Plant Biotechnology 26, 293–300 (2009)

Original Paper

SET domain-containing protein genes are involved in *Arabidopsis thaliana* embryogenesis

Naohito Hariganeya, Akira Kikuchi*, Hiroshi Kamada

Graduate School of Life and Environmental Sciences, University of Tsukuba, Ibaraki 305-8572, Japan
* E-mail: kikuike@sakura.cc.tsukuba.ac.jp Tel & Fax: +81-29-853-7729

Received November 4, 2008; accepted December 9, 2008 (Edited by Y. Ozeki)

Abstract Many genes are temporally and spatially regulated during embryogenesis in higher plants. Although many studies have examined transcriptional factors relating to gene regulation during embryogenesis, the molecular mechanisms relating to the initiation of embryogenesis are still unclear. In animals, it was reported that gene regulation by chromatin remodeling contributes to embryogenesis. In contrast, the relationship between chromatin remodeling and the initiation of embryogenesis in higher plants remains to be determined. *LEAFY COTYLEDON1* (*LEC1*) is an important factor in early embryogenesis and is ectopically expressed in the *pkl1-1* mutant, which is deficient in chromatin remodeling factor. Therefore, there is a high probability that chromatin remodeling regulates the expression of *LEC1*. To confirm this possibility, the histone methylation level, which is involved in chromatin remodeling, was examined for the genomic region of *LEC1* by chromatin immunoprecipitation analysis. In the promoter region of *LEC1*, methylation of histone H3 lysine 4 in somatic embryos was higher in rosette leaves. SET domain-containing proteins are an important factor in histone methylation. To isolate the SET domain-containing protein genes (*SET* gene) involved in *Arabidopsis thaliana* embryogenesis, expression analyses using RT-PCR were performed. Among 37 *SET* genes, seven were found to have a high probability of involvement in embryogenesis.

Key words: Chromatin remodeling, embryogenesis, SET domain-containing protein gene, somatic embryo.

In higher plants, embryogenesis is the developmental process that usually begins once the egg has been fertilized, and is temporally and spatially regulated. Although the molecular biological mechanism of embryogenesis is of interest, the zygotic embryo is not well understood. Detailed analyses of zygotic embryogenesis are hampered by the difficulty of isolating sufficient amounts of zygotic embryos in early stages. Therefore, somatic embryogenesis has been extensively used as an experimental model system to examine physiological, biochemical, and molecular biological events during zygotic embryogenesis. Somatic embryos show a similar morphological change (globular stage, heart-shaped stage, and torpedo-shaped stage) to zygotic embryos, and grow to seedlings. A number of genes involved in embryogenesis have been isolated (Meinke et al. 1995; Thibaud-Nissen et al. 2003; Takahata et al. 2004), and the transcription factors of *LEAFY COTYLEDON1* (*LEC1*), *LEC2*, *FUSCA3* (*FUS3*), and *ABSCISSIC ACID INSENSITIVE3* (*ABI3*) have been shown to play key roles in the control of early embryo development (Parcy et al. 1997). However, the molecular biological mechanism of the initiation of embryogenesis remains unclear.

Recently, it was shown that “chromatin remodeling” is involved in phase changes and cell differentiation in animals (Simon et al. 2002). Chromatin remodeling contributes to the control mechanism of inherited information, which is different from the stable information of DNA. This mechanism regulates the higher structure of chromatin switching between “heterochromatin” and “euchromatin” conditions in which DNA is tightly or loosely packaged by nucleosomes, respectively. Because tightly packed DNA cannot interact with transcriptional factors, chromatin remodeling is involved in the regulation of gene expression over the entire genome (Mellor 2005).

The relationship between embryogenesis and chromatin remodeling has already been reported in animals. For example, *Meisetz* is important for the progression

Abbreviations: ATX, Arabidopsis trithorax-like homologue; ATXR, Arabidopsis trithorax-like homologue related; ASH, absent, small or homeotic discs; ASHH, ASH homologue; ASHR, ASH homologue related; ChIP, chromatin immunoprecipitation; CLF, CURLY LEAF; 2,4-D, 2,4-dichlorophenoxyacetic acid; E(z), Enhancer of zeste; H3K4, 4th lysine from the N-terminus in histone H3; H3K9, 9th lysine from the N-terminus in histone H3; H3K27, 27th lysine from the N-terminus in histone H3; H3K36, 36th lysine from the N-terminus in histone H3; LEC1, LEAFY COTYLEDON1; MEA, MEDEA; PKL, PICKLE; Su(var)3-9, suppressor of variegation 3-9; SUVH, suppressor of variegation homologue; SUVR, suppressor of variegation homologue related; SWN, SWINGER; TRX, TRITHORAX
This article can be found at http://www.jspcmb.jp/

Copyright © 2009 The Japanese Society for Plant Cell and Molecular Biology

of early meiotic prophase in postnatal testis (Hayashi et al. 2005). In *Arabidopsis*, the relationship between embryogenesis and chromatin remodeling has also been established. *PICKLE* (*PKL*) is reportedly a chromatin remodeling factor that influences the expression of embryo-specific genes, such as *LEC1* in *Arabidopsis* (Ogas et al. 1999; Rider et al. 2003). Since the *pkl1-1* mutant shows embryonic traits due to the ectopic expression of *LEC1* after germination, it is thought that the expression of *LEC1* is regulated by the alteration of chromatin structure and that *PKL* represses embryonic identity during germination via the regulation of certain genes, such as those related to embryogenesis. Therefore, *LEC1* may be a good marker for the identification of chromatin remodeling related genes involved in embryogenesis.

Many factors are involved in regulation by chromatin remodeling. One of the most important is the histone modification enzyme (Loidl 2004). The N- and C-termini of histone are subjected to a variety of post-translational modifications including methylation, acetylation, and phosphorylation (Jenuwein and Allis 2001). A number of reports indicate that histone methylation has important functions in the epigenetic control of gene expression and chromatin packaging, especially in differentiation (Tariq and Paszkowski 2004). For example, in *Drosophila* and mice, histone methylation is important in regulating the expression of *Hox* genes, which control segmental patterning during development (Pirrotta 1998; Terranova et al. 2006).

Methylation of the 9th and 27th lysine from the N-terminus of histone H3 (H3K9 and H3K27, respectively) is a characteristic of heterochromatin, whereas methylation of the 4th and 36th lysine from the N-terminus of this protein (H3K4 and H3K36, respectively) is thought to be characteristic of euchromatin (Jenuwein and Allis 2001). In *Arabidopsis*, the expression profile of *FLOWERING LOCUS C* (*FLC*), which encodes a flowering repressor and is involved in vernalization, is reportedly associated with methylation at H3K4, H3K9, and H3K27 (Bastow et al. 2004; Sung and Amasino 2004; Finnegan et al. 2005). After vernalization, the methylation level of H3K4, surrounding the region of the transcription start site of the *FLC* gene, and the expression level of *FLC*, are reduced, whereas the methylation level at H3K9 and H3K27 are increased.

Among enzymes involved in the methylation of histone, SET domain-containing proteins have been shown to mainly contribute to methylation. The SET domain is a conserved amino acid sequence among the E(z), TRX, and Su(var)3-9 groups in *Drosophila* (Tripoulas et al. 1996; Baumbusch et al. 2001). In the *Arabidopsis* genome, 37 genes were predicted to encode SET domain-containing proteins (*SET* genes) from their sequences (Baumbusch et al. 2001). These *SET* genes are divided into four groups based on conserved domains, as typified by the ones in *Drosophila*: the enhancer of zeste (E(z)) group (MEA, CLF, and SWN); the trithorax (TRX) group (ATX1 to 5 and ATXR1 to R7); the absent, small, or homeotic discs (ASH) group (ASHH1 to H4 and ASHR1 to R3); and the suppressor of variegation 3-9 (Su(var)3-9) group (SUVH1 to H10 and SUVR1 to R5). There are a few reports that the E(z) and Su(var)3-9 groups are involved in the formation and maintenance of heterochromatin, respectively (Goodrich et al. 1997; Lindroth et al. 2004). In contrast, the TRX and ASH groups seem to be involved in the formation and maintenance of euchromatin, respectively (Goodrich et al. 1997; van Lohuizen, 1998; Alvarez-Venegas et al. 2003; Zhao et al. 2005; Saleh et al. 2007; Pien et al. 2008). However the function of *SET* genes in *Arabidopsis* is not well understood. Because the initiation of embryogenesis is regarded as a phase change from somatic to embryonic conditions, chromatin remodeling is thought to contribute to the regulation of fertilization and early embryogenesis. In this study, we examine the possibility that the expression of *LEC1* is regulated by chromatin remodeling via histone methylation and that some *SET* genes are involved in embryogenesis.

Materials and methods

Plant materials

Arabidopsis thaliana (L.) Heynh. (Ecotype Columbia) was used for the plant material. The flower buds, flowers, and siliques at various days after flowering (DAF) were harvested and stored at −80°C until use.

Induction of Arabidopsis somatic embryos from vegetative tissues

Induction of *Arabidopsis* somatic embryos from vegetative tissues was performed according to the method of Ikeda-Iwai et al. (2003). Seeds were surface-sterilized with 1% NaClO solution for 15 min and washed with sterile distilled water. They were placed on agar-solidified (0.8%) phytohormone-free B5 medium for germination at 21°C under continuous light (white light at 50 μmol photons m^{-2} s^{-1}). Germinated 5-day-old seedlings were used in the experiments. Shoot-apical-tip explants (about 1 mm in length) of 5-day-old seedlings were placed on agar-solidified, phytohormone-free B5 medium containing 0.7 M mannitol. After 8 h, the explants were washed with liquid B5 medium and transferred to agar-solidified B5 medium containing 4.5 μM of 2,4-D without 0.7 M mannitol. These were cultured for 2–3 weeks at 21°C under continuous light (white light at 50 μmol photons m^{-2} s^{-1}).

RT-PCR analysis

Total RNA was extracted using the GET Pure RNA Extraction Kit (Dojindo, Japan) according to the manufacturer's instructions. RNA was extracted from 10 mg samples of buds, flowers,

Copyright © 2009 The Japanese Society for Plant Cell and Molecular Biology

siliques, leaves, and shoot apical meristem (SAM). For RT-PCR analysis, the first-strand cDNA was synthesized from 1 μg total RNA using the SuperScript-First cDNA Synthesis System for RT-PCR (Invitrogen, USA) with an oligo dT extension (5′-GTAAAACGACGGCCAGTCCCTTTTTTTTTTTTTTTTT-3′). PCR amplification was performed using Amplitaq Gold (Applied Biosystems, USA). The primers used for RT-PCR analysis are given in Supplemental Table 1. The reactions were carried out on a thermal cycler, with denaturation starting at 94°C for 10 min, followed by 30 cycles at 94°C for 30 s, 55°C for 30 s, and 72°C for 1 min, with a final extension at 72°C for 5 min. Each experiment was repeated three times.

Chromatin immunoprecipitation analysis for the LEC1 gene

Chromatin immunoprecipitation (ChIP) assays for methylated H3K9 and H3K4 were performed according to the method of Gendrel et al. (2002) with modification. Antibodies recognizing methylated H3K9 (Upstate Biotechnology, USA) and methylated H3K4 (Abcam, UK) were used. Somatic embryo samples and 2-week-old rosette leaves of 100 mg were used in the assay. Tissues frozen in liquid nitrogen were ground to a fine powder and re-suspended in 30 ml of cold extraction buffer 1 (Supplemental Table 2). The slurry was filtered through two layers of Miracloth (MERCK, USA) and the solution was centrifuged at 3,400×*g* for 20 min. The pellets were re-suspended in 1 ml of extraction buffer 2 (Supplemental Table 2), and the solution was then centrifuged at 14,000×*g* for 10 min at 4°C. The pellets were re-suspended in 300 μl of extraction buffer 3 (Supplemental Table 2). Then 300 μl of extraction buffer 3 were added to a new, clean tube and re-suspended pellets were layered on top of the extraction buffer. These were centrifuged at 14,000×*g* for 60 min at 4°C. The pellets were re-suspended in 500 μl of nuclei lysis buffer (Supplemental Table 2) and digested with micrococcal nuclease for 2 h on ice. Then, 10 μl of 0.5 mM EDTA were added to stop digestion and the samples were centrifuged at 14,000×*g* for 10 min at 4°C. Supernatants were transferred to new tubes and centrifuged at 14,000×*g* for 10 min at 4°C, and then transferred to new tubes. These samples were diluted 10 times with ChIP dilution buffer (Supplemental Table 2). To remove the non-specific binding protein using Protein A agarose beads (Upstate Biotechnology, USA), the samples were pre-cleared by adding 40 μl of beads equilibrated by ChIP dilution buffer (Supplemental Table 2). Then, 600 μl of pre-cleared samples were divided among 4 tubes, and 5 μl of anti-methylated H3K9 and anti-methylated H3K4 were added to two tubes each, one of which had no antibody added (mock) and the other of which had only the initial material (input). The samples were incubated on a rotating mixer wheel overnight at 4°C. Immune complexes were collected by adding 40 μl of Protein A agarose beads and rotating for 1 h at 4°C. The beads were collected by centrifugation at 800×*g* for 2 min at 4°C. These were washed with 1 ml of each buffer (Supplemental Table 3) for 5 min at 4°C, and twice with 1 ml of TE. Immune complexes were eluted by 500 μl of elution buffer (Supplemental Table 2) for 15 min at 65°C with agitation. Immune complexes were then separated from the beads by centrifugation at 800×*g* for 2 min. Immune complexes were incubated in 20 μl of 5 M NaCl for 6 h at 65°C. To eliminate proteins, 10 μl of 0.5 M EDTA, 20 μl of 1 M Tris-HCl (pH 6.5), and 1 μl of 20 mg ml^{-1} proteinase K were added and incubated for 1 h at 45°C. After treatment with phenol:chloroform (1 : 1), DNA was recovered by EtOH precipitation with 15 μg of glycogen. The DNA was resuspended in 25 μl of TE, and 1.5 μl of this solution were used for quantitative PCR.

Quantitative PCR for ChIP analysis

The amount of precipitated DNA in ChIP assays was quantified by Quantitative-PCR using a LightCycler (Roche Diagnostics, Germany) with the LightCycler FastStart DNA Master SYBR Green I (Roche Diagnostics, Germany). Cycling and reaction conditions were mediated according to the manufacturer's instructions. An optimal concentration of magnesium in the PCR reaction solution was maintained for each primer set. To quantify the amount of precipitated DNA, the pGEM T Easy Vector (Promega, USA), containing DNA from each region, was used as a standard. No template controls were included in quantification to ensure that the results were not influenced by primer-dimer formation. The amount of precipitated DNA in each sample was normalized using a primer pair specific for the 5′ end of *ACTIN2/7*, which is a constitutively expressed gene assumed to be euchromatic (An et al. 1996). The primers used for the quantitative PCR analysis are given in Supplemental Table 4. Each experiment was repeated three times.

Results

Expression patterns of LEC1 during somatic embryo induction

Stress induction of somatic embryos has been reported in some plant species, such as carrot (Kamada and Harada 1979) and *Arabidopsis* (Ikeda-Iwai et al. 2003). In carrots, it is known that embryogenesis-related genes are induced by various kinds of stress treatments (Nishiwaki et al. 2000; Kikuchi et al. 2006), and novel embryogenesis-related genes have reportedly been isolated using this system (Tanaka et al. 2009). In *Arabidopsis*, somatic embryos were formed on shoot-apical-tip explants by osmotic stress and 2,4-D, but no somatic embryos were induced on 2-week-old rosette leaf explants. To verify whether embryogenesis-related genes were expressed during stress treatment in *Arabidopsis* as in carrots, we analyzed the expression of *LEC1* using material from before and after stress treatment. Expression of *LEC1* was strongly induced by stress treatment in shoot-apical-tip explants, but not in rosette leaf explants (Figure 1). From these results, we suggest that expression of *LEC1* is associated with the induction of somatic embryos. This system may be useful for the screening of candidate genes involved in somatic embryogenesis.

ChIP analysis for the location of the LEC1 gene

LEC1 expression was induced by the somatic embryo induction system (Figure 1). It was predicted that the methylation level of the genomic region of *LEC1* would

Copyright © 2009 The Japanese Society for Plant Cell and Molecular Biology

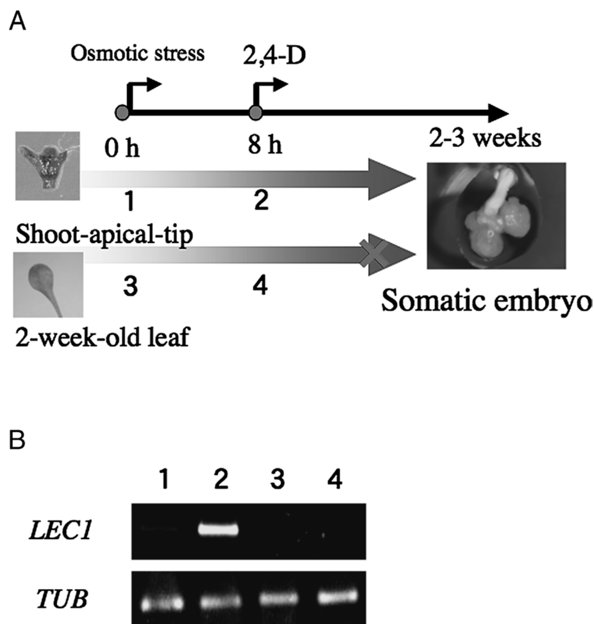

Figure 1. Expression analysis of *LEC1* genes in the stress induction system of somatic embryos. (A) Diagram of the culture system for stress-induced somatic embryogenesis. The black arrows (0 and 8 h) indicate the time course for the culture system. Red circles indicate sampling points. The red X indicates that a somatic embryo was not formed. (B) Lane 1: Shoot-apical-tip explants before osmotic stress treatment; lane 2: shoot-apical-tip explants after osmotic stress treatment; lane 3: 2-week-old leaves before osmotic stress treatment; lane 4: 2-week-old leaves before osmotic stress treatment. PCR products of 10 μl from the sample cDNA using specific primer set of *LEC1*-F and *LEC1*-R. (Supplemental Table 2) were loaded on an agarose (1.5%) gel.

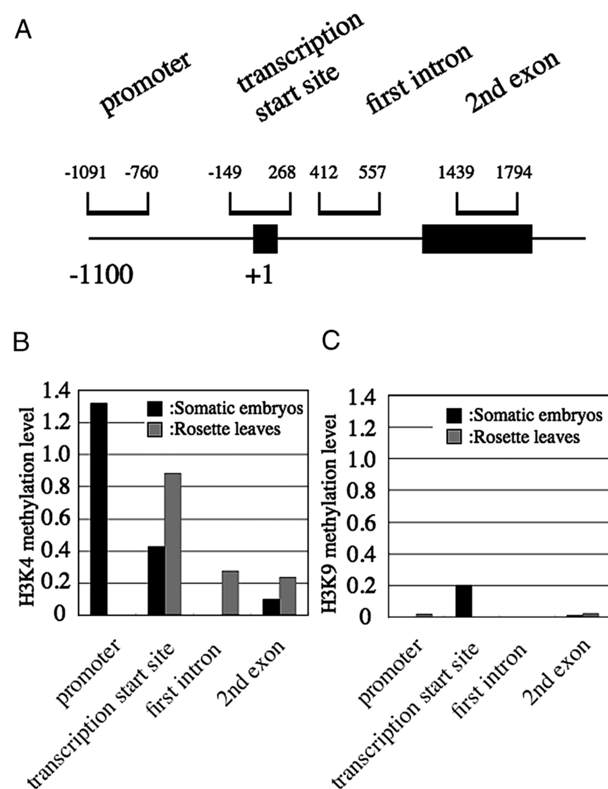

Figure 2. ChIP analysis of the genomic region of *LEC1*. (A) Schematic of the *LEC1* genomic region around the promoter from −1100 to the 3′ UTR. The box indicates the exon region. The positions from which the primer designed for ChIP comes from are indicated by bars and the primer position is shown by numbers over the bars. (B, C) Histone methylation level of H3K4 and H3K9 in the genomic region of *LEC1* by ChIP assay, respectively. Black bars indicate the methylation level in embryos, whereas gray bars indicate that in leaves.

change if chromatin remodeling regulates *LEC1* expression. Therefore, to clarify the association between *LEC1* expression and methylation of the histone in the location of *LEC1*, ChIP analysis was carried out using the somatic embryo and rosette leaves. Four parts from the genomic region of *LEC1* (promoter region, surrounding region of the transcription start site, first intron, and 2nd exon) were selected and analyzed for histone methylation (Figure 2A). In this analysis, methylation of H3K4 and H3K9 was investigated as the typical markers of chromatin status in euchromatin and heterochromatin, respectively. We observed high methylation of H3K4 in the promoter region of the somatic embryo (Figure 2B), whereas in rosette leaves, methylation of H3K4 was not detected in promoter region and higher in other parts of the genome. In contrast, low methylation of H3K9 was only seen in the surrounding region of the transcription start site in somatic embryos (Figure 2C), and was hardly detected in other regions in both of somatic embryo and rosette leaves. From these results, we suggest that H3K4 methylation at the histone of the promoter region may be involved in *LEC1* expression.

Expression profile of SET genes before and after fertilization in Arabidopsis

From the comparison of histone methylation between somatic embryos and rosette leaves, the histone methylation pattern was altered in the promoter region of *LEC1* (Figure 2B and C). To identify the *SET* genes involved in this alteration from among the 37 predicted *SET* genes in *Arabidopsis*, we performed an expression analysis using RT-PCR of inflorescences and siliques. To evaluate the expression in other stages of the life cycle of *Arabidopsis*, flower buds, flowers (1–2 days after flowering), and siliques (<5 mm), containing eggs before fertilization, fertilized eggs, and early zygotic embryos, were examined. In total, 31 *SET* genes were expressed in all tissues (Figure 3), whereas 6 of the predicted genes were not expressed. The expression levels of most genes were not altered during embryo development; only that of *MEA* increased. From these results, the 6 unexpressed or undetected *SET* genes were removed as candidates and were not used in the following experiments.

Copyright © 2009 The Japanese Society for Plant Cell and Molecular Biology

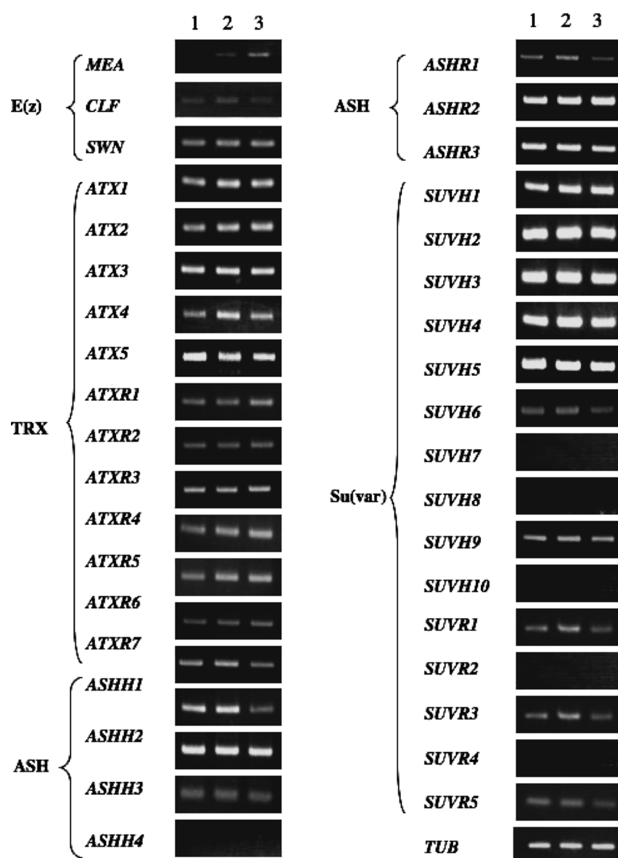

Figure 3. Expression analyses of *SET* genes in inflorescences and siliques. Results of RT-PCR from E(z), TRX, ASH, and Su(var)3-9 groups. Lane 1: flower buds; lane 2: flowers; and lane 3: siliques. PCR products of 10 μl from the sample cDNA using specific primers (Supplemental Table 1) were loaded on an agarose (1.5%) gel.

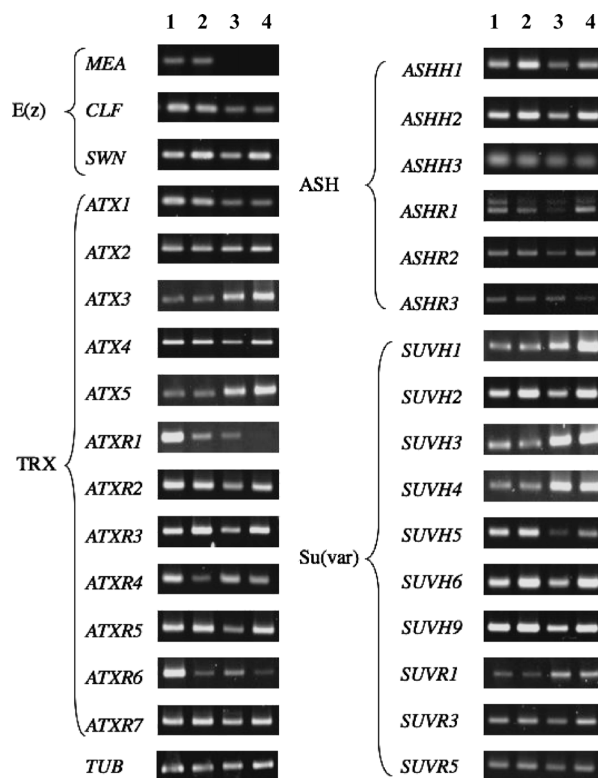

Figure 4. Expression analysis of *SET* genes in the stress induction system of somatic embryos. Results of RT-PCR of the E(z), TRX, ASH, and Su(var)3-9 groups. Lane 1: shoot-apical-tip explants before osmotic stress treatment; lane 2: shoot-apical-tip explants after osmotic stress treatment; lane 3: 2-week-old leaves before osmotic stress treatment; and lane 4: 2-week-old leaves before osmotic stress treatment. PCR products of 10 μl from the sample cDNA using specific primers (Supplemental Table 1) were loaded onto an agarose (1.5%) gel.

Evaluation of candidate genes using the stress induction system of somatic embryos

The expression profiles of 31 *SET* genes were evaluated in shoot-apical-tips and rosette leaves by RT-PCR before and after stress treatment. To confirm embryo formation in each experimental batch, a part of the explants was used as material for the induction of somatic embryos. Only *MEA* showed specific expression in shoot-apical-tip explants, but its expression did not change with stress treatment. In contrast, other *SET* genes were expressed in both explants by stress treatment (Figure 4). Among the 31 genes, the expression levels of 7 (*SWN*, *ATXR3*, *ASHH1*, *ASHH2*, *SUVH2*, *SUVH5*, and *SUVH6*) increased with stress treatment, while the expression of 3 genes (*ATXR1*, *ATXR4*, and *ATXR6*) was reduced (Figure 4). These tendencies were observed in both shoot-apical-tip and rosette leaf explants. On the other hand, the expression of 12 *SET* genes was specifically changed in leaves by stress: 7 (*ATX3*, *ATX4*, *ATXR5*, *ASHR1*, *SUVH1*, *SUVH9*, and *SUVR3*) exhibited increased expression and 5 (*ATXR1*, *ATXR2*, *ATXR4*, *ATXR6*, and *ASHR3*) had reduced expression.

Discussion

Association between embryogenesis and chromatin remodeling

Histone methylation is responsible for the higher structure of chromatin and the regulation of gene expression. Methylation of H3K4 indicates a euchromatin state and leads to active gene expression (Jenuwein and Allis 2001). On the other hand, methylation of H3K9 indicates the heterochromatin state and inactive gene expression (Jenuwein and Allis 2001). In this study, we showed that the expression of *LEC1* increased with stress treatment (Figure 1). Expression of *LEC1* is reportedly involved in chromatin remodeling because *LEC1* is ectopically expressed in *pkl1-1* mutant, which is deficient in chromatin remodeling factor (Ogas et al. 1999; Rider et al. 2003). Therefore, we expected the histone methylation status to differ between tissues expressing *LEC1* (somatic embryos) and those not expressing *LEC1* (rosette leaves), as the expression of *LEC1* is regulated by chromatin remodeling. To evaluate whether chromatin remodeling is involved in embryogenesis, we investigated

Copyright © 2009 The Japanese Society for Plant Cell and Molecular Biology

histone methylation in the genomic region of *LEC1* by ChIP analysis using somatic embryos and rosette leaves. The results showed that the H3K4 methylation level in the promoter region was higher in somatic embryos than in rosette leaves (Figure 2B). Chromatin remodeling is reported to mainly regulate the higher structure of chromatin in the promoter region (Mellor 2005). However, methylation of H3K9 was not detected in the promoter region of rosette leaves (Figure 2C). Histone in the promoter region of seedlings is reportedly modified by H3K27 methylation, which is detected in the promoter region of repressed genes (Zang et al. 2007). Therefore, repression of *LEC1* in the vegetative phase may have been involved in H3K27 methylation of the promoter region, but not in H3K9 methylation. From these results, we suggest that chromatin remodeling in the promoter region of *LEC1* may regulate gene expression. H3K9 methylation was only detected at the surroundiug region of transcription start site in somatic embryos (Figure 2C); however, H3K4 methylation was also detected at the surroundiug region of transcription start site in somatic embryos (Figure 2B). Therefore, methylation at the transcription start site may not be involved in the expression of *LEC1*.

SET genes expressed in Arabidopsis embryonic tissues

In total, 37 *SET* genes were predicted for *Arabidopsis* based on sequence information. These genes were divided into four groups based on their functional domains. From the expression analysis of inflorescences and siliques, 6 of the 37 genes were not expressed in any tissue (Figure 3). Furthermore, these 6 predicted *SET* genes were also not expressed in vegetative tissues (data not shown). From these data, these 6 predicted *SET* genes might not function in the zygotic embryo and vegetative phase, whereas 31 *SET* genes were expressed in somatic embryos, 3, 12, 6, and 10 of which belonged to the E(z), TRX, ASH, and Su(var)3-9 groups, respectively. Because some *SET* genes show redundancy in *Arabidopsis* (Baumbusch et al. 2001), it is thought that these 31 *SET* genes also work redundantly in each group.

Candidates for SET genes involved in embryogenesis

The *SET* genes were divided into 4 groups (Su(var)3-9, ASH, TRX, and E(z)) based on their functions and functional domains. We predicted that up-regulated *SET* genes in induction of somatic embryo were expressed in the embryo and function in embryogenesis. Results of expression analysis using the stress induction system of somatic embryos indicated that 7 *SET* genes had a higher probability of being involved in embryogenesis than the others (Figure 4). Among the 4 groups, Su(var)3-9 was reported to manage the maintenance of heterochromatin, which is mainly involved in the transposons and centromere regions (Lindroth et al. 2004). The Su(var)3-9 group may be indirectly involved in the expression of embryogenesis-related genes. The *SUVH2*, *SUVH5*, and *SUVH6* genes are of interest, because their expression increased in the stress induction system of somatic embryos (Figure 4). Furthermore, the *suvh2* mutation is reported to cause ectopic heterochromatization (Naumann et al. 2005) and *SUVH6* is involved in the maintenance of heterochromatin in *Arabidopsis* (Ebbs et al. 2005). In mice, the methylation level of histone throughout the whole genome was reportedly altered during the formation of germ line cells (Seki et al. 2005). Since germ line cells, as well as somatic embryos, are derived from cells in somatic tissues via re-differentiation (Surani 2001), genome-wide heterochromatization might be a necessary component of embryogenesis in plants via re-differentiation.

The expression levels of *ASHH1* and *ASHH2*, from the ASH group of genes, increased in the stress induction system in the somatic embryo (Figure 4). *ASHH2* has been reported to be involved in the transition from the vegetative to the reproductive phase, as *ashh2* showed early flowering and suppression of *FLC* (Zhao et al. 2005). Therefore, *ASHH2* may be involved in phase transition from reproductive to embryogenesis. However, *ashh2* did not have an abnormal phenotype in embryogenesis. The amino acid sequence of *ASHH1* is similar to that of *ASHH2*. Therefore, *ASHH1* and *ASHH2* may function redundantly in early embryogenesis.

The TRX and E(z) groups may function competitively and be involved in the activation and silencing of gene expression in plants, respectively (Goodrich et al. 1997), and both groups are involved in the regulation of the expression of morphogenesis-related genes in *Arabidopsis* (Goodrich et al. 1997; Saleh et al. 2007). Therefore, it is expected that genes in these two groups that exhibit increased expression under stress treatment have a high probability of involvement in the expression of embryogenesis-related genes than other *SET* genes. *MEA* is reportedly expressed in the embryo and endosperm and involved in embryo and endosperm development via genome imprinting (Autran et al. 2005; Leroy et al. 2007). This report concurs with the expression analysis that *MEA* is specifically expressed in siliques (Figure 3). However, the expression pattern of *MEA* was not greatly changed by induction of the somatic embryo (Figure 4). High expression levels of *MEA* may not be necessary to form a somatic embryo. In the TRX and E(z) groups, only the expression levels of *SWN* and *ATXR3* increased with stress treatment. *ATXR3* may function in the expression of embryogenesis-related genes, which are repressed by the E(z) group in the vegetative and reproductive phase, because the TRX and E(z) groups are competitive with each other. However, there have

Copyright © 2009 The Japanese Society for Plant Cell and Molecular Biology

been no reports on the function of *ATXR3* and the phenotype in *atxr3* plants. Therefore, it is important to analyze the double mutant of *atxr3* and other *ATXR* genes. On the other hand, for *SWN*, it was reported that *LEC1* was ectopically expressed in the double mutant of *CLF* and *SWN* after germination (Makarevich et al. 2006). *SWN* has been reported to work redundantly with *MEA*, and mutations of *SWN* enhance embryo lethality in *MEA* mutants (Wang et al. 2006). *SWN* might repress the genes that are not involved in embryogenesis, and *SWN* is an important factor in the repression of *LEC1* in the vegetative phase. Because plant SET proteins work redundantly in many cases, analysis using *clf/swn* double mutants might clarify whether *SWN* is responsible for the regulation of histone methylation in the expression of *LEC1*.

Acknowledgements

This work is supported by Grant-in-Aid Scientific Research on Priority Areas "Genome Barriers in Plant Reproduction".

References

Alvarez-Venegas R, Pien S, Sadder M, Witmer X, Grossniklaus U, Avramova Z (2003) ATX-1, an *Arabidopsis* homolog of Trithorax, activates flower homeotic genes. *Curr Biol* 13: 627–637

An YQ, McDowell JM, Huang S, McKinney EC, Chambliss S, Meagher RB (1996) Strong, constitutive expression of the *Arabidopsis* ACT2/ACT8 actin subclass in vegetative tissues. *Plant J* 10: 107–121

Autran D, Huanca-Mamani W, Vielle-Calzada JP (2005) Genomic imprinting in plants: the epigenetic version of an Oedipus complex. *Curr Opin Plant Biol* 8: 19–25

Bastow R, Mylne JS, Lister C, Lippman Z, Martienssen RA, Dean C (2004) Vernalization requires epigenetic silencing of *FLC* by histone methylation. *Nature* 427: 164–167

Baumbusch LO, Thorstensen T, Krauss V, Fischer A, Naumann K, Assalkhou R, Schulz I, Reuter G, Aalen RB (2001) The *Arabidopsis thaliana* genome contains at least 29 active genes encoding SET domain proteins that can be assigned to four evolutionarily conserved classes. *Nucl Acids Res* 29: 4319–4333

Ebbs ML, Bartee L, Bender J (2005) H3 lysine 9 methylation is maintained on a transcribed inverted repeat by combined action of SUVH6 and SUVH4 methyltransferases. *Mol Cell Biol* 25: 10507–10515

Finnegan EJ, Kovac KA, Jaligot E, Sheldon CC, Peacock WJ, Dennis ES (2005) The downregulation of *FLOWERING LOCUS C* (*FLC*) expression in plants with low levels of DNA methylation and by vernalization occurs by distinct mechanisms. *Plant J* 44: 420–432

Gendrel AV, Lippman Z, Yordan C, Colt V, Martinessen RA (2002) Plant Chromatin Immunoprecipitation. *Science* 297: 1871–1873

Goodrich J, Puangsomlee P, Martin M, Long D, Meyerowitz E, Couplant G (1997) A Polycomb-group gene regulates homeotic gene expression in *Arabidopsis*. *Nature* 383: 44–51

Hayashi K, Yoshida K, Matsui Y (2005) A histone H3 methyltransferase controls epigenetic events required for meiotic prophase. *Nature* 438: 374–378

Ikeda-Iwai M, Umehara M, Satoh S, Kamada H (2003) Stress-induced somatic embryogenesis in vegetative tissues of *Arabidopsis thaliana*. *Plant J* 34: 107–114

Jenuwein T, Allis CD (2001) Translating the histone code. *Science* 293: 1074–1080

Kamada H, Harada H (1979) Studies on organogenesis in carrot tissue culture. I. Effects of growth regulation on somatic embryogenesis and root formation. *Z Pflanzenphysiol* 91: 225–266

Kikuchi A, Sanuki N, Higashi K, Koshiba T, Kamada H (2006) Abscisic acid and stress treatment are essential for the acquisition of embryogenic competence by carrot somatic cells. *Planta* 223: 637–645

Leroy O, Hennig L, Breuninger H, Laux T, Köhler C (2007) Polycomb group proteins function in the female gametophyte to determine seed development in plants. *Development* 134: 3639–3648

Lindroth AM, Shultis D, Jasencakova Z, Fuchs J, Johnson L, Schubert D, Patnaik D, Pradhan S, Goodrich J, Schubert I, Jenuwein T, Khorasanizadeh S, Jacobsen SE (2004) Dual histone H3 methylation marks at lysines 9 and 27 required for interaction with CHROMOMETHYLASE3. *EMBO J* 23: 4286–4296

Loidl P (2004) A plant dialect of the histone language. *Trends Plant Sci* 9: 84–90

Lotan T, Ohto M, Yee KM, West MA, Lo R, Kwong RW, Yamagishi K, Fischer RL, Goldberg RB, Harada JJ (1998) *Arabidopsis LEAFY COTYLEDON1* is sufficient to induce embryo development in vegetative cells. *Cell* 93: 1195–1205

Makarevich G, Leroy O, Akinci U, Schubert D, Clarenz O, Goodrich J, Grossniklaus U, Köhler C (2006) Different Polycomb group complexes regulate common target genes in *Arabidopsis*. *EMBO Rep* 7: 947–952

Mellor J (2005) The Dynamics of chromatin remodeling at promoters. *Mol Cell* 19: 147–157

Meinke DW (1995) Molecular genetics of plant embryogenesis. *Annu Rev Plant Phys Plant Mol Biol* 46: 369–394

Naumann K, Fischer A, Hofmann I, Krauss V, Phalke S, Irmler K, Hause G, Aurich AC, Dorn R, Jenuwein T, Reuter G (2005) Pivotal role of AtSUVH2 in heterochromatic histone methylation and gene silencing in *Arabidopsis*. *EMBO J* 24: 1418–1429

Nishiwaki M, Fujino K, Koda Y, Masuda K, Kikuta Y (2000) Somatic embryogenesis induced by the simple application of abscisic acid to carrot (*Daucus carota* L.) seedlings in culture. *Planta* 211: 756–759

Ogas J, Kaufmann S, Henderson J, Somerville C (1999) *PICKLE* is a CHD3 chromatin-remodeling factor that regulates the transition from embryonic to vegetative development in *Arabidopsis*. *Proc Natl Acad Sci USA* 96: 13839–13844

Parcy F, Valon C, Kohara A, Misera S, Giraudat J (1997) The *ABSCISIC ACID-INSENSITIVE3*, *FUSCA3*, and *LEAFY COTYLEDON1* loci act in concert to control multiple aspects of *Arabidopsis* seed development. *Plant Cell* 9: 1265–1277

Pien S, Fleury D, Mylne JS, Crevillen P, Inzé D, Avramova Z, Dean C, Grossniklaus U (2008) *ARABIDOPSIS TRITHORAX1* dynamically regulates *FLOWERING LOCUS C* activation *via* histone 3 lysine 4 trimethylation. *Plant Cell* 20: 850–858

Pirrotta V (1998) Polycombing the genome: PcG, trxG, and chromatin silencing. *Cell* 93: 333–336

Rider S, Henderson J, Jerome R, Edenberg H, Romero-Severson J,

Copyright © 2009 The Japanese Society for Plant Cell and Molecular Biology

Ogas J (2003) Coordinate repression of regulators of embryonic identity by *PICKLE* during germination in *Arabidopsis*. *Plant J* 35: 33–43

Saleh A, Al-Abdallat A, Ndamukong I, Alvarez-Venegas R, Avramova Z (2007) The *Arabidopsis* homologs of trithorax (ATX1) and enhancer of zeste (CLF) establish 'bivalent chromatin marks' at the silent *AGAMOUS* locus. *Nucl Acids Res* 35: 6290–6296

Seki Y, Hayashi K, Itoh K, Mizugaki M, Saitou M, Matsui Y (2005) Extensive and orderly reprogramming of genome-wide chromatin modifications associated with specification and early development of germ cells in mice. *Dev Biol* 278: 440–458

Simon J, Tamkun J (2002) Programming off and on states in chromatin: mechanisms of Polycomb and trithorax group complexes. *Curr Opin Genet* 12: 210–218

Sung S, Amasino RM (2004) Vernalization in *Arabidopsis thaliana* is mediated by the PHD finger protein VIN3. *Nature* 427: 159–163

Surani MA (2001) Reprogramming of genome function through epigenetic inheritance. *Nature* 414: 122–128

Takahata K, Takeuchi M, Fujita M, Azuma J, Kamada H, Sato, F (2004) Isolation of putative glycoprotein gene from early somatic embryo of carrot and its possible involvement in somatic embryo development. *Plant Cell Physiol* 45: 1658–1668

Tanaka M, Kikuchi A, Kamada H (2009) Isolation of putative embryo-specific genes using stress induction of carrot somatic embryos. *Breed Sci* 59: 37–46

Tariq M, Paszkowski J (2004) DNA and histone methylation in plants. *Trends Genet* 20: 244–251

Terranova R, Agherbi H, Boned A, Meresse S, Djabali M (2006) Histone and DNA methylation defects at *Hox* genes in mice expressing a SET domain-truncated form of Mll. *Proc Natl Acad Sci USA* 103: 6629–6634

Thibaud-Nissen F, Shealy RT, Khanna A, Voldkin LO (2003) Clustering of microarray data reveals transcript patterns associated with somatic embryogenesis in soybean. *Plant Physiol* 132: 118–136

Tripoulas N, LaJeunesse D, Gildea J, Shearn A (1996) The *Drosophila* ash1 gene product, which is localized at specific sites on polytene chromosomes, contains a SET domain and a PHD finger. *Genetics* 143: 913–928

van Lohuizen M (1998) Functional analysis of mouse Polycomb group genes. *Cell Mol Life Sci* 54: 71–79

Wang D, Tyson MD, Jackson SS, Yadegari R (2006) Partially redundant functions of two SET-domain polycomb-group proteins in controlling initiation of seed development in *Arabidopsis*. *Proc Natl Acad Sci USA* 103: 13244–13249

Zhang, X, Clarenz, O, Cokus, S, Bernatavichute, YV, Pellegrini, M, Goodrich, J, Jacobsen, SE. (2007) Whole-genome analysis of histone H3 Lysine 27 trimethylation in *Arabidopsis*. *PLoS Biol* 5: e129

Zhao Z, Yu Y, Meyer D, Wu C, Shen WH (2005) Prevention of early flowering by expression of *FLOWERING LOCUS C* requires methylation of histone H3K36. *Nat Cell Biol* 7: 1256–1260

Copyright © 2009 The Japanese Society for Plant Cell and Molecular Biology