An Epigenetic Integrator: New Insights into Genome Regulation, Environmental Stress Responses and Developmental Controls by HISTONE DEACETYLASE 6

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Introduction

Gene silencing via histone deacetylation is a universally conserved epigenetic regulation system in eukaryotes (Wolffe 1996, Yang and Seto 2003, Horn 2008). In several biological processes, such as ontogenesis, oncogenesis and genome maintenance, epigenetic silencing has important roles in the regulation of gene activity (Ropero and Esteller 2007, Horn 2008). Histone deacetylases (HDACs) catalyze the removal of acetyl groups from acetylated lysine (K) residues in the N-termini of histone proteins that are active epigenetic marks, such as histone H3 Lys9 acetylation (H3K9ac) (Shahbazian and Grunstein 2007). Histone deacetylation functions in the repression of genes and is correlated with repressive epigenetic marks of histone methylation, such as histone H3 Lys9 di-methylation (H3K9me2), histone H3 Lys27 tri-methylation (H3K27me3) and DNA methylation (Richards and Elgin 2002, Fuks 2005, Moss and Wallrath 2007, Shahbazian and Grunstein 2007, Zhou 2009).

In Arabidopsis, there are 16 genes encoding HDACs (Pandey et al. 2002). RPD3 (REDUCED POTASSIUM DEFICIENCY 3)-type HDACs are important as regulators of chromatin maintenance and of the activity of housekeeping genes in yeast, fruit flies, nematodes and metazoans (Pandey et al. 2002, Yang and Seto 2008). There are four HDAC genes (HDA6, HDA7, HDA9 and HDA19) which belong to the RPD3-type HDACs in Arabidopsis. HDA19 functions in development, embryogenesis and light response in plants (Long et al. 2006, Tanaka et al. 2008, Jang et al. 2011). Functional analyses of HDA7 and HDA9 have not been reported yet.

HDA6 has been well studied with respect to its regulation of gene activity and genome maintenance in plants (May et al. 2005, Earley et al. 2006, Aufsatz et al. 2007, Pontes et al. 2007, Tessadori et al. 2009, To et al. 2011a, To et al. 2011b, Zhu et al. 2011). Initially, HDA6 was identified as a genetic repressor of transgene expression (Furner et al. 1998, Murfett et al. 2001, Aufsatz et al. 2002). More recently, several functional features of HDA6, such as gene silencing of heterochromatic regions and
regulation of gene activities in response to developmental and environmental signals, have been reported (Fig. 1). In this review, we summarize recent findings on the silencing mechanism and the biological functions of HDA6 in plants.

**Regulation of genes, transposons and genome integrity by HDA6**

Previous genetic screens for mutants with increased expression of transgenes showed that HDA6 is required for transgene silencing (Furner et al. 1998, Murfett et al. 2001, Aufsatz et al. 2002). Some retrotransposons, such as ATCOPIA, ATLINE1-4, ATLANTYS, ATGP1 and Sadhu, are also suppressed by HDA6 (Lippman et al. 2003, May et al. 2005, Rangwala and Richards 2007). Genetic screens have suggested that HDA6 is a component of the RNA-directed DNA methylation (RdDM) pathway. So far, HDA6 is the sole HDAC cooperating with small interfering RNAs (siRNAs) that are generated via the RdDM pathway to repress gene activity in Arabidopsis (Aufsatz et al. 2002, Aufsatz et al. 2007).

Nucleolar dominance is an epigenetic phenomenon in genetic hybrids that involves the selective expression of rRNA genes inherited from one progenitor due to the silencing of the other progenitor’s rRNA genes (Chen et al. 1998, Earley et al. 2006, Vaillant et al. 2007). In *Arabidopsis suecica*, the allotetraploid hybrid of *A. thaliana* and *A. arenosa*, the *A. thaliana*-derived rRNA genes are selectively silenced. Previous studies using *A. suecica* showed that transcription of the silenced *A. thaliana*-derived rRNA genes was derepressed and decondensation of *A. thaliana*-derived nucleolar organizer regions (NORs) was observed in the HDA6 RNA interference (RNAi) lines, suggesting that HDA6 is required for repression of rRNA transcription and NORs in nucleolar dominance (Earley et al. 2006, Pontes et al. 2007). The condensation and inactivation of NORs are developmentally regulated and linked to transition from the accumulation of H3K4m3, an active histone modification mark, to H3K9m2 and 5-methylcytosine-enriched chromocenters. However, in HDA6 RNAi lines, the developmentally regulated condensation and the inactivation of *A. thaliana* NORs were disrupted, indicating that HDA6 is required for developmentally regulated silencing of rRNA genes (Pontes et al. 2007).

At the intergenic spacer (IGS) region of 45S rRNA genes, spurious transcription by RNA polymerase II (Pol II) occurs in *hda6* mutants, suggesting that RNA polymerase I (Pol I), which transcribes the rRNA gene repeat, and Pol II cooperate under the control of HDA6. In the *hda6* mutants, CG and CHG methylation was reduced and siRNAs on the IGSs were consistently overproduced. It is possible that siRNA overproduction compensates for the loss of HDA6 function to regulate rRNA regions in the *hda6* mutants (Earley et al. 2010). HDT1/AtHD2A, a member of HD2-type HDACs, is also involved in the condensation and inactivation of *A. thaliana*-derived NORs (Lawrence et al. 2004, Pontes et al. 2007). These results indicate that multiple HDACs and DNA methyltransferase might be required for silencing of NORs in nucleolar dominance.

Similar to mammalian cells, centromeric regions harboring 180 bp repeats are epigenetically regulated in Arabidopsis. Loss of silencing from reverse-strand centromeric 180 bp repeats was observed in *sil1* (an allele of *HDA6*; Furner et al. 1998) and *met1* mutants, but not in *ago1* and *dcl1* mutants, indicating that HDA6 and MET1 are required for the silencing of centromeric regions (May et al. 2005).
**HDA6-mediated silencing and its direct targets**

HDA6 has a strong preference to repress repetitive sequences and multicopy genes. The sil1 and axe1-5 alleles of hda6 reactivated rDNA repeats in Arabidopsis (Probst et al. 2004). On the rDNA repeats in hda6 mutants, the chromatin was changed to an active status, which was seen as enrichments of histone acetylation, H3K4m3 and DNA hypomethylation, suggesting that HDA6-mediated gene silencing is correlated with histone deacetylation and DNA methylation. However, the HDA6-mediated silencing mechanism on other genomic region was unclear and the direct targets of HDA6 were not reported until recently.

Recently, HDA6 direct targets were identified by transcriptome and chromatin immunoprecipitation (ChIP) analysis, and the model of HDA6-mediated gene silencing on its target genes was proposed (To et al. 2011a). From the results of transcriptome analysis using a tiling array and ChIP assay using an antibody specific to the C-terminus of the HDA6 protein, HDA6 was found to target several transposable elements, including a Sadhu-type transposable element and various genes of unknown function. Within these regions, all lysine acetylations on the N-termini of histones H3 and H4, except histone H4K16, were significantly enriched in the axe1-5 mutant (To et al. 2011a). This observation is consistent with the results of an in vitro assay showing that recombinant HDA6 protein can deacetylate H3K14ac, H4K5ac and H4K12ac (Early et al. 2006).

A role for HDA6 in DNA methylation has been suggested. Recent extensive studies of DNA methylation of the direct targets of HDA6 revealed the loss of CHG and CHH DNA methylations in the hda6 mutant in all loci tested (To et al. 2011a). More interestingly, two patterns of CG DNA methylation in the mutant were found depending on the locus. CG methylation was sustained in the hda6 mutant at some HDA6 target loci that were surrounded by flanking DNA-methylated regions. In contrast, complete loss of CG methylation occurred in the hda6 mutant at the HDA6 target loci that were isolated from flanking DNA methylation (To et al. 2011a). The analysis suggests that the effects of the DNA methylation surrounding the HDA6 targets could account for the sustained CG DNA methylation, because the loss of CG DNA methylation was correlated with the existence of other DNA-methylated regions around the targets. More importantly, HDA6 was shown to be essential for MET1-derived CG DNA methylation at the loci where the other DNA-methylated region is absent. In addition, the transcriptome analysis identified the significant overlap between the hda6 and met1 mutants. Furthermore, HDA6 deficiency resulted in the loss of heterochromatic epigenetic marks and the enrichment of euchromatic marks at HDA6 direct targets, along with ectopic expression of these loci (To et al. 2011a). Quite recently, it was reported that HDA6 physically interacts with MET1 (Liu et al. 2012). Therefore, it has been suggested that HDA6 and MET1 function cooperatively in heterochromatic gene silencing on the HDA6 direct targets (Fig. 2).

Although the evidence for HDA6-mediated silencing involving siRNAs has been reported for some regions, such as on 180 bp repeats (May et al. 2005), the proposed model may still not be sufficient to explain the silencing of all HDA6 target genes. In addition, results from the transcriptome analysis suggested that HDA6 also affects the repression of several endogenous genes encoding known proteins in Arabidopsis (To et al. 2011a). To gain a more complete understanding of HDA6-mediated gene silencing mechanisms, genome-wide identification of HDA6 direct binding sites and profiling studies of methylation and histone modification status of HDA6 direct target regions will be necessary.

**HDA6 functions in plant growth and development**

HDACs have important roles in plant development and growth. By adding the HDAC inhibitor trichostatin A during growth, some mutant plants and seeds have shown altered expression profiles and changes in plant growth and development (Tai et al. 2005, Fukaki et al. 2006, Ueno et al. 2007, Tanaka et al. 2008). hda6 mutant plants showed apical defects in embryogenesis similar to the topless-1 mutant (Long et al. 2006). HDA6 is also involved in the regulation of embryonic properties after germination (Tanaka et al. 2008). HDA6 and HDA19 redundantly regulate the repression of key genes expressed during embryogenesis [leafy cotyledon 1 (LEC1), fusca 3 (FUS3) and abscisic acid insensitive 3 (ABI3)].

LEC1 encodes a transcriptional activator of genes required for embryo maturation and FUS3 encodes a transcriptional factor involved in embryo development ending in seed dormancy (Parcy et al. 1997). The hda6/hda19 double RNAi line arrested growth after germination and formation of an embryo-like structure, and its phenotype was rescued by a pickel (Pkl) mutation. In a mutant lacking PICKLE (Pkl), a CHD3 (CHROMODOMAIN-Helixcase-DNA-Binding 3)-type chromatin remodeling factor, the transcription of LEC1 and FUS3 was ectopically uncontrolled (Ogas et al. 1999, Rider et al. 2003). Post-germination growth, such as cotyledon expansion and vegetative growth, was arrested in an HDA6:RNAi pkl1-1-doubt mutant (Tanaka et al. 2008). The expression of LEC1, FUS3 and ABI3 was higher in the HDA6:RNAi pkl1-1 double mutant than in each single mutant. Repression of the formation of an embryo-like structure was partially released in the HDA6:RNAi pkl1-1-doubt mutant by long-term cultivation on Murashige and Skoog (MS) medium. These results suggest that HDA6 enhances derepression of embryonic properties in the pkl mutant.

HDA6 also functions as a component of repressors for master regulators during seed maturation, LEC2 and FUS3, and acts downstream of microRNAs (miRNAs; Willmann...
et al. 2011). Although there was no morphological defect, sil1-1 mutants showed retardation of maturation during an early embryonic stage as measured by Chl fluorescence of the embryo. Microarray analysis showed that expression of HDA6, as well as of ASIL1 (ARABIDOPSIS 6B-INTERACTING PROTEIN 1-LIKE 1), a trihelix transcription factor, and CLF (CURLY LEAF) genes, was down-regulated in the miRNA-deficient mutant dcl1-15, suggesting that HDA6 represses the maturation program during early embryogenesis at a point downstream of miRNA targets.

Overexpression of AtHD2A resulted in morphological defects of leaves and flowers, delayed flowering and aborted seed development (Zhou et al. 2004). The hda6 mutants exhibited late flowering and increased leaf longevity (Wu et al. 2008, Yu et al. 2011). Flowering timing of an axe1-5/flc-3 double mutant was earlier than that of the axe1-5 single mutant. Co-immunoprecipitation assays showed that the HDA6 protein interacted with FLD, a histone demethylase, and a ChIP assay showed that H3K9K14ac and H3K4m3 were enriched in FLC (FLOWERING LOCUS C) MAF4 (MADS AFFECTING FLOWERING 4) and MAF5 gene regions in axe1-5 and fld-6 mutants (Yu et al. 2011). These results show that HDA6 is involved in the control of flowering.

HDA6 functions in several environmental stress responses

Environmental stresses affect plant growth and productivity. Plants have adapted multiple mechanisms to aid their survival against the harmful effects of abiotic and biotic stresses. Cold and freezing temperatures are stresses that can cause serious damage to plants. In order to withstand cold stress, many plants have the ability to increase their freezing tolerance through a process termed cold acclimation, whereby gene expression of cold stress-responsive genes is altered in response to low but non-freezing temperatures (Thomashow 1999). It is well known that a rapid response to cold through the action of transcription factors, such as DEHYDRATION-RESPONSIVE ELEMENT-BINDING PROTEIN 1A (DREB1A)/C-REPEAT-BINDING FACTOR 3 (CBF3), DREB1B/CBF1 and DREB1C/ CBF2, plays an important role in cold acclimation (Gilmour et al. 1998, Shinwari et al. 1998). Interestingly, HDA6 regulates the expression of several long-term cold stress-responsive genes and plays a role in the acquisition of freezing tolerance (To et al. 2011b). Cold-acclimated hda6 mutant plants showed a freezing-sensitive phenotype compared with cold-acclimated wild-type plants. Although the hda6 mutation did not affect the expression of several cold-responsive genes involved in the

**Fig. 2** HDA6-mediated gene silencing mechanism. To establish gene silencing, HDA6 and MET1 are recruited on the HDA6 direct target regions. HDA6 deacetylates histone acetylation on histone H3 lysine 9, 14, 23 and 27 sites and histone H4 lysine 5, 8 and 12 sites on the target regions. At the same time, CG DNA methylation is added by MET1.
rapid response, such as DREB1A and RD29A/COR78/LTI78, the expression of several genes that are induced by long-term cold treatment, such as fatty acid desaturase and lipid transfer protein genes, was changed in the axr1-5 mutant (To et al. 2011b). These results suggest that the cold acclimation process consists of two phases, the rapid response phase, which is driven by transcription factors, and the slow response phase, which is driven by HDA6-mediated chromatin regulation.

Light is also a very important environmental factor in plant life cycles. Chromatin organization was recently shown to be controlled by light intensity (Tessadori et al. 2009). A quantitative trait locus (QTL) analysis, coupled with microscopic observations, showed that HDA6 and PHOTOCROME B (PHYB) are positive regulators of light-controlled chromatin compaction (Tessadori et al. 2009). Light-dependent compaction of NORs is regulated by HDA6 and PHYB. These observations suggest that HDA6 functions in chromatin organization and plasticity in response to several environmental changes.

The jasmonate (JA) signaling pathway functions in defense and wounding responses triggered by insects and other pathogens, plant development and stress-related growth inhibition (Balbi and Devoto 2008, Memelink 2009). CORONATINE INSENSITIVE 1 (COI1), an F-box protein that forms an SCF ubiquitin-ligase complex, is an essential component of the JA signaling pathway (Xie et al. 1998, del Pozo and Estelle 2000). JASMONATE ZIM-DOMAIN 1 (JAZ1), a key regulator of JA signaling, interacts with COI1 in the presence of jasmonylisoleucine (JA-Ile) conjugate (Thines et al. 2007). Interaction between HDA6 and COI1 in Arabidopsis cells was shown by co-immunoprecipitation and yeast two-hybrid assays (Devoto et al. 2002). Ethylene signaling also has an important role in the regulation of plant development and tolerance to necrotrophic fungi. A recent study indicated that JAZ1 recruits HDA6 to repress ETHYLENE INSENSITIVE 3 (EIN3)/EIN3-LIKE 1 (EIL1)-dependent transcription, inhibits JA signaling and that HDA6 might be involved in the modulation of JA-ethylene-regulated processes (Zhu et al. 2011). These results suggest that HDA6 has an integrative function in several biological processes in plants.

Conclusions and future perspectives

Chromatin status is intimately connected with the delicate regulation of gene activity in eukaryotes. HDA6 is a well studied chromatin-modifying factor in plants that is involved in the regulation of several biological processes, such as transcriptional regulation, genome maintenance and environmental stress responses. A complete set of direct targets and functional complexes involving HDA6 has not yet been identified and it is not known whether these targets and complexes change in response to environmental and developmental phases. To understand HDA6 function in more depth, ChIP-on-chip or ChIP-seq analyses, that are very advantageous and useful, aimed at acquiring this information are essential. We believe that such intensive studies will lead to a better understanding of the universally conserved regulatory mechanisms and functions of epigenetic information in eukaryotes.

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References


