Temporal dynamics of gene expression and histone marks at the *Arabidopsis* shoot meristem during flowering

Yuan You¹, Aneta Sawikowska², Manuela Neumann¹, David Posé¹, Giovanna Capovilla¹, Tobias Langenecker¹, Richard A. Neher³, Paweł Krajewski² & Markus Schmid¹,⁴

Plants can produce organs throughout their entire life from pluripotent stem cells located at their growing tip, the shoot apical meristem (SAM). At the time of flowering, the SAM of *Arabidopsis thaliana* switches fate and starts producing flowers instead of leaves. Correct timing of flowering in part determines reproductive success, and is therefore under environmental and endogenous control. How epigenetic regulation contributes to the floral transition has eluded analysis so far, mostly because of the poor accessibility of the SAM. Here we report the temporal dynamics of the chromatin modifications H3K4me3 and H3K27me3 and their correlation with transcriptional changes at the SAM in response to photoperiod-induced flowering. Emphasizing the importance of tissue-specific epigenomic analyses we detect enrichments of chromatin states in the SAM that were not apparent in whole seedlings. Furthermore, our results suggest that regulation of translation might be involved in adjusting meristem function during the induction of flowering.

¹Department of Molecular Biology, Max Planck Institute for Developmental Biology, Spemannstrasse 35, 72076 Tübingen, Germany. ²Department of Biometry and Bioinformatics, Institute of Plant Genetics, Polish Academy of Sciences, Strezszyńska 34, 60-479 Poznań, Poland. ³Evolutionary Dynamics and Biophysics Group, Max Planck Institute for Developmental Biology, Spemannstrasse 35, 72076 Tübingen, Germany. ⁴Umeå Plant Science Centre, Department of Plant Physiology, Umeå University, SE-901 87 Umeå, Sweden. †Present address(es): Zentrum für Molekularbiologie der Pflanzen, Eberhard-Karls-Universität Tübingen, Auf der Morgenstelle 32, Tübingen D-72076, Germany (Y.Y.); Department of Statistical and Mathematical Methods, Poznań University of Life Sciences, Wojska Polskiego 28, 60-637 Poznań, Poland (A.S.); Facultad de Ciencias, Departamento de Biología Molecular y Bioquímica, Instituto de Hortofruticultura Subtropical y Mediterránea, Universidad de Málaga—Consejo Superior de Investigaciones Científicas, Universidad de Málaga, 29071 Málaga, Spain (D.P.); Biozentrum, University of Basel, Klingelbergstrasse 50/70, CH—4056 Basel, Switzerland (R.A.N.). Correspondence and requests for materials should be addressed to M.S. (email: markus.schmid@umu.se).
In contrast to most animals, plants have the capacity to produce new organs due to the life-long maintenance of populations of pluripotent stem cell in specialized reservoirs called shoot apical meristems (SAM), from which the aerial parts of a plant, stems, leaves and flowers, are derived. This endows plants with the ability for continued growth, and also allows them to adjust rapidly and flexibly to changes in their environment, which is of particular importance considering the sessile life style of plants. At the time of flowering, the SAM of Arabidopsis thaliana undergoes a remarkable phase transition and becomes an inflorescence meristem (IM) that induces on its flanks flower meristems, from which floral organs will differentiate (Supplementary Fig. 1a). At the same time the central IM needs to remain in an undifferentiated state to ensure continued growth.

The correct timing of flowering is crucial to ensure reproductive success and is therefore of both adaptive and economic value. Genetic analyses have identified a number of molecular pathways that perceive and integrate diverse endogenous and environmental signals to ensure that flowering commences under the most favourable conditions. Prominent examples are the vernalization pathway that regulates flowering in response to prolonged cold (overwintering) and the photoperiod pathway that in A. thaliana promotes flowering in long-day (LD) conditions. Day length is perceived in the leaves and through a complex regulatory mechanisms that involves light signalling and the circadian clock results in the stable expression of the transcription factor (TF) CONSTANS (CO) protein specifically at the end of the LD. CO in turn activates the expression of FLOWERING LOCUS T (FT) and TWIN SISTER OF FT (TSF) in the phloem companion cells of the leaf vasculature.

While we understand the function of many of the individual genes in this circuit relatively well, genome-wide analyses have been complicated by the fact that flowering time signals are integrated in specific cell types such as the phloem companion cells in leaves and the SAM. Several studies have investigated the transcriptional changes during the initiation of flowering and early flower development at the SAM by either hand dissection or laser-capture micro-dissection of meristems, followed by transcription profiling using either microarrays or RNA-seq. These studies provided insights into the changes in expression at the SAM during the floral transition in a tissue-specific manner and identified previously uncharacterized genes with a potential role in flowering time regulation. However, samples generated by these methods are not well suited for epigenomic analyses as they generally contain only small amounts of chromatin. This is unfortunate, as epigenetic factors clearly play an important role in flowering time control. For example, expression of the important flowering time integrator genes FT, FLC in the phloem companion cells of leaves and FLOWERING LOCUS C (FLC) in response to vernalization have both been shown to be controlled in part at the chromatin level. However, these studies have been performed on complex tissues or whole seedlings and little is known about the epigenetic processes that regulate flowering time locally, mostly because of the poor accessibility of tissues such as the SAM. It is challenging to isolate enough high quality SAM samples for multiple study approaches in spatio-temporal developmental programs.

To overcome this limitation we have adopted INTACT, which employs in vivo biotinylation of nuclear envelopes to facilitate purification of nuclei from specific tissues, to describe the temporal dynamics of the chromatin modifications H3K4me3 and H3K27me3, which have been implicated in promoting and repressing gene expression, respectively, and their correlation with transcriptional changes at the SAM during photoperiod-induced flowering. Emphasizing the importance of tissue-specific analyses we observe narrow peaks of H3K27me3 close to or even covering the transcription start sites (TSS) of thousands of loci, a chromatin feature rarely observed in epigenomic studies conducted on complex tissues. Our results also suggest that control of translation could be an important factor in regulating meristem function during the induction of flowering.

### Results

#### Establishment of INTACT for the SAM

In order to study transcriptional responses and the dynamic of epigenetic modifications at the shoot meristem during the floral transition we first established a modified INTACT reporter line that employs two promoters to biotinylate nuclear envelopes in the entire SAM and IM (Fig. 1a,b and Supplementary Fig. 1c; Methods section). Enrichment of SAM marker genes such as SHOOT MERISTEMLESS (STM), CLAVATA3 (CLV3) and FD in nuclei isolated from the INTACT line was verified by semi-quantitative RT–PCR (Fig. 1c, Supplementary Figs 1e and 2). Synchronous flowering of the INTACT reporter line was accomplished by shifting 21-day-old plants from non-inductive short days (SD) to inductive LD conditions (Supplementary Fig. 1b). Two to three days after the shift, we consistently detected by RNA in situ hybridization robust expression of the flower marker gene AP1, indicating floral commitment (Fig. 1d,e and Supplementary Fig. 1d). Furthermore, quantitative reverse transcription PCR (qRT-PCR) using nuclear RNA isolated from meristems of plants transferred from SD to LD demonstrated a significant increase in the expression of the meristematic flowering time integrators SOC1 and AGAMOUS-LIKE 24 (AGL24) within 24 h after the shift (Fig. 1f). Taken together these initial results indicate that our INTACT line constitutes a system suitable for the study of photoperiod-controlled floral transition at the SAM.

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**Figure 1 | Establishment of the INTACT system for the meristem.**

(a,b) Detection of biotinylation and (d,e) expression of the floral marker gene AP1 at the meristem of the INTACT line before (a,d) and 3 days after (b,e) the shift to LD. Scale bar, 50 μm. (c) Semi-quantitative RT-PCR detecting expression of STM, CLV3 and FD in the total nuclei from the samples enriched for shoot apical region by manual dissection (input) and in the nuclei isolated by INTACT from the meristem (INTACT). (f) Quantitative RT-PCR of SOC1 (light grey) and AGL24 (dark grey) in the nuclei from the meristem before (O), and 1, 2 and 3 days after the shift to LD. Error bars, values of two biological replications.
Differential gene expression at the SAM during flowering.

Having established INTACT for the SAM we next performed RNA-seq of the nuclear RNA pool isolated from the meristem for four biological replicates 0, 1, 2 and 3 days after the shift to LD. In total we detected expression (FPKM > 1) of ~60% (19,940) of all genes annotated in *Arabidopsis thaliana* at the SAM at least at one time point (Supplementary Data 1), which is comparable to previous reports. The meristem identity genes (KI, KNO, STM, KNOTTED-LIKE FROM ARABIDOPSIS THALIANA 1 (KNAT1), CLAVATA 1 (CLV1) and CLV3) were stably detected in all samples (Fig. 2a), indicating the presence of mRNA originating from meristems and confirming results from the semi-quantitative RT–PCR analysis (Fig. 1c). In contrast, the early floral homeotic genes (AP1, APETALA 3 (AP3) and CAULIFLOWER (CAL)), and genes expressed in the centre or abaxial side of developing leaves such as JAGGED (JAG), WUSCHEL RELATED HOMEBOX 1 (WOX1) and WOX3, were basically undetectable (Fig. 2b,c), indicating strong enrichment for mRNA from the SAM.

Pairwise comparison at the four time points identified 298 significantly differentially expressed genes, including 148 and 146 genes that were consistently up- or down-regulated, respectively, and 4 genes that exhibited more complex expression patterns (Supplementary Data 2). Interestingly, expression of the morning-expressed genes of the central oscillator of the circadian clock (LATE ELONGATED HYPOCOTYL (LHY) and CIRCADIAN CLOCK ASSOCIATED 1 (CCA1)) was increased significantly 1 day after the shift to LD (Fig. 2d). In contrast, the evening-expressed clock gene (PSEUDO-RESPONSE REGULATOR 5 (PRR5) was significantly down-regulated. LUX ARRHYTHMO (LUX) and EARLY FLOWERING 4 (ELF4), which encode components of the evening complex, displayed a similar trend as PRR5, but the expression changes were not statistically significant after correction for multiple testing (Fig. 2d). These rapid changes in transcript levels most likely reflect a change in the phase of clock gene expression in response to the shift from SD to LD rather than a change in amplitude. Increased expression following the shift to LD was also detected for SOCI, FD and the CCAAT-binding TF NF-YA4, which are known to promote flowering (Fig. 2e). The expression changes determined by RNA-seq for SOCI confirm previous results from qRT-PCR experiments (Fig. 1f), emphasizing the reproducibility of the INTACT method. In addition to induction of floral promoters we also observed decreased expression for the flowering-time repressor *IMJ30* (ref. 25; Fig. 2e). Interestingly, expression of SHEPHERD (SHD), which is required for stem cell functions and affects the expression of CLV3 and WUSCHEL (WUS) and is responsible for the

**Figure 2 | Differential gene expression in the meristem during flowering.** (a–h) Expression (counts of reads) of marker genes for the meristem (a), leaf primordia (b), flower primordia (c), circadian clock (d), flowering time (e), stem cell (f), translation (g) and lateral organ boundary (h) at the SAM before (blue), and 1 (green), 2 (red) and 3 (purple) days after the shift to LD. Error bars ± s.e. based on four biological replicates; *P<0.05, **P<0.01 (Deseq2 P values corrected for multiple testing using the Benjamini-Hochberg method). (i) Biological GO functions enriched for differentially regulated genes with increased (red) and decreased (blue) expression. Only significantly enriched GO terms (P<0.05) are reported.
formation of functional CLAVATA proteins, was also significantly decreased upon shift to LD (Fig. 2f). The shd mutant is characterized by an increase in stem cell number and an enlarged meristem and a decrease in SHD expression would thus be in agreement with the growth of the meristem that accompanies the floral transition. Taken together these findings indicate that it is possible to capture changes in gene expression of important flowering time and meristem identity genes in the nuclear RNA pool isolated by INTACT from the SAM.

Gene ontology (GO) analysis confirmed that genes related to long-day photoperiodism (GO:0048574) and regulation of circadian rhythm (GO:0042754) were significantly overrepresented among the genes induced at the SAM (Fig. 2i and Supplementary Data 3). In contrast, down-regulated transcripts were significantly enriched for genes related to ribosome biogenesis (GO:0042254) and the control of translation (GO:0006412; Fig. 2i). Affected genes include the translation initiation factor EIF4A1, the ribosomal RNA (rRNA) processing factor DIFFERENTIATION AND GREENING-LIKE (DAL), and the ribosomal structural constituents PIGGYBACK 1 (PGY1), PGY2 and RPL23aA (Fig. 2g, Table 1). Interestingly, mutations in the PGY genes and RPL23aA have previously been shown to cause (or enhance) developmental phenotypes including defects in leaf polarity and inflorescence architecture, and late flowering. Taking together these results suggest a connection between the control of translation and the regulation of developmental phase transition at the SAM.

### Changes in H3K4me3 at the SAM correlate with gene expression

To study the correlation between gene expression and chromatin features during floral transition we next analysed the genome-wide distribution of tri-methylation on lysine 4 or 27 of histone 3 (H3), epigenetic marks that are often associated with transcriptional activation (H3K4me3) and repression (H3K27me3), respectively. ChIP-seq was performed on nuclei isolated from the SAM at the four time points for two biological replicates using H3K4me3- and H3K27me3-specific antibodies (Supplementary Fig. 3).

H3K4me3 was detected on 61.6% (16,746) of protein-coding genes at least at one time point, and was mostly (81.5%; 13,647) associated with the TSS (Fig. 3a, Supplementary Fig. 4a and Supplementary Data 4). We observed a significant positive association between the presence of H3K4me3 on the TSS and gene expression, as (on average over four time points) the proportion of genes marked with H3K4me3 on the TSS was 5.02% (± 0.31%; ± refers to s.e., n = 4) and 66.13% (± 1.68; ± refers to standard error, n = 4) for the non-expressed and expressed genes (FPKM >1), respectively (Fig. 3b and Supplementary Fig. 5b). Furthermore, for genes expressed at a given point in time, the level of H3K4me3 signal in the TSS was significantly (Spearman rank correlation ranging from 0.29 to 0.33 over the four time points, P <0.001, test based on t approximation) correlated with the level of gene expression (Supplementary Fig. 4b). For example, H3K4me3 was significantly enriched at the TSS of the SAM marker gene STM in the SAM samples (Fig. 4d). Over the course of the floral induction, 2,024 genes displayed significant changes in H3K4me3 signal over the gene body at the SAM (Supplementary Data 5). The levels of H3K4me3 changes were positively correlated with the significance of gene expression changes (Fig. 3c and Supplementary Fig. 4b), and 56 genes changed significantly both in H3K4me3 signal and in expression, mostly (89.3%) in the same direction (Supplementary Fig. 6a). The mean expression of genes that increased in H3K4me3 upon shift from SD to LD (pairwise

#### Table 1 | Significantly differentially expressed structural constituents of ribosome and genes for RNA-binding proteins.

| ID      | Gene     | Expression change | Chromatin state* | Annotation                        |
|---------|----------|-------------------|------------------|-----------------------------------|
| AT1G01000 | RPP1A    | Dec               | E                | 60S acidic ribosomal protein P1-1 |
| AT1G01930 | RPS12A   | Dec               | E                | 40S ribosomal protein S12-1       |
| AT1G41880 | RPL35A-B | Dec               | E                | 60S ribosomal protein L35a-2      |
| AT2G19730 | RPL28A   | Dec               | E                | 60S ribosomal protein L28-1       |
| AT2G36160 | RPS14A   | Dec               | E                | 60S ribosomal protein S14-1       |
| AT2G39460 | ATRPL23A | Dec               | E                | 60S ribosomal protein L23a-1      |
| AT3G1920  | EIF4A1   | Dec               | E                | Eukaryotic initiation factor 4A-1 |
| AT3G22230 | RPL27B   | Dec               | E                | 60S ribosomal protein L27-2       |
| AT3G49910 | RPL26A   | Dec               | E                | 60S ribosomal protein L26-1       |
| AT3G53460 | CP29     | Dec               | E                | 29 kDa ribonucleoprotein, chloroplastic |
| AT3G53740 | RPL36B   | Dec               | E                | 60S ribosomal protein L36-2       |
| AT4G15000 | RPL27C   | Dec               | E                | 60S ribosomal protein L27-3       |
| AT4G16390 | SVR7     | Dec               | E                | Pentatricopeptide repeat-containing protein, |
| AT4G18100 | RPL32A   | Dec               | E                | 60S ribosomal protein L32-1       |
| AT5G09320 | NFD1     | Dec               | E                | 50S ribosomal protein L21, mitochondrial |
| AT5G02870 | RPL4D    | Dec               | E                | 60S ribosomal protein L4-2        |
| AT5G16130 | RPS7C    | Dec               | E                | 40S ribosomal protein S7-3        |
| AT5G18380 | RPS16C   | Dec               | E                | 40S ribosomal protein S16-3       |
| AT5G23740 | RPS11-BETA| Dec              | E                | 40S ribosomal protein S11-3       |
| AT5G27850 | RPL18C   | Dec               | E                | 60S ribosomal protein L18-3       |
| AT3G02830 | ZFN1     | Inc               | E                | Zinc finger CCCH domain-containing protein, |
| AT1G33120 | RPL9B    | Dec               | None             | 60S ribosomal protein L9-1        |
| AT1G33400 | P5G2     | Dec               | None             | 60S ribosomal protein L9-B        |
| AT2G27530 | PGY1     | Dec               | None             | 60S ribosomal protein L10a-2      |
| AT3G55570 | RPL35AD  | Dec               | None             | 60S ribosomal protein L35a-4      |
| AT3G60770 | RPS13A   | Dec               | None             | 40S ribosomal protein S13-1       |
| AT3G62250 | UBQ5     | Dec               | None             | Ubiquitin-40S ribosomal protein S27a-3 |
| AT3G13740 | MMM17.15  | Dec/Inc           | None             | Ribonuclease III family protein   |
| AT3G12915 |         | Inc               | None             | Ribosomal protein S5/Elongation factor G/III/V |

*Genes that were in H-state, E-state (E) or none of the two (None) at least at one time point.
comparisons: 0 –> 1; 0 –> 2; and 0 –> 3) increased significantly (P<0.05; test based on rejecting zero slope of linear regression of mean expression against time; Supplementary Fig. 6b), whereas the expression changes observed in the remaining comparisons, while generally following the same trend, were not significant. In summary, our findings suggest a general congruence between H3K4me3 dynamics and gene expression.

GO analysis of the 2,024 genes that exhibited significant changes in H3K4me3 identified 12 overrepresented biological processes (Fig. 3d, Supplementary Data 6). For example, H3K4me3 changed within 24 h after the shift to LDs on genes that form the central oscillator of the circadian clock, as also seen at the transcript level (Fig. 2d), followed by signal receptors, TFs (Supplementary Fig. 7), chromatin remodelers, and categories which play important regulatory roles in cell division, differentiation and developmental phase transitions (Fig. 3d, Supplementary Data 6). Together these data establish changes in H3K4me3 abundance as a fast and important response in the regulation of genes at the SAM during the transition to flowering.

H3K27me3 is a weak predictor of gene expression at the SAM. Opposing the effect of H3K4me3, which is often enriched at the TSSs of genes and promotes expression (Fig. 3a,b and Supplementary Fig. 5), is H3K27me3, a canonical repressive epigenetic mark16,17. At the SAM H3K27me3 was detected on 63.69% (17,328) of protein-coding loci at least at one time point (Fig. 5a and Supplementary Data 7). This is in contrast to reports from analyses of complex tissues or whole seedlings, which had reported H3K27me3 on less than 10,000 loci31. In agreement with published findings16,17 broad regions of H3K27me3 were
observed on 40.56% ± 0.95% (average over the four time points; ± refers to s.e., n = 4) transcriptionally silent genes (FPKM < 1, for example Fig. 4a–c). In addition, we also detected an enrichment of H3K27me3 on the TSS of 15.84% ± 0.93% (average over the four time points; ± refers to s.e., n = 4) of expressed genes in the SAM (Fig. 5a,b, and Supplementary Fig. 8a,b). The fraction of non-expressed genes (FPKM < 1) was increased in the group of genes carrying H3K27me3 mark on the TSS when compared to the other groups (Fig. 5b, Supplementary Fig. 8b). For expressed genes (FPKM > 1), the H3K27me3 signal at the TSS was formally negatively correlated with the level of gene expression, though the Spearman rank correlation coefficient was rather low (0.07–0.08 over the four time points, P < 0.001, test based on t approximation). Changes in H3K27me3 at the TSS were not significantly correlated with changes in gene expression between time points (Fig. 5c and Supplementary Fig. 8c). The 472 genes that displayed significant changes in H3K27me3 signal over the gene body during the course of the floral transition (Supplementary Data 8) were not enriched for any biological and molecular functions in GO analysis.

Previous studies had suggested a negative correlation between gene expression level and H3K27me3 abundance31,32. However, our data indicate that during the transition to flowering changes in gene expression at the SAM are not always reflected in changes

Figure 4 | Examples of H3K4me3 and H3K27me3 dynamics at the SAM during the floral transition. (a–i) Integrative Genomics Viewer traces of AP1 (a), JAG (b), FT (c), STM (d) KNAT2 (e), KNAT6 (f), PTL (g), FLC (h), DAL (i), RPL27C (j), SOC1 (k), SNZ (l). H3K4me3, H3K27me3 and H3 signals are depicted in blue, green and red, respectively. Horizontal bars indicate regions and signals of significant enrichment of H3K4me3 and H3K27me3 (according to analysis in MACS2).
in H3K27me3 signals (Fig. 4). For example, the activation of SOC1 expression in response to LD is accompanied by deposition of H3K4me3 mark whereas H3K27me3 levels remain essentially unchanged (Fig. 4k).

Opposing H3K4me3-H3K27me3 modification states at the SAM. Overlapping regions of H3K27me3 and H3K4me3 have been described in plants before, both on individual genes as well as in genome-wide analyses. In the meristem, co-occurrence of H3K4me3 and H3K27me3 was mainly detected within ±500 bp around the TSS of expressed genes (Fig. 5a; Supplementary Fig. 9a), and two categories of H3K4me3-H3K27me3 states could be distinguished (Fig. 6c,d; Supplementary Fig. 9b, Supplementary Fig. 10, Supplementary Data 9): broad H3K27me3 regions containing a narrow H3K4me3 peak around the TSS (±500 bp), to which we refer as ‘harbouring’ state (H-state); and an ‘embedded’ state (E-state) with opposite characteristics, that is, narrow H3K27me3 peaks within broader H3K4me3 domains. 28.3% of regions that displayed H-state characteristics in the SAM were also detected in a control data set obtained by performing ChIP-seq in whole seedlings (Fig. 6a,c; Supplementary Data 10). In contrast, the embedded chromatin state was almost exclusively detected in INTACT samples obtained from the SAM and was rarely detected in whole seedlings, demonstrating the power of tissue-specific epigenomic studies (Fig. 6b,d; Supplementary Data 10).

Functions and dynamics of H- and E-chromatin state genes. To gain a better understanding of the potential differences in molecular functions of the H- and E-chromatin states at the TSS we performed GO enrichment analyses (Supplementary Data 11). We found that genes with TF (GO:0003700) and DNA binding activity (GO:0003677) were overrepresented among the genes that were in H-state (Fig. 7a). Over the course of floral induction about half of the H-states present in the vegetative meristem resolved into non-overlapping, partial overlapping or E-states, respectively, demonstrating the dynamic nature of this chromatin state (Fig. 7b). Genes include, for example, the homeobox TFs KNAT2 and KNAT6, and the trihelix TF PTL that are involved in meristem and lateral organ boundary development. We observed that during floral transition H3K4me3 signal levels for these genes increased, while H3K27me3 domains decreased in length, and that these epigenetic changes were accompanied by increased expression (Fig. 2h). At the end of the floral transition the H-state of PTL became partial overlapping (H3K27me3 > H3K4me3), and KNAT2 and KNAT6 had converted to E-state (Fig. 4e–g).

In contrast, GO term analysis revealed that structural constituents of ribosome (GO:0003735), involved in translation (GO:0006416) and genes that encode for RNA-binding proteins (GO:0003732; GO:0044822) were overrepresented among the E-state genes (Fig. 7a). In this context it is worth remembering that genes involved in ribosome biogenesis, rRNA processing, and in the initiation of translation were significantly enriched among the genes whose expression in the SAM decreased during the transition to flowering (Table 1 and Fig. 2i).

Interestingly, several ribosomal protein genes were reported to express at the highest levels in meristems and young dividing tissues and at the lowest in non-dividing tissues, and mutants of these r-protein genes exhibit multiple developmental lethal/defect phenotypes. Our result suggested that regulation at the translation level might play important roles for fate decision of the IM, however changes in expression of these translation factors are unrelated with H3K4me3 and H3K27me3 modification levels. Overall, the E-chromatin state was mostly stable (Figs 4i,j and 7b).

Discussion

Meristems are of interest to plant developmental biology as they harbour stem cells that divide slowly and give rise to daughter cells that eventually differentiate into all other cell types. Despite their importance it is still unclear what exactly distinguishes undifferentiated meristem cells from their differentiated descendants. It seems likely that regulation at the epigenetic and chromatin level contributes to the differentiation of cells derived from the meristem and there is mounting evidence that different cell types exhibit distinct epigenetic features. For example, unique DNA-methylation patterns have recently been reported for different cell types in the root apical meristem. How other epigenetic marks such as acetylation or methylation of histones contribute to the function of meristems and how dynamic these modifications respond to change in the environment is, however, poorly understood.
Our analysis of the dynamic changes of two histone modifications, H3K4me3 and H3K27me3, and their correlation with transcription during the transition to flowering has identified two unusual chromatin states at the SAM. Both of these chromatin states, to which we refer as H- and E-state, display high levels of H3K27me3 at the TSS. In particular the E-state, which is characterized by narrow peaks of H3K27me3 within wider regions of H3K4me3, and which we observe at the four time points analysed in total on more than 9,000 genes stands apart, as H3K27me3 at the TSS has previously been only observed at much lower frequency. Importantly, the E-state might be rather specific to the undifferentiated cells at the SAM as it was not apparent in another INTACT study, which had analysed hair and non-hair cell in the root epidermis, and was also largely absent from the whole-seedling data. Together, these findings provide evidence for SAM-specific chromatin states at the TSS, emphasizing the value of and need for tissue- (or even cell type-) specific epigenomic analyses.

Genes in E-state were significantly enriched for GO categories related to ribosome function and translation. Similarly, ribosomal proteins and translation initiation factors were also overrepresented among the genes whose expression decreased during flowering. Several examples demonstrate the importance of regulation of translation in animal and plant development, and some ribosomal proteins have even been suggested to possess a non-ribosome function. In Arabidopsis thaliana, many ribosomal proteins, including PGY1, PGY2 and RPL23aA, are preferentially expressed in meristems and young dividing tissues, and loss of function of these genes causes pleiotropic developmental defects. Taken together, our results could suggest that control of translational activity might contribute to the regulation of fate transition of the SAM. Finally, RNA-binding proteins that mediate 18S rRNA biogenesis have been shown to be preferentially expressed in mouse embryonic stem cells (ESC) where they play a critical role in sustaining the protein levels of labile pluripotency factors and are required for efficient reprogramming of induced pluripotent stem cells. It is tempting to speculate that, similar to the situation in animal ESCs, control of global translation rates could be involved in maintaining pluripotency of the plant meristem.

Reprogramming of epigenetic states is critical for initial establishment and subsequent maintenance of lineage-specific transcriptional programs during the differentiation of cells. In agreement with the important role of transcriptional reprogramming, we have identified 226 TFs, many of which are known to play important roles in regulation flowering and other developmental processes, among the genes that display significant changes in H3K4me3 at the SAM during the transition to flowering (Supplementary Fig. 7). This includes TFs related to circadian clock function such as REVEILLE (RVE1), CCA1 and LHY, whose expression significantly change together with H3K4me3 changes (Supplementary Fig. 7) and that all affect long-day photoperiodism and flowering. However, for most of these TFs we observe only small changes in expression (Supplementary Fig. 11), including the important flowering time regulator SOCI, which are not statistically significant after correcting for multiple testing (Supplementary Data 2). Moreover, a few of these TFs do not change in their expression, for example, the flowering time repressor SCHNARCHZAPFEN (SNZ), whose H3K4me3 level was significantly decreased at the TSS (Figs 2e and 4 and Supplementary Data 2). Our gene expression data and qRT-PCR experiments support the notion that expression of developmentally regulated genes in the SAM is usually rather low. However, if expression of at least some of these genes were confined to a small sub-population of cells in the SAM this could also result in an underestimation of their actual expression changes. Also we cannot completely rule out the possibility that our low-input RNA-seq method, which involves 2-step cDNA amplification, limits our ability to detect changes of expression changes. Also we cannot completely rule out the possibility that our low-input RNA-seq method, which involves 2-step cDNA amplification, limits our ability to detect changes of weakly expressed genes. Nevertheless, our results suggest that changes in H3K4me3 are important early events in the regulation of (and might even precede) changes in gene expression at the SAM during the initiation of flowering.

Other known flowering time genes do change significantly in their expression at the SAM in response to LD. One example is JMJ30, which encodes a Jumonji-C domain-containing H3K6me2 demethylase. One of the confirmed targets of JMJ30 is FT. However, since FT is not expressed at the SAM, JMJ30 likely exerts its effect on flowering at the SAM through other targets. JMJ30 has also been shown to be under direct transcriptional control of CCA1 and LHY, two key components of the central circadian oscillator, which directly bind to JMJ30 and are required for efficient reprogramming of induced pluripotent stem cells. Another example is the CCAAT-motif binding TF, NF-YA4, which has
been shown to mediate H3K27me3 demethylation of the SOC1 promoter to facilitate flowering\(^1\). In our analysis, expression of NF-YA4 was significantly decreased at the SAM, however, no changes were observed for H3K27me3 on SOC1, which was broadly covered from TSS to TTS throughout the entire experiment (Fig. 4k). Instead, our data suggest that activation between plants and animals. In our study the H-state is reminiscent of the bivalent situation in ESCs, and TFs were overrepresented among the genes that were in H-state. Unfortunately, sequential ChIP-seq (or -PCR) is at this point not really feasible given the very limited amount of starting material available from INTACT, so that the nature of the overlapping H3K4me3 and H3K27me3 signals at the SAM cannot be answered conclusively at this time.

**Methods**

**Oligonucleotides.** Sequences of oligonucleotide primers used in this work are listed in Supplementary Table 1.

**Plant growth conditions.** *Arabidopsis thaliana* accession Col-0 was used in this work. To synchronize germination and floral transition, all seeds were stratified before sowing in 0.1% agar at 4°C for 3 days in the dark. Seeds were sown on soil and all plants were grown in growth chambers in a controlled environment, at 23 °C with 65% relative humidity. The light condition was constructed by a mixture of Cool White and Gro-Lux Wide Spectrum fluorescent lights, with a fluence rate of 125–175 μmol m\(^{-2}\) s\(^{-1}\). LDs were 16 h of light and 8 h of dark, and SDs were 8 h of light and 16 h of dark. In shift experiments for floral induction plants were grown in SDs for 21 days before shifting to LDs.

**Plasmid constructs and the SAM-specific INTACT reporter line.** The *E. coli* biotin ligase (BirA) gene was cloned in a modified Gateway entry plasmid pCR8/GW-TOPO (pDEP084), and subsequently recombined into a pGREEN-IIS based destination vector (pFK273) that harbours the *Arabidopsis thaliana* pUBQ10 (At4g05320) promoter, using a modified Gateway recombination system\(^1\). The pUBQ10::BirA construct (pDP085) was transformed into Col-0 plants making use of Agrobacterium tumefaciens strain A5E and the floral dip method\(^3\). Homozygous pUBQ10::BirA lines were identified by selective germination on soil watered with 0.1% glucose (BASTA). For INTACT, a modified red nuclear envelope-targeting protein (RedNTF), consisting of the WPP domain (amino acids 1-111) of the *Arabidopsis thaliana* RAN GTPASE ACTIVATING PROTEIN 1 (RanGAP1; At3g63310) at the N terminus, followed by red fluorescent protein (mCherry) for visualization, and the biotin ligase recognition peptide at the C terminus was prepared by PCR using GL2p::NTF\(^3\) as template. The RedNTF open reading frame was cloned in a modified Gateway entry plasmid (pPLSmart) to create pYY1204. For expression of RedNTF at the SAM, two pGREEN-IIS based destination vectors were used. pTY64 consisted of a 1,393 bp promoter fragment of At3g59270, which was cloned in front of the attR1-attR2 Gateway recombination cassette, followed by the At3g59270 terminator sequence (1,404 bp) and drives expression in the entire meristem except for the stem cells. pFK321 consisted of a 1,444 bp promoter sequence of CLAVATA 3 (CLV3) in the front of the attR1-attR2 cassette, followed by the CLV3 terminator sequence (1,253 bp) and drives expression of the reporter in the stem cell population at the shoot meristem. pAt3g59270::RedNTF::pAt3g59270 construct (pYY1208) and pCLV3p::RedNTF::CLV3 construct (pYY1301) were generated by recombination of

Figure 7 | H- and E-state genes at the SAM during floral transition. (a) Molecular GO functions enriched for H-state, E-state and H/E-state genes. Only significantly enriched GO terms (P < 0.05; fold-change > 1.5) are reported. (b) Matrix of chromatin state transitions from 0-3 LDs for non-overlapping (Non), H-state, and E-state genes, and genes with partial overlapping H3K27me3-H3K4me3 marks (K27 > K4; K4 > K27). Colours indicate the fraction of genes in a given category on 3 LD, starting from the 0 LD sample (n = number of genes in each category in the 0 LD sample).
pY1204 into pY364 and pK621, respectively. pY1208 and pY1301 were subsequently transformed into homologous pUSB16-B:Rb plants to generate CV3 and At3g59270 RedNTF reporter lines. The individual RedNTF reporters were identified by selective germination of T2 seeds on ½ MS agar plates containing 50 μg ml⁻¹ of kanamycin. INTACT lines that carried both reporters were created by genetic crossings and double-homozygous lines that expressed RedNTF in the meristem were selected by PCR-based genotyping and used in subsequent experiments. PCR reactions were performed using Phusion polymerase or Taq polymerase (New England Biolabs), and all constructs were verified by Sanger sequencing after cloning.

In situ RNA hybridization and Immunohistochemistry. The digoxigenin-labeled anti-sense RNA probe to APETALA 1 (API) mRNA was prepared using a published plasmid25. Shoot apices from SD-grown plants (21 days) and plants shifted to LD for 2, 3, or 7 days were fixed and embedded in formaldehyde/acetic acid/ethanol (3.7%/5%/50%). Paraffin-embedded material was sectioned to 8 μm thickness and RNA in situ hybridization was performed as described27. To detect in vivo biotinylation by immunohistochemistry sections were dewaxed and rehydrated by applying 1:2,000 diluted streptavidin alkaline phosphatase (Promega) in 1x TBS (0.1% Triton X-100). Permeabilization and immunohistochemical staining were carried out at the room temperature for 1 h 30 min. The signal was developed by a color reaction using NBT-BCIP solution (Roche).

Microscopy. A ZEISS Axioplan 2 imaging system was used for imaging the histological sections and for examining the quality and quantity of purified nuclei after each INTACT experiments using DAPI and Mcherry filters. In addition, nuclei isolated from the INTACT reporter line were also imaged using a Zeiss LSM510 confocal laser-scanning microscope.

INTACT purification of SAM nuclei. For each biological replicate, ~6,000 INTACT reporter plants were grown in SDs for 21 days before shifting to LDs. The 0 LD, 1 LD, 2 LD and 3 LD samples were collected on the 21st day in SDs and 1, 2 and 3 days after the shift to LD. For each sample, the shoot apical region was manually dissected from ~1,300 INTACT reporter plants and frozen immediately in Eppendorf tubes suspended in liquid nitrogen. Each sample collection took about 3 h from ZTS to 8 and samples were stored at ~80°C from processing. The INTACT experiments were carried out as described23 with the following modifications. Briefly, the 1,300 frozen apices were homogenized to a fine powder with mortar and pestle pre-chilled in liquid nitrogen. Approximately 1/3 of the powder was suspended in lysis buffer (NP-20 lysis buffer; NP-20 M MOPS (pH 7), 40 mM NaCl, 90 mM KCl, 2 mM EDTA, 0.5 mM EGTA, 0.5 mM spermidine, 0.2 mM spermine, 1 × Complete protease Inhibitors (Roche)), for isolating non-fixed nuclei by INTACT for transcriptome analysis by RNA-seq. The remaining approximately 2/3 of the ground tissue was suspended in 10 ml of ice-cold NP buffer containing 1% (vol/vol) formaldehyde (NPB) and placed under vacuum at RT for 10 min to cross-link proteins to DNAs. The cross-linking was stopped by adding 2 M glycine to a final concentration of 125 mM and incubated for an additional 5 min under vacuum. The fixed nuclei from the SAM were isolated by INTACT and subsequently used to analyze histone modifications by ChiP. Nuclei were filtered through 40 μm cell strainers (BD Falcon) and pelleted at 1,000 × g for 5 min. Nuclei were washed with 1 ml of NPB, pelleted again by centrifugation and finally resuspended in 1 ml of NPB. For control ChIP-seq experiments using 3-weeks-old LD-grown seedlings, nuclei were suspended in 1 ml NPB, C (Thermo Scientific) before the RNA was purified a second time using the RNeasy Micro kit (Qiagen). The concentration and yield of the purified cDNA was determined using the RNA 6,000 Pico Kit (Agilent). The double-stranded cDNA was synthesized from ~500 pg RNA by linear amplification using the SMARTer Ultra Low Input RNA Kit (Clontech) according to the manufacturer’s instructions. The concentration and yield of the amplified cDNA was determined using the High Sensitivity DNA Kit (Agilent).

Quantitative PCR. The enrichment of the meristic genes in the isolated nuclei after the INTACT purification was examined by semi-quantitative PCR. ~100 pg amplified double-stranded cDNA prepared from the input and purified 3 LD samples was used in PCR reactions to examine the expression of STM, CLV3 and APETALA 1 (API) mRNA, for and the expression of non-fixed nuclei after the INTACT purification was examined by semi-quantitative PCR. After 40 cycles the PCR products were separated on a 1.5% agarose gel followed by ethidiumbromide staining to visualize DNA. Real-time qPCR of SOC1 and AGL24 was performed from ~100 pg amplified double-stranded cDNA, using the Platinum SYBR Green qPCR Supermix-UDG (Invitrogen, Life Technologies) and gene-specific oligonucleotides (Supplementary Table 1) on a BioRad C1000 Touch Thermal Cycler. The relative expression values were normalized over the mean expression value of 0 LD samples and calculated by the ΔΔCT method using TUB2 as control. Because amplification signals from the 0 LD SOC1 samples did not cross the detection threshold, the Ct values was set to 40, the number of cycles used in the PCR programme, to be able to conservatively estimate the differences in expression. Two biological replications with 3 technical replications per sample were used for qPCR analysis.

RNA sequencing. For transcriptome profiling, 1–2 ng of amplified double-stranded cDNA was sheared to 200–500 bp fragments in water using a focused ultrasonicator (Covaris S2 system), using microtubes at a power of 10, 35, 500 cpsi for 40 s. Subsequently the Low Input Library Prep Kit (Clontech) was used for preparation of sequencing libraries. Libraries were validated using the High Sensitivity DNA Kit (Agilent), and sequencing was performed on an Illumina HiSeq 2,000 system. Approximately 30 million 2 × 101 base-pair-end reads that passed the Illumina quality control were collected for each sample in four biological replications (Supplementary Table 2).

Chromatin immunoprecipitation and sequencing. For ChiP-seq experiments using INTACT-purified nuclei, chromatin was prepared from ~10,000 purified fixed nuclei per sample as described above. ChiP was performed as described25 with the following modifications. Chromatin was sheared to 100–500 bp fragments in 200 ng lysis buffer (50 mM Tris, pH 8.0, 10 mM EDTA, 1% SDS, 1 × complete protease inhibitors (Roche)) using a focused ultrasonicator (Covaris S2 system), using microtubes at a power of 10, 5, 200 cpsi for 3 min. The sheared chromatin was divided into three aliquots at the ratio of 1:2:3 for immunopurification with 0.5 μl anti-H3 histone (Millipore, Cat.17-10254, Lot. 2051404), 1.5 μl anti-H3K4me3 antibody (Millipore, Cat.17-614, Lot.1973237), and 2.5 μl anti-H3K27me3 antibody (Active Motif, Cat. 39155, Lot. 2582104), respectively. The specificity and sensitivity of the antibodies was verified by western blot using nuclear protein extracts as described25, and by dot-blot analysis using peptide containing non-, mono-, di- and tri-methylated versions of the respective residues (Abcam, ab2623, ab2903, ab1340, ab7768, ab1342 and ab1780–1782), according to a protocol provided by the manufacturer. Approximately 50 pg of ChiP DNA per sample was isolated using the MinElute Reaction Cleanup Kit (Qiagen), the ChiP-seq libraries were generated using an Illumina MiSeq Chip Prep Kit (Illumina), and linear amplification was monitored by quantitative PCR including SYBR Green 1 (Invitrogen). Libraries were quantified using the KAPA Library Quantification Kit (KAPABIOSYSTEMS), and further evaluated on a high sensitivity DNA chip (Agilent).

For control ChiP-seq experiments using 3-weeks-old LD-grown seedlings, chromatin was prepared from ~2 g of fresh material as described25. The ChiP experiments were performed as described above and libraries were prepared from ~5 ng of ChiP DNA using the TruSeq ChiP Library Prep Kit (Illumina). Libraries were quantified on a high sensitivity DNA chip (Agilent).

Libraries were sequenced on Illumina MiSeq, HiSeq 2,000 and HiSeq 3,000 systems for collecting 50 base-pair single-end or 150 base-pair paired-end reads to high sequencing depth60 (Supplementary Table 2). Only Read 1 of 150 base-pair-paired end reads was used and further trimmed to 50 base pair. Uniquely mapped paired-end reads were used for ChiP-seq analyses.

RNA-seq data analysis. Next generation sequencing (NGS) reads were mapped to Arabidopsis thaliana reference transcriptome TAIR10 ver. 24, with ribosomal RNA regions (2,3471–9557; 3,149,175–3,149,203,988) masked, using TopHat 2.0.13 (and Falcon alignments; up to 48 alignments per genome)21. Counts of NGS reads covering transcripts were computed using the function summarizeOverlaps25 in R. Expressed genes were defined as those having the value of FPKM > 1 at least one time point. Read counts were submitted to differential gene expression analysis in Deseq2 (default parameters, FDR < 0.05)24. Regularized logistic regression followed by read count comparison was performed by the function limma in the package llimma for the analysis of relationships between gene expression level and histone modifications signal.
Histone modification data analysis. NGS reads were mapped to Arabidopsis thaliana reference genome TAIR10 ver. 24 using Bowtie2 2.2.4 (minimum alignment score −12.5; uniquely mapped reads only)44. The sets of aligned H3K27me3 and H3 reads were down-sampled to minimum number of reads, respectively, for peak-calling from the same sequencing depth in all samples85. Significant enrichment regions in H3K4me3 and H3K27me3 samples, relative to H3 control samples, were identified using MACS2 2.1.0 (default settings for H3K4me3: FDR < 0.05; broad-peak mode for H3K27me3—FDR < 0.1)46,67. Regions with signal level measured by FPKM CHIP/FPKM(H3 control) differing between biological replications by more than 2 s.d. were filtered out. Consensus regions found as a sum of regions from two replications were taken as histone modification marks. Differential modification analysis for H3K4me3 and H3K27me3 marks was performed using DiffBind66 (Bioconductor 3.2; min overlap = 2; FDR < 0.01). The modification marks were intersected with annotated genes to obtain lists of genes with specific coverage by H3K4me3 and H3K27me3. Spearman rank correlation coefficients were computed and tested for significance in Genstat 18 (ref. 69).

GO term analysis. AmiGO 2 versions 2.3.2 and 2.4.24 (ref. 70) were used for GO analysis to assess the over- and underrepresentation in biological processes of significantly differentially-H3K4me3-modified genes and molecular functions of GO analysis to assess the over- and underrepresentation in biological processes of significantly differentially-modified genes and molecular functions of GO term analysis. Coefficients were computed and tested for significance in Genstat 18 (ref. 69).

Data availability. ChIP-seq and RNA-seq data have been deposited with ArrayExpress database (www.ebi.ac.uk/arrayexpress), accession numbers E-MTAB-4680, E-MTAB-5130, and E-MTAB-5136. The authors declare that all other data supporting the findings of this study are available within the manuscript and its supplementary files or are available from the corresponding author upon request.

References.

1. Weigel, D. & Jürgens, G. Stem cells that make stems. Nature 415, 751–754 (2002).
2. Wilczek, A. M. et al. Effects of genetic perturbation on seasonal life history plasticity. Science 323, 930–934 (2009).
3. Kwiatkowska, D. Flowering and apical meristem growth dynamics. J. Exp. Bot. 59, 187–201 (2008).
4. de Montaigu, A. et al. KNAT6: an Arabidopsis homeobox gene involved in meristem maintenance of pluripotent stem cells. Proc. Natl Acad. Sci. USA 112, 905–910 (2015).
5. Corbesier, L. et al. FT protein movement contributes to long-distance signaling in floral induction of Arabidopsis. Science 316, 1030–1033 (2007).
6. Tomita, K. et al. A MYB-domain protein EFM mediates flowering responses to environmental cues in Arabidopsis. Dev. Cell 30, 437–448 (2014).
7. Ishiguro, S. SHEPHERD is the Arabidopsis GRP94 responsible for the plasticity. Development 135, 1315–1324 (2008).
8. Daum, G. et al. Flowering and apical meristem growth dynamics. EMBO J. 30, 1928–1938 (2011).
9. Deal, R. B. & Henikoff, S. The INTACT method for cell type–specific gene expression and chromatin profiling in Arabidopsis thaliana. Plant Physiol. 180, 653–667 (2016).
10. Rawat, R. et al. Integrative epigenomic mapping defines four main chromatin states in Arabidopsis. EMBO J. 30, 58–68 (2010).
11. Yang, H. et al. FT protein movement contributes to long-distance signaling in floral induction of Arabidopsis. J. Exp. Bot. 59, 187–201 (2008).
12. Yan, Y. et al. A MYB-domain protein EFM mediates flowering responses to environmental cues in Arabidopsis. Dev. Cell 30, 437–448 (2014).
13. Schmid, M. et al. Network discovery pipeline elucidates conserved time-of-day–specific cis-regulatory modules. PLoS Genet. 4, e14 (2008).
14. Pinon, V. et al. Three PIGGYBACK genes that specifically influence leaf patterning encode ribosomal proteins. Development 135, 1315–1324 (2008).
15. Yan, Y. et al. Repression of the floral transition through a distinct polycomb repressive complex. Proc. Natl Acad. Sci. USA 112, 14619–14624 (2015).
16. Bugner, V., Tecza, A., Gessert, S. & Kuhl, M. Peter Pan functions independently of SMZ and complements smz-1 and smz-2 mutants. Development 138, 2369–2378 (2011).
17. Yang, W. et al. Arabidopsis ribosomal protein S13-homologous gene is expressed in the shoot apical meristem and regulates flowering. Development 140, 512–519 (2013).
18. Daum, G., Medizhradszky, A., Suzuki, T. & Lohmann, J. U. A mechanistic framework for noncell autonomous stem cell induction in Arabidopsis. Proc. Natl Acad. Sci. USA 111, 14619–14624 (2014).
19. Abe, M. et al. FT protein movement contributes to long-distance signaling in floral induction of Arabidopsis. J. Exp. Bot. 59, 187–201 (2008).
20. Schmid, M. et al. Dissection of floral induction pathways using global expression analysis. Development 130, 6001–6012 (2003).
21. McCloud, C. R. Wheels within wheels: new transcriptional feedback loops in the Arabidopsis circadian clock. F1000Prime Rep. 6, 2 (2014).
22. Michael, T. P. et al. A mechanistic framework for cell type–specific gene expression and chromatin profiling in Arabidopsis thaliana. Plant Physiol. 155, 906–915 (2010).
49. Hou, X. et al. Nuclear factor Y-mediated H3K27me3 demethylation of the SOC1 locus orchestrates flowering responses of Arabidopsis. *Nat. Commun.* 5, 4601 (2014).

50. Farrona, S. et al. Tissue-specific expression of FLOWERING LOCUS T in Arabidopsis is maintained independently of polycystin group protein repression. *Plant Cell* 23, 3204–3214 (2011).

51. Jeong, J.-H. et al. Repression of FLOWERING LOCUS T chromatin by functionally redundant histone H3 lysine 4 demethylases in Arabidopsis. *PLoS ONE* 4, e8033 (2009).

52. Voigt, P., Tee, W. W. & Reinberg, D. A double take on bivalent promoters. *Genes Dev.* 27, 1318–1338 (2013).

53. Mikkelsen, T. S. et al. Genome-wide maps of chromatin state in pluripotent and lineage-committed cells. *Nature* 448, 553–560 (2007).

54. Mathieu, J., Warthmann, N., Küttner, F. & Schmid, M. Export of FT protein from phloem companion cells is sufficient for floral induction in Arabidopsis. *Curr. Biol.* 17, 1055–1060 (2007).

55. Clough, S. J. & Bent, A. F. Floral dip: a simplified method for Agrobacterium-mediated transformation of Arabidopsis thaliana. *Plant J.* 16, 735–743 (1998).

56. Galvao, V. C., Horrer, D., Kuttner, F. & Schmid, M. Spatial control of flowering by DELLAs proteins in Arabidopsis thaliana. *Development* 139, 4072–4082 (2012).

57. Wang, J.-W., Czech, B. & Weigel, D. miR156-regulated SPL transcription factors define an endogenous flowering pathway in Arabidopsis thaliana. *Cell* 138, 738–749 (2009).

58. Copenhagen, G. P. et al. Polycomb repressive complex 2 controls the embryo-to-seedling phase transition. *PLoS Genet.* 7, e1002141 (2011).

59. Gendrel, A.-V., Lippman, Z., Martienssen, R. & Colot, V. Profiling histone modification patterns in plants using genomic tiling microarrays. *Nat. Methods* 2, 213–218 (2005).

60. Sims, D., Sudbery, I., Iltott, N. E., Heger, A. & Ponting, C. P. Sequencing depth and coverage: key considerations in genomic analyses. *Nat. Rev. Genet.* 15, 121–132 (2014).

61. Trapnell, C., Pachter, L. & Salzberg, S. L. TopHat: discovering splice junctions with RNA-Seq. *Bioinformatics* 25, 1105–1111 (2009).

62. Lawrence, M. et al. Software for computing and annotating genomic ranges. *PLoS Comput. Biol.* 9, e1003118 (2013).

63. Love, M. I., Huber, W. & Anders, S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biol.* 15, 550 (2014).

64. Langmead, B. & Salzberg, S. L. Fast gapped-read alignment with Bowtie 2. *Nat. Methods* 9, 357–359 (2012).

65. Jung, Y. L. et al. Impact of sequencing depth in ChIP-seq experiments. *Nucleic Acids Res.* 42, e74 (2014).

66. Zhang, Y. et al. Model-based analysis of ChIP-Seq (MACS). *Genome Biol.* 9, R137 (2008).

67. Kellis, M. et al. Defining functional DNA elements in the human genome. *Proc. Natl Acad. Sci. USA* 111, 6131–6138 (2014).

68. Stark, R. & Brown, G. D. DiffBind: differential binding analysis of ChIP-seq peak data. Bioconductor http://bioconductor.org/packages/release/bioc/vignettes/DiffBind/inst/doc/DiffBind.pdf (2011).

69. VSN International. GenStat for Windows18th edn http://www.vsni.co.uk/ (2015).

70. Carbon, S. et al. AmiGO: online access to ontology and annotation data. *Bioinformatics* 25, 288–289 (2009).

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**Author contributions**

Y.Y. and M.S. designed the experiments. Y.Y. performed most experiments and analysed data; A.S. analysed RNA-seq and ChIP-seq data; M.N. selected homozygous transgenic INTACT line, assisted Y.Y in INTACT experiments and quantified sequencing samples; D.P. prepared UBQ10:BiRa and redNFT entry constructs; G.C. performed in situ RNA hybridization to detect API transcript; T.L. generated the meristem-specific expression vector; R.N. participated in data analyses and discussion; P.K. supervised RNA-seq and ChIP-seq analyses; Y.Y. and M.S. supervised the work and wrote the manuscript with input from A.S., P.K. and R.N.

**Additional information**

Supplementary Information accompanies this paper at http://www.nature.com/naturecommunications

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