DNA Methylation and Histone Modifications Regulate De Novo Shoot Regeneration in Arabidopsis by Modulating WUSCHEL Expression and Auxin Signaling

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Abstract

Plants have a profound capacity to regenerate organs from differentiated somatic tissues, based on which propagating plants in vitro was made possible. Beside its use in biotechnology, in vitro shoot regeneration is also an important system to study de novo organogenesis. Phytohormones and transcription factor WUSCHEL (WUS) play critical roles in this process but whether and how epigenetic modifications are involved is unknown. Here, we report that epigenetic marks of DNA methylation and histone modifications regulate de novo shoot regeneration of Arabidopsis through modulating WUS expression and auxin signaling. First, functional loss of key epigenetic genes—including METHYLTRANSFERASE1 (MET1) encoding for DNA methyltransferase, KRYPTONITE (KYP) for the histone 3 lysine 9 (H3K9) methyltransferase, JMJD14 for the histone 3 lysine 4 (H3K4) demethylase, and HAC1 for the histone acetyltransferase—resulted in altered WUS expression and developmental rates of regenerated shoots in vitro. Second, we showed that regulatory regions of WUS were developmentally regulated by both DNA methylation and histone modifications through bisulfite sequencing and chromatin immunoprecipitation. Third, DNA methylation in the regulatory regions of WUS was lost in the met1 mutant, thus leading to increased WUS expression and its localization. Fourth, we did a genome-wide transcriptional analysis and found out that some of differentially expressed genes between wild type and met1 were involved in signal transduction of the phytohormone auxin. We verified that the increased expression of AUXIN RESPONSE FACTORS3 (ARF3) in met1 indeed was due to DNA demethylation, suggesting DNA methylation regulates de novo shoot regeneration by modulating auxin signaling. We propose that DNA methylation and histone modifications regulate de novo shoot regeneration by modulating WUS expression and auxin signaling. The study demonstrates that, although molecular components involved in organogenesis are divergently evolved in plants and animals, epigenetic modifications play an evolutionarily convergent role in this process.

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Introduction

Differentiated somatic tissues of plants can be reprogrammed to generate various organs, a process called de novo organogenesis. This feature is not only critical for in vitro plant propagation and application of biotechnology, but also provides a good experimental system for understanding regulatory mechanisms underlying organogenesis.

Recent studies have revealed some molecular mechanisms underlying de novo shoot regeneration in Arabidopsis [1–4], in which WUS, a transcription factor, plays a key role [5,6]. WUS is a master regulator of stem cell fate determination in shoot apical meristem (SAM), on which many signaling pathways converge [7]. It turned out to be also critical for de novo shoot regeneration. During de novo shoot regeneration in Arabidopsis, expression of WUS is sufficient to specify the organizing center, which is required for stem cell induction and subsequent shoot regeneration [5,6,8]. WUS induction is also essential for shoot formation during de novo somatic embryogenesis [9]. Induction of the WUS expression during de novo shoot regeneration was regulated by the master phytohormone auxin [2,5]. Recently, WUS expression in the organizing center of the Arabidopsis plant SAM was shown to be regulated by epigenetic modifications [10].

Epigenetic modifications, including DNA methylation and histone modifications, occur extensively during cellular differentiation and development in mammals [11–13]. In mammals, the patterns of DNA methylation are established by de novo DNA methyltransferase 3 (DNMT3) family and maintained by methyltransferase DNMT1 [14]. DNMT1 plays a vital role in controlling the self-renewal and differentiation of stem cells during hematopoiesis and leukemogenesis and is critical for progenitor maintenance and self-renewal in mammalian somatic tissues [15,16]. DNA methylation and histone modifications regulate gene expression through changing chromatin structure and transcriptional activities [17–19]. For instance, transcriptional repression is associated with hypermethylation of DNA, histone deacetylation and histone H3K9 methylation, whereas active chromatin is linked with hypomethylation of DNA, histone acetylation and histone H3K4 methylation [17,20].
Plants have a strong ability to generate organs from differentiated somatic tissues. Due to this feature, shoot regeneration in vitro has been used as an important way for producing whole plants in agriculture and biotechnolog- 

phytoregulate the transition from vegetative phase to reproductive phase. A key regulator of shoot regeneration is WUSCHEL (WUS). WUS expression is critical for stem cell formation during de novo shoot regeneration [5,6]. Here, we show that induction of wild-type calli on SIM for 4 days (S4) and 6 days (S6) was accompanied by a significant increase of WUS expression. This increase was correlated with the development of shoot primordia on SIM to reflect the capacity of de novo shoot regeneration. However, it took different time for wild-type calli to reach half of the maxima as compared to the wild type (Figure 1A–C). We obtained similar results indicating more development of shoots in these mutants using either pistils or roots as explants (Figure 1A–C, Figure S1). Interestingly, calli of met1 cultured on SIM developed differently from those of the wild type (Figure 1D). At 4 days on SIM, around 70% of met1 calli contained green regions from which the shoots would differentiate, but these green regions could not be identified in the wild-type calli. At 6 to 14 days on SIM, more shoots emerged from the met1 calli than those from the wild-type calli (Figure 1D). At 18 days on SIM, the shoots from the met1 calli were much more developed compared to those from the wild-type calli. These results indicated that the regulation of WUS expression during de novo shoot regeneration may have resulted from dynamic DNA methylation.

Regulation of WUS expression during de novo shoot regeneration may have resulted from dynamic DNA methylation.

It was established that WUS expression is critical for stem cell formation during de novo shoot regeneration [5,6]. Here, we showed that induction of wild-type calli on SIM for 4 days (S4) and 6 days (S6) was accompanied by a significant increase of WUS expression during de novo shoot regeneration. This increase was correlated with the development of shoot primordia on SIM to reflect the capacity of de novo shoot regeneration. However, it took different time for wild-type calli to reach half of the maxima as compared to the wild type (Figure 1A–C). We obtained similar results indicating more development of shoots in these mutants using either pistils or roots as explants (Figure 1A–C, Figure S1). Interestingly, calli of met1 cultured on SIM developed differently from those of the wild type (Figure 1D). At 4 days on SIM, around 70% of met1 calli contained green regions from which the shoots would differentiate, but these green regions could not be identified in the wild-type calli. At 6 to 14 days on SIM, more shoots emerged from the met1 calli than those from the wild-type calli (Figure 1D). At 18 days on SIM, the shoots from the met1 calli were much more developed compared to those from the wild-type calli. These results indicated that the regulation of WUS expression during de novo shoot regeneration may have resulted from dynamic DNA methylation.

To test this possibility, we first compared DNA methylation of the ~10 kb WUS genomic sequences between the calli of wild type on CIM (C16 and S0) and those on SIM (S6) by bisulfite genomic
sequencing. Three regions within the WUS genomic sequences were hyper-methylated in S0 calli but substantially decreased in S6 calli (Figure 4A and 4B). Among the three regions, region I was previously proposed to regulate WUS expression [36]. Both CpG dinucleotide motifs and non-CG motifs in the three regions of the WUS genomic sequences showed induced demethylation upon induction on SIM (Figure 4B). These results showed that de novo shoot regeneration was accompanied with demethylation on methylated WUS genomic sequences. That could partially contribute to the regulation of WUS expression during de novo shoot regeneration.

Demethylation and regulation of WUS expression in met1 mutant

Because DNA methylation was significantly reduced in met1 mutant [27], we wondered whether DNA methylation in the WUS genomic sequences would be affected in met1 mutant. To find out, we used two approaches. First, we compared the expression patterns of WUS in wild-type calli and met1 calli at different induction points. Indeed, the met1 mutant showed much higher WUS level than that in the wild type at each time point by qRT-PCR (Figure 2A). Then, in situ hybridization analysis demonstrated that localization of WUS in the met1 calli on SIM was earlier than that in the wild-type calli on SIM (Figure S3A–S3F, Table S1). GUS staining confirmed that the pattern of WUS expression is similar to that in situ hybridization (Figure 3), and the number of GUS signal distribution in both the met1 calli and the wild-type calli on SIM is consistent to percentages of shoot primordia on SIM at different induction points (Figure 3, Figure S3, Table S2). Thus, the results indicated that WUS expression and corresponding developmental rate of de novo shoot regeneration were mediated by reduced DNA methylation.

Next, we tested whether MET1 loss of function affected the methylation status of WUS genomic region by bisulfite genomic sequencing. We found that the calli of met1 mutant on CIM (C16 and S0) and on SIM (S6) showed much lower level of DNA methylation in the WUS genomic region than those of wild type under the same condition (Figure 4B). WUS expression was
Figure 2. DNA methylation and histone modifications regulate *WUS* transcript levels. A) Transcript levels of *WUS* in calli of the wild type (*Ws*) and the mutant *met1*. B) Transcript levels of *WUS* in calli of the wild type (*Ler*) and the mutant *kyp-2*. C) Transcript levels of *WUS* in calli of the wild type (*Col*) and the mutants, *hac1-3*, *hac1-5*, *jmj14-1* and *jmj14-2*. Total RNAs were isolated from calli of wild type (*Ws, Ler* and *Col*) and various mutants (*met1, kyp-2, jmj14-1, jmj14-2, hac1-3* and *hac1-5*) cultured on SIM at the indicated time points, respectively. *WUS* transcript levels were quantified by qRT-PCR. The results are shown as mean values of three biological replicates with standard errors. The relative expression level of *WUS* gene, corresponding to the expression level of *TUBULIN2*, was calculated using the comparative C(T) method. After incubating on CIM for 20 days (S0), some of the calli were transferred onto SIM for further induction for 4 days (S4) and 6 days (S6), other calli were still cultured on CIM as controls (C24, C26). C16, C24, C26 indicated that pistils as explants were cultured on CIM for 16 days, 24 days and 26 days, respectively.

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detected in met1 calli earlier than in wild type based on in situ hybridization and GUS reporter analysis (Figure 3 and Figure S3). In addition, met1 contained more WUS-expressing regions than wild type, indicating that increased WUS expression level contributed to elevated the number of organizing centers (Figure 3 and Figure S3). These results suggested that the regulation of WUS expression in met1 mutant during de novo shoot regeneration could at least partially be contributed by DNA demethylation on methylated WUS genomic sequences.

Dynamic changes of histone modifications at the genomic regions of WUS during de novo shoot regeneration

Higher WUS level in the met1 mutant suggested the involvement of MET1-mediated DNA methylation in the regulation of WUS expression. However, the expression of WUS still responded to the induction by incubation on SIM in met1 mutant (Figure 2A), indicating additional pathways that regulated the dynamic expression of WUS. Because we showed that histone modifications were also important for de novo shoot regeneration (Figure 2B and 2C), we next tested whether histone modifications played a role in mediating WUS expression during de novo shoot regeneration.

We analyzed several histone modifications for the WUS genomic sequences using chromatin immunoprecipitation at two developmental stages: S0 and S6. Methylation at histone H3 at lysine 4 (H3K4me3) was shown to occur in euchromatin undergoing active transcription [37]. Whereas methylation at histone H3 at lysine 9 (H3K9me2) was shown to inhibit transcription [38]. Additionally, acetylation at histone H3 at lysine 9 (H3K9ac) is one of the most characterized epigenetic marks invariably associated with active transcription in all species investigated so far [18]. It also plays a crucial role in plant development [39].

Our results showed that these three histone modifications were dynamically regulated at the WUS genomic sequences during de novo shoot regeneration. Compared with S0, S6 showed an increase in the levels of H3K4me3 at region a and d, but not at b and c (Figure 5A and 5B); H3K1me3 occurred in euchromatin undergoing active transcription [37], therefore increased H3K1me3 levels were consistent with WUS induction during de novo shoot regeneration (Figure 1G, Figure 2C). A mark for chromatin acetylation, H3K9ac, also showed increased levels at all four regions during induction (Figure 5C). In contrast to these epigenetic marks associated with active transcription, H3K9me2,
which is associated with transcription suppression [37] were reduced during de novo shoot regeneration in all four regions (Figure 5B). The changes at these epigenetic marks around WUS genomic region explained the active state of WUS chromatin structure, and might well contribute to the regulation of WUS expression during de novo shoot regeneration.
**WUS expression was changed in mutants defective in histone modifications**

Dynamic histone modifications at the genomic regions of **WUS** during de novo shoot regeneration indicated that histone modifications contributed to regulation of **WUS** expression during de novo shoot regeneration. To provide further evidence that histone modifications regulated **WUS** expression in this process, we examined transcript level of **WUS** in mutants that were defective in histone modifications by qRT-PCR. As stated before, **KYP**, **JMJ14** and **HAC1** encoded enzymes for histone modification, mutations of which affected the developmental rate of de novo shoot regeneration (Figure 1B and 1C, Figure S1). Comparing with the wild-type calli, levels of **WUS** expression in the calli of the mutant **kyp-2** were significantly enhanced compared to those of wild type for 6 days on SIM (Figure 2B). Similar results were obtained for the mutants **jmj14-1** and **jmj14-2** (Figure 2C). Contrast to the mutants **kyp** and **jmj14**, the levels of **WUS** transcripts in two different allelic **hac1** mutants were reduced compared to that of wild type (Figure 2C).

Then, we used **kyp-2** calli on SIM (S0, S4, and S6) to do in situ hybridization analysis. The results showed that localization of **WUS** signals in **kyp-2** calli on SIM occurred early comparing to that in wild-type calli on SIM (Figure S3G–S3L). Also, the number of localized **WUS** signals in **kyp-2** calli on SIM (S4 and
S6) was more than that in wild-type calli at the same time points (Table S1). Similar to the case of met1, expression of WUS appeared earlier in kyp-2 calli than in wild type (Figure S3). Thus, changes of WUS expression in these mutants correlated with their different developmental rates of de novo shoot regeneration, suggesting that WUS expression was regulated by histone modifications.

**SIM-induced as well as MET1-dependent transcriptional changes during de novo shoot regeneration**

Our results showed that DNA methylation and histone modifications regulated WUS expression during de novo shoot regeneration. To get a whole picture of epigenetic modifications during this process, we decided to do a genome-wide expression profiling using the Affymetrix ATH1 full genome array. We analyzed the transcriptomes of wild-type calli being transferred to CIM for 20 days (S0) and to SIM for 6 days (S6). Because met1 calli showed significantly different developmental rate from wild-type calli, we also analyzed transcriptomes of met1 calli being transferred to CIM for 20 days (M0) for comparison. Significance Analysis of Microarrays software package analysis was conducted for three biological samples replicates between wild-type calli, we also analyzed transcriptomes of met1 calli being transferred to CIM for 20 days (M0) for comparison. This criterion gave 1334 upregulated genes, and 501 downregulated genes by induction on SIM (S6 versus S0) (Table S3). 768 candidate genes showed over 2 fold difference between M0 and S0, suggesting that they might be regulated by MET1-dependent DNA methylation (Table S4). 306 candidate genes showed over 2 fold difference both between S6 versus S0 and between M0 versus S0, suggesting that they might be induced on SIM and be regulated by MET1-dependent DNA methylation (Table S5). By qRT-PCR analysis, we confirmed the microarray data (Figure S4).

Because auxin and cytokinin are essential for de novo shoot regeneration [2,5], we selected genes involved in cytokinin and auxin signaling for bisulfit sequencing analysis. Indeed, some displayed differential methylation patterns during de novo shoot regeneration, such as AUXIN RESPONSE FACTORS (ARFs), AUXIN RESPONSE FACTOR4 (ARF4), INDOLE-3-ACETIC ACID INducible18 (AIL18) and BELL1-LIKE HOMEODOMAIN (BLH7) (Figure 6B-6E). A loss of DNA methylation occurred in these genes, along with increased levels of their transcription in induced wild-type calli (Figure S4). Their expression levels were also higher in met1 than those in the wild type, suggesting that the expression of these genes might be regulated by a MET1-dependent dynamic DNA methylation during shoot regeneration.

On the other hand, some candidate genes selected from SIM-induced and MET1-dependent pathways displayed no methylation alterations, such as ASYMMETRIC LEAVES1 (AS1), ARABIDOPSIS RESPONSE REGULATOR13 (ARR13), CYTOKININ OXIDASE/DEHYDROGENASE1 (CKX1), INDOLE-3-ACETIC ACID27 (AIL27) and PINOID2 (PID2), but they displayed great changes in their transcriptional levels upon SIM-induction, implying that those genes might not be directly regulated by MET1 (Table S5).

**Epigenetic modifications: evolutionary recurring themes for reprogramming**

DNA methylation and histone modifications are critical epigenetic processes that control chromatin structure and gene expression during development and differentiation [17,18], and there are likely complicated interactions between these processes [20,40]. In human, a crosstalk between DNA methylation and histone modifications has been proposed to regulate gene transcription in tumors [20]. Similarly, DNA methylation controls histone H3K9 methylation and further affect heterochromatin assembly in Arabidopsis [41]. Recent study has indicated that chromatin status regulates the accessibility of transcription factor to FLOWERING LOCUS T (FT) in Arabidopsis, and distant regulatory regions are required for FT transcription [42]. WUS transcription is regulated through a fairly complicated chromatin remodeling mechanism in the SAM of the Arabidopsis plant [43]. It was shown that WUS expression was positively correlated with FASCIATA1 (FAS1)/FAS2, subunits of ASSEMBLY FACTOR-1 (CAF-1), and BRUSHY1 (BRU1), both of which regulate post-replicative stabilization of chromatin structure [44,45]. Another study showed that the chromatin remodeling factor SPLAYED (SYD) directly regulated WUS to maintain proper WUS transcript levels in its spatial expression domain [46]. It has been demonstrated that at least 3.5 kb fragment upstream of WUS is required for its spatiotemporal expression during plant development [36]. Here, we showed that the 5’ and 3’ regions of WUS were regulated by SIM-induced changes of DNA methylation and histone modifications. Because the met-1 kyp-7 double mutant displayed more severe phenotypes than each single mutant [19], we propose that regulation of WUS by DNA methylation and histone modifications may function in a partially redundant manner during de novo shoot regeneration. To understand mechanism of the in vitro organogenesis mediated by the factors involved in both DNA methylation and histone modifications, knocking out both DNA methylation and histone modifications remains to be investigated in the future.

It has long been thought that animal cells, once committed to a specific lineage, can no longer change their fate. However, recent studies suggested that differentiated animal cells do maintain plasticity and can be induced to undergo reprogramming [47,48]. Further studies have shown that differentiated cells in mouse can be reprogrammed to pluripotent stem cells by introducing four transcription factors [49]. Plant cells can easily regenerate organs from the differentiated tissues under proper cultured conditions [1]. Previously, we used Arabidopsis ptstils as explants on CIM to obtain the callus, a mass of pluripotent cells [26], and by transferring calli onto SIM, the expression of WUS was induced in a group of cells termed the organizing center as a self-renewing source of stem cells within calli. The induced organizing center and stem cells were responsible for subsequent shoot regeneration. Here, we showed that expression of many genes was induced by SIM-induction (Figure 6A). Those genes were divided into either MET1-dependent or MET1-independent. Among MET1-dependent genes, WUS is a key transcription factor to regulate shoot regeneration [1]. ARF3 was required for shoot induction (Cheng et al., unpublished data). Previous study showed that ARF3 and ARF4 act redundantly to establish the abaxial cell fate of the Arabidopsis leaves [50]. Thus, ARF3 and ARF4 may function on de novo meristem formation mediated by epigenetic modifications. MET1-independent genes might also be involved in the process of shoot induction. Our results suggested that pluripotent cells of the callus can be reprogrammed to stem cells and subsequent, shoot formation through the regulation of both MET1-dependent genes, such as WUS and ARFs, and some MET1-independent genes.

In conclusion, our results indicate that dynamic DNA methylation and histone modifications contribute to the control of stem-cell formation and subsequent shoot regeneration. These epigenetic modifications regulate WUS and probably hormone-related genes, whose spatiotemporal expression was critical for de
novo shoot regeneration. In mammals, epigenetic modifications of transcription factors and of components in hormone signaling pathways also play crucial roles in cell differentiation and organogenesis [51,52]. Our results thus provide an interesting scenario in which epigenetic modifications were adopted as recurring themes during evolution for de novo organogenesis.

Figure 6. Identification of the candidate genes regulated by DNA methylation. A) The overlap between differentially-expressed genes of S6 versus S0 (Table S3) and M0 versus S0 (Table S4) were identified as candidate genes, and were listed in Table S5. A two-fold difference in the expression level of genes with a q value≤0.05 between S6 versus S0 and M0 versus S0 was set as the threshold for the selection of differentially-expressed genes. B–E) Cytosine methylation levels of ARF3, ARF4, IAA18 and BLH7 genes in calli of wild type (S0, S6), and calli of met1 (M0) were determined by bisulfite genomic sequencing. H represents A, T or C.

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Materials and Methods

Plant materials

The met1 mutant in the Wassilewskija (Ws) background was a kind gift from Dr. J. Bender (The MCB Department of Brown University) [27]. The hyp-2 [28] mutant in the Landsberg (Ler) background, jmj14-1, jmj14-2 [29], hac1-3, and hac1-5 [31] mutants in the Columbia (Col) background were generously provided by Dr. Xiaofeng Cao (Institute of Genetics and Developmental Biology, Chinese Academy of Sciences).

Plant growth and shoot regeneration

Plants were grown as previously described [9]. Arabidopsis seeds were surface sterilized and plated on germination medium [53]. After cold treatment for 2 days at 4°C in the dark, they were transferred to sterile conditions or the growth chamber at 22°C in a 16 h light/8 h dark cycle. Shoot regeneration procedures used in this study were based on the previously described protocols [26,54]. Pistils were excised from sterile Arabidopsis plants and transferred onto callus induction medium (CIM, MS medium [53] with 0.5 mg/L 2, 4-dichlorophenoxyacetic acid (2, 4-D) and 1.0 mg/L 6-benzylaminopurine (6-BA)). The explants were incubated for 20 days on CIM to induce callus production, and calli were then transferred onto shoot induction medium (SIM, MS medium with 0.01 mg/L indole-3-acetic acid (IAA) and 2 mg/L zeatin (ZT)). Root explants of 5–10 mm length were excised from 7-day-sterile seedlings, then transferred onto callus induction medium (CIM, Gamborg’s B5 medium [53] with 0.5 g/L MES, 2% glucose, 0.2 μmol/L kinetin, and 2.2 μmol/L 2, 4-dichlorophenoxyacetic acid (2, 4-D), 0.8% agar), and incubated for 6 days in continuous light. Finally, explants were transferred onto shoot-inducing medium (SIM, Gamborg’s B5 medium with 0.5 g/L MES, 2% glucose, 0.9 μmol/L 3-indoleacetic acid, 0.5 μmol/L 2-isopentenyladenine) and incubated in continuous light.

The morphology of calli was examined and photographed with an Olympus microscope. We defined the number of regenerated shoots as the number of at least 2 mm long shoots on each callus.

In situ hybridization

Probes were labeled using digoxigenin RNA labeling kit (Boehringer Mannheim). An antisense probe from a full-length WUS cDNA clone was generated using T7 RNA polymerase, and an sense probe was synthesized using SP6 RNA polymerase. The detailed protocol was carried out as described previously [56]. Primer sequences used for probes amplification are summarized in Table S6.

β-glucuronidase (GUS) assay

Plant tissues were incubated in GUS assay solution [50 mmol/L Na2HPO4, 50 mmol/L KH2PO4, pH 7.2, 10 mmol/L Na2EDTA, 0.5 mmol/L K3Fe(CN)6, 0.5 mmol/L K4Fe(CN)6, 1% Triton X-100 and 2 mmol/L X-Gluc (Bio. Basic Inc., Canada)] at 37°C for 12 h. To further investigate WUS expression pattern, some GUS-stained tissues were embedded in paraffin (Sigma) and sectioned. To display the outline of cells clearly, ruthenium red (200 mg/L) was used to stain cell walls.

Genomic bisulfite sequencing

DNA methylation assays were performed by bisulfite sequencing as previously described [57]. PCR products were cloned into the pMD19-T Simple Vector (Takara), and 12 clones were sequenced to determine the methylation status of a locus in each genotype. Primer sequences are shown in Table S6. Bisulfite sequencing data were analyzed by the CyMATE software [58].

The results returned by CyMATE were input into SigmaPlot 10.0 to illustrate DNA methylation frequencies at CG, CHG and CHH (where H = A, C or T) at the various cultured stages of each genotype.

Chromatin immunoprecipitation assay

The Arabidopsis calli grown on CIM for 20 days (S0) and on SIM for 6 days (S6) were vacuum-infiltrated with formaldehyde crosslinking solution. Chromatin immunoprecipitation was performed according to manufactures’ instructions (Epigentek Group Inc. USA, Catalogno. P-2014). Chromatin samples were immunoprecipitated with antibodies against a negative control normal mouse IgG and H3 dimethyl Lys 9 (both included in EpiQuik™ Plant ChIP Kit), or with antibodies against H3 trimethyl Lys 4 (Abcam USA, Catalogo. ab1012) and H3 acetyl Lys 9 (Abcam USA, Catalogo. ab10812). PCR amplification was performed in 25 μL volumes for 32 to 37 cycles to determine the appropriate conditions for the PCR products of each region. Primer sequences are shown in Table S6. The PCR products were electrophoresed in a 2% agarose gel. Three biological replicates were analyzed and each was tested by three technical replicates.

Total RNA isolation and quantitative real-time PCR analysis

Total RNAs were isolated from callus tissues 2 to 3 mm deep from the surface. Quantitative real-time PCRs (qRT-PCRs) were performed as described previously [9]. To check the specificity of amplification, the melting curve of the PCR products was detected. The expression levels of specific genes were standardized to the housekeeping gene TUBULIN2. Each reaction was carried out in three biological replicates. The relative expression level of each gene, corresponding to the expression level of TUBULIN2, was calculated using the comparative Ct method [59]. Primer sequences used for qRT-PCR are summarized in Table S6.

DNA microarray analysis

RNA of three plant samples was prepared from each of the following tissue types: the wild-type calli cultured on CIM for 20 days (S0), and on SIM for 6 days (S6); the met1 mutant calli cultured on CIM for 20 days (M0). RNA purification, probe labeling, chip hybridization, probe array scanning and data pre-processing normalization were performed by the Affymetrix custom service (CapitalBio, Beijing, China). Significance Analysis of Microarrays software package analysis was conducted for three biological samples replicates between the Ws and met1. When all replicates clustered together, further analysis was performed based on mean values. A two-fold change in the gene expression levels between one versus another samples with a q value≤0.05 was set as the threshold for altered gene expression. Microarray data are available in the ArrayExpress database (www.ebi.ac.uk/arrayexpress) under accession number E-MEXP-3120.

Supporting Information

Figure S1 Frequency of shoot regeneration of met1 mutant and the mutants defective in histone modifications. Frequency of shoot regeneration of the wild type (Col) and the mutants jmj14-2 and hac1-5 was shown, using pistils as explants. Frequency of shoot regeneration of the wild type (Ws, Ler and Col) and the mutants met1, hyp-2, jmj14-1, jmj14-2, hac1-3 and hac1-5 was shown, using roots as explants. Standard errors were calculated from three sets of biological replicates. In each replicate, at least 60 calli were examined. (TIFF)
Figure S2  *MET1* mutation promotes shoot regeneration in *Arabidopsis* using roots as explants. Calli of the wild type (Ws) and the *met1* mutant were cultured on SIM for 6 to 18 days. Scale bars, 1 mm. (TIF)

Figure S3  Expression patterns of *WUS* were changed in *met1* and *kyp-2* mutants. *In situ* hybridization of *WUS* expression in calli of the wild type (Ws) cultured on SIM for A) 0 day, B) 4 days and C) 6 days, and that of *met1* mutant cultured on SIM for D) 0 day, E) 4 days and F) 6 days. *In situ* hybridization of *WUS* expression in calli of the wild type (Let) cultured on SIM for G) 0 day, H) 4 days and I) 6 days, and that of *kyp-2* mutant cultured on SIM for J) 0 day, K) 4 days and L) 6 days. Scale bars, 50 μm. (TIF)

Figure S4  Expression patterns of candidate genes validated by qRT-PCR. Total RNAs were isolated from calli of wild type and the *met1* mutant cultured on SIM at the indicated time points, and the transcripts of genes *ARR3*, *ARR4*, *LAX1*, *BLH7*, *ANT*, *AS1*, *CKX1*, and *ARR15* were measured by qRT-PCR. Three independent RNA preparations were analyzed for each time point. Mean values were calculated from triplicate qRT-PCR analysis with standard errors. The relative expression level of each gene, corresponding to the expression level of *TUBULIN2*, was calculated using the comparative G(T) method. (TIF)

Table S1  The percentage of *WUS* expressing signals detected by *in situ* hybridization. (DOC)

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**Table S2**  The number of β*WUS::GUS* signal distribution detected in each callus. (DOC)

**Table S3**  List of 1334 up-regulated genes and 501 down-regulated genes in S6 as compared to S0. (XLS)

**Table S4**  List of 768 genes showing more than two-fold difference between M0 and S0. (XLS)

**Table S5**  List of 308 genes showing more than two-fold difference both between S6 and S0 and between M0 and S0. (XLS)

**Table S6**  Sequences of primers used in this study. (XLS)

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**Author Contributions**

Conceived and designed the experiments: XSS WL. Performed the experiments: XSS WL HL ZJG YHS HNH. Analyzed the data: XSS WL HL. Contributed reagents/materials/analysis tools: XSS WL HL ZJG YHS. Wrote the paper: XSS YZ.
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