dexamethasone or DMSO as RNA extraction for 24 h, 6 h, or 1 h before the end of the 5-day incubation on CIM plates. RNA from each sample (ca. 0.2 g) was purified using the RNeasy mini kit (Qiagen, 74104) after TRIzol (Invitrogen, 15596026) extraction.129 RNA secondary structure was removed by a 5 min 65°C incubation followed by immediate cool down. mRNA was selected with OligoT25 dynabeads (Invitrogen, 610-02). Reverse transcription was performed using the SSII RT kit (Invitrogen, 18800-044) followed by end repair of cDNA using an enzyme mixture of T4 PNK and T4 DNA polymerase (Enzymatics Y9140-LC-L). After generating a 3 A-overhang by Klenow HC (Enzymatics, P7010-HC-L), adapters were ligated with T4 DNA ligase (Enzymatics T4 DNA ligase (Rapid) #1603-HC-L, 600 U/µl). One-sided selection with SPRI select beads (Beckman Coulter, B23317) was conducted before library ampliﬁcation with P5 and P7 index primers. Library quantiﬁcation was performed with the Nextseq Library Quant Kit for Illumina (NEB, E7630). Single-end sequencing was conducted using Nextseq 550/500 High Output Kit v2 (Illumina, TG-160-2005) on the NextSeq500 platform (Illumina). Quality control and ﬁltering were identical to the ChIP-seq analysis. Mapping of reads was performed using the Bowtie software.125,126,127 The experimental data was analyzed using the ‘non-linear regression’ function with ‘One Site – Total’ in GraphPad Prism (v8.0) software.14,15,128 Bmax less than 1 and R2 values between 0.8 and 0.99 were met to ensure actual fit of data.14,15,128 Kd was either computed from the reduction in DNA or nucleosome bound fractions (designated total KD) or based on the first appearance of a DNA or nucleosome bound complex (designated specific KD) as in Ref.14,15.

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expressed genes 1, 6, and 24 h after dexamethasone relative to mock treatment, respectively.

Peak annotation and dataset comparisons. Significant LFY ChIP peaks were annotated to release 11 of Arabidopsis genome annotation (Araport11)131. Two rounds of annotation were performed. First, all peaks that were 3 kb upstream, or within 100 kb regions were annotated to that gene. Second, orphan peaks were annotated to the nearest LFY dependent gene within 10 kb of the peak. LFY dependent genes are defined as genes that displayed rapid changes in gene expression after LFY-GR activation (this study and Ref. 12). Plant GOSim analyses were performed in AgrIGO v2.0.135.

Structural analysis of DNA binding domains. Structures of linker histone H1 (PDB: 5NL0), LFY (PDB: 2YF1), FoxA2 (PDB: 5X07) and ARF1 (PDB: 4LDX) were visualized and aligned using Pymol v2.3 (method=super, 5 cycles, cutoff = 2.0)136.

Statistical analysis and replication. For all qPCR data, the Kolmogorov–Smirnov (K–S)136 test was implemented to assess normal distribution of the data. Since all data were normally distributed, unpaired one-tailed t-tests were used to test whether changes in one specific direction were statistically significant and two-tailed t-tests were used to test changes in any direction. Error bars represent standard error of the mean (SEM) of at least two independent biological replicates. The hypergeometric test137 was used to test whether two datasets significantly overlapped.

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 the paper and its supplementary information files. The ChIP-seq, MNA-seq and RNA-seq datasets generated in this study are available at the GEO repository under accession number GSE141706. Source data are provided with this paper.

Code availability

Scripts for peak to gene annotation: https://github.com/kladveld/ChIP_Annotation.

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Author contributions

D.W., R.J., and S.K. conceived of the study. S.K. and R.J. conducted the bioinformatic analyses. R.J. and Y.Z. performed all wet lab experiments. J.X. contributed to genomic analyses, M.E.G. assisted with the nucleosomal EMISAs and A.K. contributed to HPY purification. S.-K.H. generated the gSWI3R-SHHA construct. D.W. wrote the paper with input from all authors.

Competing interests

The authors declare no competing interests.

Additional information

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Correspondence and requests for materials should be addressed to D.W.

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