Histone Deacetylase Complexes: Implications for Plants

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Summary

Post-translational modifications of histone tails have dramatic ramifications on a variety of vital cellular functions. Removal of the acetyl groups from lysine residues is catalyzed by histone deacetylases (HDs). Many HDs are known to be components of multiprotein complexes such as SIN3 and NuRD that are involved in chromatin condensation and gene regulation. Plants contain a highly elaborated set of HDs with four distinct classes of these enzymes. Plant HDs have been implicated to play roles in transgene silencing, rDNA regulation, gene expression, and many developmental processes. Seventeen *Arabidopsis* HDs are apparent in Genbank as are numerous putative HD-interacting partners. Maize HDs have been extensively characterized biochemically, and the use of powerful genetic tools currently available in *Arabidopsis* is rapidly accelerating the base of knowledge on the control circuitry of plant chromatin.

Key words:
transcriptional regulation, chromatin, histone deacetylation, repressor.

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1. Chromatin

The packaging of the genome into the eukaryotic nucleus is a remarkable feat involving extensive folding and wrapping of the DNA into compact chromatin structures. This folding and wrapping are necessary to pack the 100 cm to 100 m of DNA into a nucleus that is only 5-10 mm in diameter (1). The most basic unit of chromatin structure is the nucleosome, composed of 146 bp of DNA wrapped around an octamer of proteins (2-5). This

octamer comprises of four core histones (H2A, H2B, H3, and H4) that are organized as dimers of H2A-H2B attached to both sides of an H3-H4 tetramer (5-8). Histone H1 (the linker histone) binds the internucleosomal DNA. The histone N-termini are described as tails because they appear unstructured in nucleosome x-ray crystallography. However, the histone tails have vital functions; they mediate internucleosomal contacts and are required for repression of basal transcription (9,10).

Tight packing of DNA into chromatin structures prevents essential interface between DNA and proteins, such as RNA polymerase, DNA polymerase, DNA repair enzymes, etc. Therefore, dynamic alterations in chromatin structure are necessary to allow such processes as transcription, replication, recombination, and repair to occur (11-15). Two types of chromatin remodeling are known: physical displacement of nucleosomes by ATP-driven motors, and posttranslational modification of histone tails. ATP-driven motors that displace nucleosomes are large complexes, such as DNA and RNA polymerases, and the SWI/SNF (switch/sucrose non-fermenting) proteins (16-19). Examples of posttranslational modifications include four classes of histone-tail alteration: methylation, phosphorylation, ADP-ribosylation, and acetylation (10,20). Acetylation of histones by histone acetyltransferases (HATs) is correlated with variation in chromatin structure *in vivo* (21,22). About twenty-eight lysines per nucleosome have a capacity to be acetylated with a remarkable spatial and temporal heterogeneity (20). Most importantly, there is a correlation between hyperacetylation of chromatin and transcriptional activation (23,24).

Two models explain this correlation. The "acetylation signaling" model suggests that histone acetylation is a signal, similar to that of protein phosphorylation (25,26). This signal is proposed to alter recruitment of chromatin remodeling complexes. This model is supported by the relatively low level of histone acetylation necessary to facilitate transcription. Only 12 of 28 possible lysine residues per histone octamer must be acetylated to increase *in vitro* transcription more than 15-fold (9,10). The "loose chromatin" model proposes that acetylation causes histone-DNA interactions to weaken, resulting in a loosening of chromatin (23,27,28). The conversion of positively charged lysine ε-amino groups into neutral ε-acetamido groups alters chromatin structure, and increases transcription (29) by increasing accessibility to transcription factors (8,23,27,28). The acetylated mono-nucleosomes appear to wrap DNA less tightly than those deacetylated (9). On the other hand, deacetylation of histone tails catalyzed by histone deacetylases (HDs) causes chromatin tightening, inducing transcriptional repression (24).

2. HDs and their complexes

A connection between histone deacetylase activity and repression was indicated by deacetylase inhibitor studies in yeast, but direct proof was not provided (24). Nonetheless, a HD inhibitor was used in the initial molecular identification of an HD,

bovine HDAC1, which was recognized as a homolog to the yeast transcriptional repressor RPD3 (30). This finding solidified the connection between repression and histone deacetylation. Many eukaryotic HDs are now known to show homology to yeast RPD3, indicating that they are members of an ancient gene family (31,32). Originally, the mechanism by which RPD3 mediated HD activity was unclear since it did not bind DNA and had no defined interactions with other proteins (23). However, it is now clear that RPD3 is a constituent of both the yeast SIN3 (SWI independent 3) and HDB complexes (33,34). With these findings, it appears that neither HATs nor HDs work alone, as many are known to exist in multiprotein complexes (20,24).

3. Four classes of HDs

HDs can be grouped into four classes (35): (I) yeast RPD3-related proteins; (II) yeast HDA1-related proteins; (III) *Zea mays* HD2-related proteins; and (IV) yeast

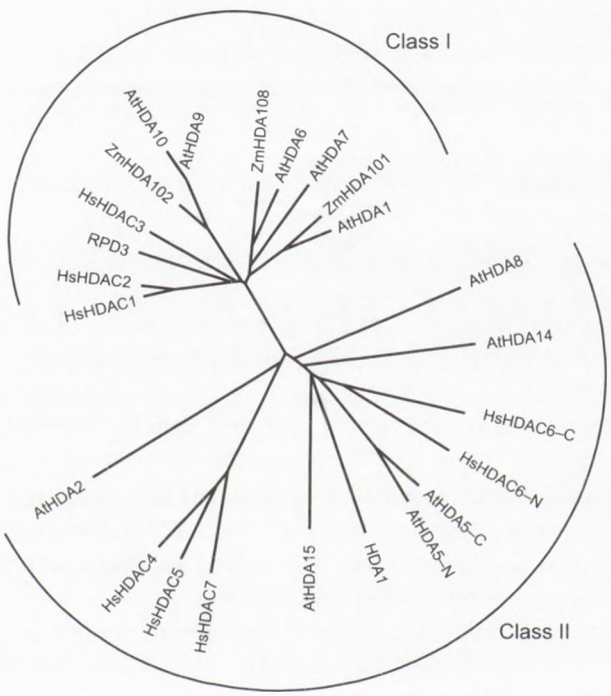

Fig. 1. Phylogenetic analysis of class I and class II HDs from yeast, humans (Hs), and *Arabidopsis* (At). Class I HDs (top) include yeast RPD3 and six *Arabidopsis* genes (note that AtHDA17 appears to be a recent duplication of AtHDA10 and was omitted from the phylogenetic tree for clarity). Class II HDs (bottom) include yeast HDA1 and 5 *Arabidopsis* genes. HsHDAC6 and AtHDA5 were divided at amino acids 450 and 560, respectively, to demonstrate the relationships of their N- and C-terminal HD domains. The phylogenetic tree was constructed using CLUSTALW and PHYLIP software (86,87).

Sir2-related proteins (20,32,36-41). Classes I, II, and III are hydrolytic enzymes that do not appear to require co-factors. However, the newly elucidated class IV enzymes require energy from hydrolysis of the NAD^+ glycosidic bond (38). Classes I and II are related in their catalytic domains (Fig. 1), but classes III and IV share no homology with class I, II, or each other. Interestingly, HD2 shares sequence similarity with peptidyl-prolyl *cis-trans* isomerases, a *Trypanosoma brucei* nucleolar RNA binding protein, and *Spodoptera* FK506-binding protein 46 (42,43). Class IV enzymes are phylogenetically related to Sir2 proteins, Sirtuins, from prokaryotes and eukaryotes (44,45). Human class I HDs (HDAC1, 2, 3, and 8) comprise of 400-500 amino acids and are primarily located in the nucleus (46,47). When immunopurified, the class I HDs can deacetylate all four core histones with little substrate specificity *in vitro* (24). The mammalian class II enzymes (HDAC4, 5, 6, and 7) are larger proteins, approximately 1000 amino acids in length and are also unspecific as to substrates (46-48). The class IV member, Sir2, shows a specificity for deacetylating lysines 9 and 14 of histone H3, and lysine 16 of H4 (three of the four critical residues for transcriptional silencing; 38); however, a *Drosophila* homolog does not share the same specificity (49). Since their initial discovery in maize, class III HDs have been identified in other organisms, and they will be discussed in greater detail below (see section 5).

3.1. The SIN3 complex

The class I HDs in mammals, HDAC1 and HDAC2, both exist in two complexes: the SIN3 complex (Fig. 2) and the nucleosome remodeling HD complex, NuRD (24). The SIN3 complex (see Table 2) has a minimum of seven subunits: the two deacetylases HDAC1 and HDAC2, Sin3, RbAp48, RbAp46, Sin3-associated-protein 30 (SAP30), and SAP18 (50,51). The Sin3 protein is a likely candidate for a scaffold of the SIN3 complex due to its paired amphipathic helices, a known motif for protein-protein interactions (24,52,53). In support of this role for Sin3, transcriptional co-repressors N-CoR (nuclear receptor co-repressor) and SMRT (silencing mediator for retinoid and thyroid receptor) indirectly bind HDAC1 through Sin3, and the Sin3-associated protein SAP30 appears to target the SIN3 complex to some promoter-repressor elements that use N-CoR (24,46).

The SIN3 complex components RbAp48 and RbAp46, identified by their association with retinoblastoma protein, are capable of binding directly to helix 1 of histone H4 possibly classifying them as the histone-binding subunits of the SIN3 complex (50,54,55). RbAp48 also directly binds a complex of HDAC1 and HDAC2 and may enhance the HD activity by securing the enzyme near the lysine moieties being modified (30,54). However, the helix 1 of histone H4 is deep within the nucleosome and RbAp48 and RbAp46 can not attach to assembled nucleosomes without assistance, as seen *in vitro* where the SIN3 complex does not deacetylate nucleosomal

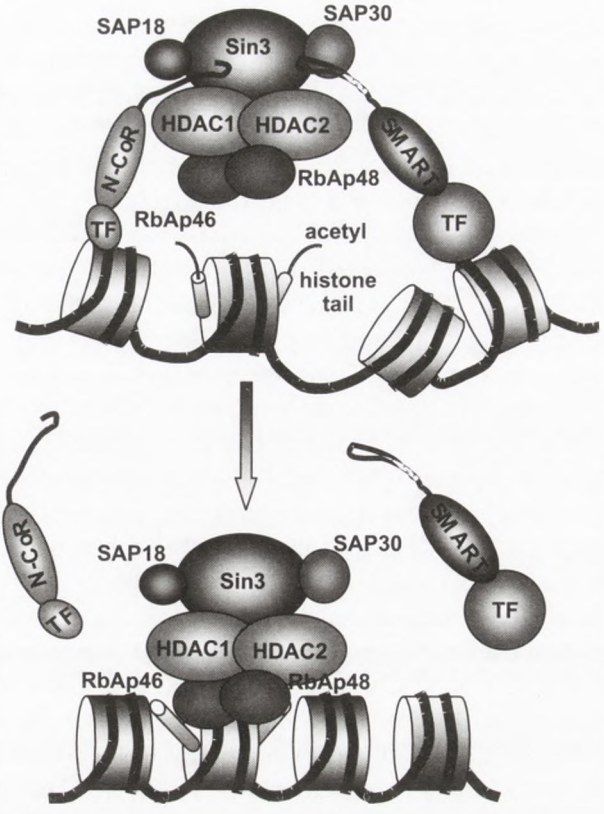

Fig. 2. An example of a histone deacetylase complex, the SIN3 complex, recruited by transcriptional repressors or co-repressors (i.e. N-CoR or SMRT) to acetylated chromatin. RbAP46 and RbAp48 may gain access to the histone H4 helix 1, and HDAC1 and HDAC2 deacetylate the histone tails resulting in a repressed chromatin state. TF, transcription factor.

histones (56). Thus, other factors appear to be required for loosening the nucleosomes, because nucleosomes are apparently deacetylated by the yeast SIN3 complex *in vivo* (57).

3.2. The NuRD complex and DNA methylation

Mammalian nucleosome remodeling HD complex assembles from 10 to 40 proteins (58-60). These include two class I HDs (HDAC1, and HDAC2), RbAp46, and RbAp48 as core constituents; however, no other components of the SIN3 complex are present (24). Instead, NuRD contains p66 (serine- and proline-rich protein that targets NuRD to specific loci), metastasis-associated protein 1 (MTA1), MTA2, Mi-2, and methyl-CpG-binding-domain protein 3 (MBD3; 52,58,60-62).

Mi-2 has several recognizable motifs, including plant homeodomain (PHD) type zinc fingers, chromodomains, and a SWI2/SNF2 helicase/ATPase domain (an enzymatic component common to all known ATP-driven chromatin remodeling complexes; 24,50,58). Sequence-specific DNA-binding proteins (Ikaros and Aiolos) recruit Mi-2, suggesting a general link of NuRD to transcription factors (52). The Mi-2 ATPase activity disrupts the nucleosome, allowing access of RbAp48 to helix 1 of histone H4, and assisting the HD activity on nucleosomal histones (9,24,58,60).

Human MTA1 and MTA2 regulate activity of the HD core complex through interaction with MBD3 (50,61). MBD3 is a member of a MBD family of proteins and is able to bind NuRD to CpG-methylated DNA (61). This implies that the nucleosome remodeling and HD activities of NuRD are targeted to methylated chromatin sites (50). Similarly, the methyl-CpG-binding protein MeCP2 binds the SIN3 complex and recruits it to methylated DNA (10). Another MBD protein, MBD2, is a component of the methyl-CpG-binding complex MeCP1 that contains or interacts with HDs (50). *In vitro,* MBD2 is capable of interacting with NuRD, suggesting that MBD2 recruits NuRD to methylated DNA (50). Thus, at least two HD containing complexes (SIN3 and NuRD) are recruited to methylated DNA presumably to aid in repression of these transcriptionally down-regulated regions.

Fuks et al. (63) proposed a model linking the HD and DNA methyltransferase activities. DNA methyltransferase 1 (Dnmt1) would help deacetylate histones on methylated DNA by recruiting a HD through a MBD protein and lock the chromatin into a repressed state (63). The model linking Dnmt1 methylation of DNA to chromatin remodeling is augmented by the identification of the SWI/SNF2-like *Arabidopsis thaliana* protein DDM1 (63). Knockout of DDM1 severely decreases methylation, implying that the proposed remodeling activity may be required for efficient methylation of DNA (63). De-methylation of DNA appears to be involved in vernalization, the induction of early flowering by cold treatment, suggesting an enticing connection between DNA methylation, HDs, and development (64).

3.3. Other HD Complexes

The mammalian class II HDs (as well as HDAC3, a class I HD) also act in multiprotein complexes. For example, the myocyte enhancer factor (MEF2A) binds with HDAC4 and HDAC5 (65). Similarly, N-CoR and SMRT interact *in vitro* with class II HDs such as HDAC4, HDAC5, and HDAC7, as well as class I HDs HDAC1, HDAC2, and HDAC3 (46,66). Interestingly, *in vivo* HDAC3, N-CoR, and SMRT form a tight complex while HDAC4 was shown not to interact with N-CoR (66). This implies that N-CoR conformational changes (such as those that may occur *in vitro*) are necessary for interactions with HDs other than HDAC3 (66). It has been detected that Sin3 also interacts with the class II HD HDAC7, as well as the class I HDs found in the SIN3 complex (67). These data indicate that the recruitment of a variety of enzymatic activi-

ties mediates transcriptional repression, similarly to transcriptional activation (46,67).

Interestingly, the molecularly promiscuous mammalian 14-3-3 proteins associate with the class II proteins HDAC4 and HDAC5 at three phosphorylation sites, segregating them to the cytoplasm (65,68). 14-3-3 proteins block the nuclear localization sequences of HDAC4 and 5 from interaction with the nuclear transport machinery, eliminating translocation to the nucleus (68). When in the cytoplasm, HDAC4 and HDAC5 are restricted from interactions with nuclear proteins, such as MEF2 and class I HD HDAC3 (48), and are unable to directly repress transcription (68). Disruption of the 14-3-3 interaction allows HDAC4 and HDAC5 to be imported into the nucleus, form a complex with HDAC3, and putatively repress transcription (46,68). Such 14-3-3 interactions may also regulate RbAp48 function, suggesting that 14-3-3-based cytoplasmic segregation may be a shared mechanism for negative regulation of HD activity (68).

Class III HDs from maize have been shown to form a ~400 kDa complex (69). This complex was highly purified and appeared initially to be composed of three class III HD isoforms (p39, p42, and p45; 69). Further study found that this complex was actually a homopolymer of a single protein in multiple phosphorylation states (36).

Class IV HDs (Sir2 and homologs) have long been recognized as transcriptional repressors (39). Sir2 and other Sir proteins are critical components of silent chromatin in yeast. This silent chromatin (including telomers) is coated by Sir3 that binds to hypoacetylated histone tails blocking access of polymerase proteins to the DNA. Sir4 appears to play a similar role as Sir3, and both are recruited to silent chromatin by sequence-specific DNA-binding repressors such as Rap1 (39). Early studies showed evidence of a Sir2 complex with Sir3 and Sir4 (70,71). Sir2 is now known to be present in two distinct, multiple-subunit HD complexes in yeast cells (72,73). Interestingly, neither of these two complexes include the Sir3 protein (73). One of these Sir2 complexes (TEL) contains Sir4 and has a strong NAD^+-dependent HD activity (73). The second complex (regulator of nucleolar silencing and telophase exit, RENT) contains the cell division control protein 14 (Cdc14), Net1 and Nan1 (Net1-associated nucleolar protein 1) proteins (73). Interestingly, RENT displays a strong HD activity, but only a weak NAD^+-dependent HD activity indicating another HD may be a subunit in this complex (73).

4. HDs and development

A striking number of histone deacetylases and their interacting proteins appear to be involved in development. Even early physiological studies utilizing HD inhibitors showed that normal development was altered, indicating that histone acetylation states are developmentally significant (52). Examples include *Drosophila* Rpd3 that is vital for proper embryo segmentation, and nematode *C. elegans* HDAC complexes

that are important for embryonic patterning (52). In multiplying cells, mouse HDAC6 was found exclusively in the cytoplasm; however, at arrest of cell division and the beginning of differentiation a portion of the protein migrated to the nucleus – a possible 14-3-3 connection (discussed in section 3.3; 74). Human HDAC4 and HDAC5 are highly expressed in muscle tissue and are proposed to function in muscle cell differentiation through contacts with MEF2 (68).

Histone deacetylase-interacting proteins also show developmental roles. *Drosophila* Mi-2 mutants halt at the first or second larval instar without defects (52). *C. elegans* MTA1 homologs have partially overlapping functions in embryonic body patterning (52). The embryos deficient in MTA1 homologs are disorganized, but have orderly mitosis and tissue differentiation (52). Furthermore, a comparable phenotype is observed after inactivation of HDA1 by RNAi, further supporting the hypothesis that the MTA1 homologs function through a HD complex (52).

5. Plant HDs and putative binding partners

In recent years, the four classes of HDs have been identified in plants, with the most thorough biochemical characterization done in maize. The maize HDs are designated as follows: the class I, HD1-B1 and HD1-B2; non-RPD3-like HD1-A (75); and the first class III to be identified, HD2. It has been found that maize HDs differ from each other in their biochemical and enzymatic properties and subcellular localization (36). For example, HD2 has multiple phosphorylation states and is chromatin-bound in the nucleolus (36). HD1-A is phosphorylated and soluble; copurified HD1-Bs can be either soluble or chromatin bound and have an unknown phosphorylation state (36,76). Furthermore, the maize HDs deacetylate all core histones tails, but with variable preferences (42). For example, each maize HD preferred histone H3, but HD1-A and copurified HD1-Bs deacetylated histones H2A and H4 with equal bias and had the least substrate specificity for histone H2B (42). The class III nucleolar HD2 deacetylated histones H2A and H2B with equal preference, but had least specificity for histone H4 (42).

A change in phosphorylation states of maize HD2 and HD1-A alters their biochemical properties. Dephosphorylation *in vitro* destroyed HD2 activity, yet had an opposite effect on activity of HD1-A (42). Native HD1-A from maize embryos can be isolated in both phosphorylated and nonphosphorylated forms (42). Dephosphorylation changes HD1-A substrate specificity so that histones H2A and H4 became totally deacetylated after phosphatase treatment of the enzyme (42). Interestingly, it was found that the deacetylases had activity specific to certain acetylation patterns on the core histones (42).

Since the completion of the *Arabidopsis* Genome Initiative (77), a total of 17 AtHDs have been identified in the public database (Table 1). Recently, two cDNA clones encoding HD2-like proteins have been identified in *Arabidopsis thaliana* (32).

The first clone, HDA3 has a predicted zinc finger at the C-terminal domain and is highly expressed in flowers and young siliques (32). The second clone, HDA4, appears to be ubiquitously expressed and is lacking the zinc finger motif (32). HDA3 represses transcription of a β-glucuronidase (GUS) reporter when artificially recruited to the promoter in a tobacco leaf transient expression system, suggesting that the repression function of HDs is conserved in plants (32). Deletion studies demonstrated that the zinc finger motif of HDA3 is not necessary for repression; whereas, an acidic region near the HD domain is required (32). It is proposed that this acidic region could be required for interactions with the histone tails (32). HDA3 is involved in embryo development similarly to many metazoan HDs. Anti-sense repression of HDA3 resulted in aborted seed development, distorted or aborted siliques and reduced seed number in remaining siliques (32) indicating that this HD may play a role in developmental gene regulation, or in chromatin condensation during meiosis or mitosis in seeds.

Table 1

Classification of expressed and putative *Arabidopsis thaliana* histone deacetylases

Arabidopsis Gene	Relatedness (E-value)				cDNA Accession	Reference
	class I (RPD3)	class II (HDA1)	class III (HD2)	class IV (Sir2)		
HDA1 (AtRPD3A, AtHD1)	**e-137**	2e-23	–	–	AF014824	(79-81)
HDA6 (AtRPD3B)	**e-136**	3e-22	–	–	AF195548	(79,81)
HDA7	**1e-98**	8e-11	–	–	NM_122951*	(79)
HDA9	**e-125**	8e-19	–	–	NM_114336*	(79)
HDA10	**7e-19**	–	–	–	NM_114334*	
HDA17	**5e-19**	–	–	–	NM_114317*	
HDA2	1e-04	**3e-06**	–	–	AF428336	
HDA5	2e-23 2e-22	**3e-69** **2e-67**	–	–	2351061*	
HDA8	5e-22	**6e-31**	–	–	NM_100719*	
HDA14	1e-16	**1e-42**	–	–	AY052234	
HDA15	4e-26	**1e-71**	–	–	4757414*	
HDA3 (HD2A)	–	–	**1e-20**	–	AF195545	(32,43)
HDA4 (HD2B)	–	–	**9e-22**	–	AF195546	(32,43)
HDA11 (HD2C)	–	–	**2e-26**	–	AF255712	(43)
HDA13 (HD2D)	–	–	**2e-12**	–	AF255713	(43)
HDA12	–	–	–	**5e-11**	AF283757	
HDA16	–	–	–	**1e-10**	AY045873	

* predicted coding sequence. The BLASTP program calculated the degree of relatedness to other known histone deacetylases (yeast RPD3, yeast HDA1, *Zea mays* HD2, and yeast Sir2) using the amino acid sequence encoded by the respective mRNA. The naming system used is that of the Plant Chromatin Database (http://www.chromdb.org/). E-values are in regular font when HDS are compared across classes.

Although no Sir2-like HDs have been extensively studied in plants, Sir2 HD inhibitors have been applied to germinating *Arabidopsis* seedlings (78). Application of one of these inhibitors, sirtinol, created a phenotype manifested by a dramatic reduction in apical-basal axis (root and stem) formation and vascularization of the seedling (78). This phenotype is similar to that caused by inhibition of auxin transport and mutations in the transcriptional regulator *MONOPTEROS* (78). These similarities suggest a role in the auxin signaling pathway or a related pathway (78). In other organisms, the Sir2 HDs are believed to play a role in rDNA regulation in the nucleolus. Since plants contain the class III enzymes putatively regulating rDNA in the nucleolus, the class IV HDs may have been developed for other functions in plants.

Several RPD3-like HDs have been studied in *Arabidopsis* recently as well. HDA6 was identified in a mutant screen for repressors of transgenic auxinresponse element-driven hygromycin phosphotransferase and GUS reporter genes (79). Interestingly, the HDA6 mutants seemed to only derepress the methylated transgenes used in the screen, and not natural auxin responsive genes (79). Another *Arabidopsis* RPD3 homolog (HDA1) that can repress transcription when recruited to a promoter appears to be involved in developmental processes, such as the developmental switch to flowering (80,81). *Arabidopsis* plants with antisense-repressed HDA1 accumulated tetra-acetylated histone H4 and displayed pleotropic non-heritable phenotypes such as early senescence, asymmetrical primary leaf formation, secondary aerial rosettes, floral abnormalities, and reduced fecundity (80). At least some of these phenotypes can be attributed to ectopic expression of specific genes. The *SUPERMAN* gene was found to be constitutively expressed in antisense HDA1 plants, and ectopic expression of *SERRATE* is likely to have caused the leaf serration phenotype (80). Serration was heritable for several generations suggesting an epigenetic alteration, possibly by methylation (however, general DNA methylation alterations were not observed; 80). Both of these studies support the speculation that a connection between DNA methylation and HDs persists in plants.

One interesting example, HDA5, contains two HD domains and similarly to human HDAC6 is predicted to form an intramolecular dimer (47,67). Likewise, NuRD, Sin3, and other HD complexes contain two HDs. This common theme among HD complexes suggests an advantage to multiple co-localized HD domains. Perhaps this allows higher affinity of binding to histones, more rapid deacetylation of multiple sites in the histone octamer, or demonstrates the need for multiple substrate specificities to produce the proper acetylation code.

Many putative HD class I- and II-interacting partners are encoded in the *Arabidopsis* genome (Table 2). Three Mi-2 (CHD3/CHD1) orthologs are present: PICKLE (PKL), PICKLE Related 1 (PKR1); and PICKLE Related 2 (PKR2) with E-values from e-90 to 0.0 (82). These proteins (like Mi-2) contain chromodomains, SNF2-related helicase/ATPase domains, and DNA-binding domains (82). PKL and PKR1 also contain PHD zinc-finger domains (82). It is possible that these PHD zinc finger domains mediate an interaction with other zinc finger containing proteins such as HDA3. The *Arabidopsis* tran-

scription factor LEC1 is specific to seeds and vital for embryo tissue differentiation from endosperm (82). LEC1 is de-repressed in maturing PKL (Mi-2 homolog) mutants, along with seed storage protein and storage lipid deposition genes (82). This mutant has a gibberellin-suppressed, dwarfed and embryonic appearance, and a yellow-green, lipid-swollen root, hence the "pickle" phenotype (82). These mutant phenotypes suggest that PKL represses embryonic development, allowing transition to maturation in normal plants (82).

Table 2

Human SIN3 and NuRD complex subunits and related proteins from *Arabidopsis thaliana*

Human Complex		*Arabidopsis*	Characteristics
SIN3	NuRD		
HDAC1, 2	HDAC1, 2	HDA1, 6, 7, 9, 10, 17	Class I HDs
HDAC7	–	HDA2, 5, 8, 14, 15	Class II HD, interacts with Sin3 (not a member of the SIN3 complex)
RbAp46/48	RbAp46/48	MSI1, 2, 3, 4, MSIR1	Interact with histone H4, and retinoblastoma protein; may direct HD specificity
mSin3A	–	AtSIN3-1,-2,-3,-4,-5,-6	Possible protein scaffold
SAP30	–	–	Sin3 Associated Protein, targets SIN3 complex to promoters
SAP18	–	AtSAP18	Sin3 Associated Protein
–	Mi-2α, Mi-2β	PICKLE, PKR1, PKR2	SNF2-related ATPase chromatin remodeling factor
–	MTA1	–	Regulates NuRD through interaction with MBD3
–	MTA2	–	Regulates NuRD through interaction with MBD3
–	MBD3	MBD2, 4, 5, 6, 7, 12	Binds NuRD to CpG-methylated DNA
–	p66	–	Targets NuRD to specific loci

Six Sin3 orthologs are present in the *Arabidopsis* genomic sequences, all with E-values from e-25 to e-35. In support of conservation of HD protein complexes from yeast to plants, a maize RbAp46 homolog is known to form a complex with the maize RPD3 homolog HD1B (20). Furthermore, five RbAp46/48 relatives are present in *Arabidopsis* (multicopy suppressor of *ira1* 1-4, AtMSI1-1 to -4; and AtMSI-related 1, AtMSIR1) all with E-values from e-55 to e-166 (83,84). *Arabidopsis* plants expressing antisense AtMSI1 display vegetative and reproductive irregularities, strongly indicating its role in growth and development (83). Chromatin remodeling, in general, may be a common method of developmentally-dependent transcriptional regulation

because anti-sense disruption of *Arabidopsis* BSH, a putative component of the SWI/SNF complex, gives a seedless phenotype (85).

In this early stage of plant HD-complex study, it seems clear that HDs are functionally involved in the developmental progression of plants. This apparent connection may be due to many elements such as ectopic expression of tissue specific transcription factors or to a more global physiological role in chromatin control. Transgene silencing, rDNA regulation, chromatin condensation, and formation of virtually every plant organ have all been linked to HD function. In plants, as in metazoan systems, DNA methylation and HDs seem to work in concert to regulate gene expression (79). Intriguingly, plants have evolved a unique set of histone deacetylase enzymes, the HD2-like class III HDs. This novel class of the enzyme is not present in metazoans and may represent the consequence of unique regulatory functions developed in plants or simply reflects their unique evolutionary history. In addition, studies of class IV HDs in plants also imply that they play elaborated roles in developmental events. Together, these findings indicate that novel signaling pathways for plant gene regulation may be on the brink of discovery.

Abbreviations

Abbreviations in the current text have been shown alphabetically:
GUS – β-glucuronidase
HD, HDA, HDAC – histone deacetylase
HDB – histone deacetylase B
NuRD – nucleosome remodeling HD complex
RbAp – retinoblastoma associated protein
Sin – SWITCH-independent
Sir – silent information regulator

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