

*Full Length Research Paper*

# Analysis of histone deacetylase families of *Arabidopsis thaliana* and *Oryza sativa*

Joseena Mariam Iype, Rashmi Mishra, S. Karthikeyan, S. Babu and K. M. Gothandam\*

School of Bio Sciences and Technology, VIT University, Vellore-632014, TamilNadu, India.

Accepted 17 December, 2012

**Histone deacetylases (HDACs) plays critical roles in the regulation of chromatin structure and gene expression. HDACs are highly conserved in yeast, animals and plants, suggesting a fundamental mechanism in transcriptional regulation. Sequence characterization and phylogenetic relationships reveals the existence of several distinct families. In plants, the genome-wide identification of HDACs has been performed in several species. These genes are emerging as crucial players in all aspects of plant development, including embryogenesis, abaxial/adaxial polarity determination, flowering and senescence as well as responses to day length and environmental stresses. In this study, we analysed the HDACs from *Oryza sativa* and *Arabidopsis thaliana*. This analysis revealed the presence of 21 and 82 genes coding for putative HDACs in rice and arabidopsis, respectively and found out Poly amphipathic helix domain (PAH) and HDAC interact domains are present in these genes. In this work, we analysed the HDAC families and classified based on their structure.**

**Key words:** Histone deacetylases, *Oryza sativa*, *Arabidopsis thaliana*, poly amphipathic helix domain (PAH) domain, Histone deacetylases (HDACs) domain.

## INTRODUCTION

Gene expression in eukaryotes involves a complex interplay among transcription factors and chromatin proteins that pack chromosomal DNA into the confined space of the nucleus while poising genes for activation or repression (Kadonaga, 1998). The basic unit of chromatin is the nucleosome core particle, a structure in which ~146 bp of DNA is wrapped around a protein octamer made up of two subunits each of the core histones H2A, H2B, H3 and H4 (Kornberg and Lorch, 1999). Core histones can exist in multiple alternative states of acetylation, methylation, phosphorylation, ubiquitination or ADP-ribosylation (Strahl and Allis, 2000). The regulatory significance of these modifications for processes including gene repression, gene activation and replication is increasingly clear (Grunstein, 1997; Ng and Bird, 2000; Struhl et al., 1998).

Acetylation and deacetylation of histones are known to play important roles in the regulation of gene expression in eukaryotes (Meyer, 2000; Alinsug et al., 2009; Aquea et al., 2010). Histone acetyltransferases (Grant and Berger, 1999; Strahl and Allis, 2000) and histone deacetylases (Johnson and Turner, 1999; Knoepfler and Eisenman, 1999; Ng and Bird, 2000) have been identified in large, multisubunit complexes that target these enzymes to specific sites in nuclear DNA. In the nucleosomes of tightly coiled, condensed chromatin, positively charged lysine residues in the N-terminal tails of core histones interact with DNA and other chromosomal proteins. Acetylation of the lysine residues by histone acetyltransferases disrupts these interactions, resulting in relaxation of the chromatin structure, which allows gene expression to occur (Meyer, 2000). Conversely, the deacetylation of lysine residues in histones by HDACs results in the condensation of chromatin structure and the repression of gene expression (Meyer, 2000). HDAC genes and proteins have been isolated and characterized from a variety of animals, fungi, and plants. There are two subgroups of HDACs found in all eukaryotes. One subgroup is most closely related to the yeast RPD3 HDAC,

\*Corresponding author. E-mail: gothandam@yahoo.com.

**Abbreviations:** HDAC, Histone deacetylase; PAH, Poly amphipathic helix domain.

and the other is more closely related to yeast HDA1 (Johnson and Turner, 1999). In addition to these two subgroups of HDACs, plants contain a family of HDACs (HD2) that appear to be unique and unrelated to yeast RPD3 (Lusser et al., 1997; Kölle et al., 1999; Lechner et al., 2000; Wu et al., 2000). HDACs generally are found as part of multiprotein complexes that contain transcriptional repressors, corepressors, and a variety of other proteins (Ng and Bird, 2000). In some cases, HDAC complexes contain ATP-dependent chromatin-remodeling proteins and proteins that bind to methylated DNA (Ahringer, 2000).

## MATERIALS AND METHODS

### Protein identification and domain prediction

Thorough NCBI ([www.ncbi.nlm.nih.gov](http://www.ncbi.nlm.nih.gov)) searches with several divergent HDACs proteins of rice and arabidopsis were performed to retrieve HDACs genes of Arabidopsis through and of rice. Using the *Arabidopsis thaliana* and *Oryza sativa* sequences of HDACs, we performed a blast search for each sequence using BLASTp algorithm in the uniprot browser (<http://www.uniprot.org/>). The similar sequences obtained of all HDAC proteins were analysed for recognizable domains in PFAM (<http://pfam.sanger.ac.uk/>).

### Phylogenetic analysis

Phylogenetic analyses were performed using *Arabidopsis thaliana* and *O. sativa* HDAC protein sequences. The evolutionary history was inferred using the neighbour joining method; made by using ClustalW ([align.genome.jp/](http://align.genome.jp/)). Conserved domains were obtained from the multiple sequence alignment.

## RESULTS AND DISCUSSION

### Identification and domain prediction of HDACs from *A. thaliana* and *O. sativa*

Histone acetylation is a reversible process that plays a vital role in epigenetic regulation. Therefore, histone acetylation and deacetylation are of particular importance to plant growth, development, defense and adaptation. HDAC proteins sequences retrieved from different databases as described in the Materials and Methods. Totally, 21 and 82 genes coding for putative HDACs in rice and Arabidopsis were identified. The individual domain structures and number of identical proteins from other plants are presented in Table 1. The retrieved sequences were subjected to blastclust (<http://toolkit.tuebingen.mpg.de/bblastclust>) to make them into clusters. The different clusters for rice and Arabidopsis are given Tables 2 and 3 respectively. PAH: poly amphipathic helix repeat, this family contains the paired amphipathic helix repeat. The family contains the yeast SIN3 gene (also known as SDI1) that is a negative regulator of the yeast HO gene. The family contains the

eukaryotic Sin3 proteins, which have at least three PAH domains (PAH1, PAH2, and PAH3). Sin3 proteins are components of a co-repressor complex that silences transcription, playing important roles in the transition between proliferation and differentiation. Sin3 proteins are recruited to the DNA by various DNA-binding transcription factors such as the Mad family of repressors, MeCP2, p53, and Ume6. Sin3 acts as a scaffold protein that in turn recruits histone-binding proteins RbAp46/RbAp48 and histone deacetylases HDAC1/-HDAC2, which deacetylate the core histones resulting in a repressed state of the chromatin. The PAH domains are protein-protein interaction domains through which Sin3 fulfils its role as a scaffold. The PAH2 domain of Sin3 can interact with a wide range of unrelated and structurally diverse transcription factors that bind using different interaction motifs; HDAC INTERACT: HDAC\_interact super family, this domain is found on transcriptional regulators. It forms interactions with histone deacetylases.

### Classification of HDAC in rice and arabidopsis

To determine the evolutionary relationship of HDAC proteins of *A. thaliana* and *O. sativa* sequences, all HDAC protein sequences from these plants were used to construct a neighbour-joining phylogenetic tree. The tree is shown in Figures 1 and 2. The HDACs in *O. sativa* were divided into 2 classes:

Class I with 4 domains- 3 PAH and 1 HDAC interact  
Class II with 2 domains- 1 PAH and 1 HDAC interact

The HDACs in *Arabidopsis thaliana* were divided into 4 classes:

Class I with 3 domains- 2 PAH and 1 HDAC  
Class II with 2 domains- 1 PAH and 1 HDAC  
Class III with 4 domains- 3 PAH and 1 HDAC  
Class IV with 1 domains - 1 HDAC

### Multiple sequence alignment

Amino acid sequence of histone deacetylases were used for multiple sequence alignment using clustalW. Highly conserved regions were obtained from the alignment. As shown in Figure 3. The HDAC sequence is conserved in all the 21 sequences. This domain represents the HDAC\_interact super family as shown in Figure 4.

### Function of histone deacetylases genes in plants

The homeostatic balance of nucleosomal histone acetylation is maintained by actions of histone

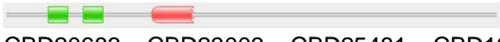
**Table 1.** PFAM structure and domains of *O. sativa*'s HDAC gene.

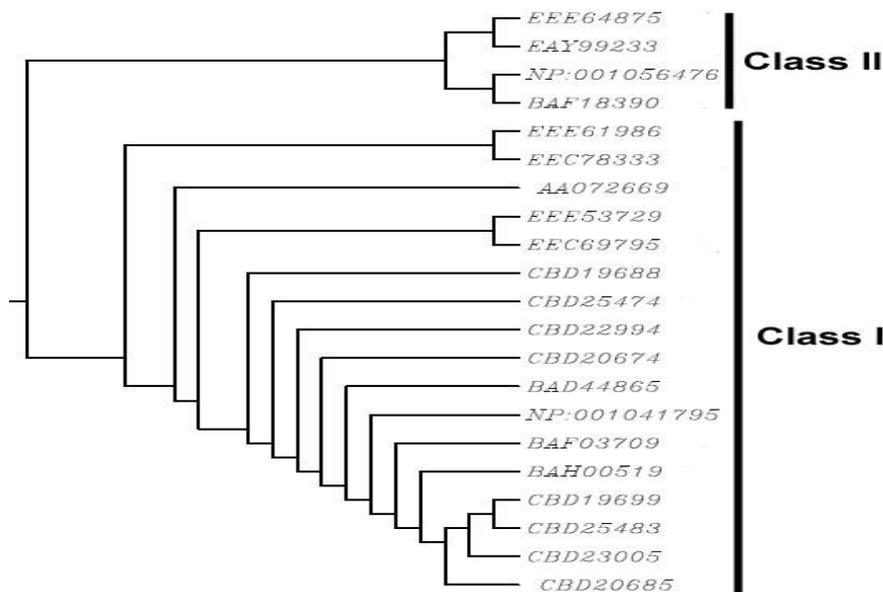
S/N	Accession No.	PFAM structure	Domains
1	CBD19699		3 PAH; 1 HDAC
2	CBD25483		3PAH; 1 HDAC
3	CBD23005		3 PAH; 1 HDAC
4	CBD20685		3 PAH; 1 HDAC
5	EEE64875		2 PAH; 1 HDAC
6	EEE61986		3 PAH; 1 HDAC
7	EEE53729		3 PAH; 1 HDAC
8	EEC78333		3 PAH; 1 HDAC
9	EEC69795		3 PAH; 1 HDAC
10	EAY99233		2 PAH; 1 HDAC
11	NP_001056476		1 PAH; 1 HDAC
12	NP_001041795		3 PAH; 1 HDAC
13	AAO72669		3 PAH; 1 HDAC
14	BAF18390		2 PAH; 1 HDAC
15.	BAF03709		3 PAH; 1 HDAC
16	CBD19688		3 PAH; 1 HDAC
17	CBD25474		3 PAH; 1 HDAC
18	CBD22994		3 PAH; 1 HDAC
19	CBD20674		3 PAH; 1 HDAC
20	BAD44865		3 PAH; 1 HDAC
21	BAH00519		3 PAH; 1 HDAC

**Table 2.** Rice HDAC clusters and their pfam structure.

S/N	Number of similar structures	Domain structures and Accession no:	Number name and of domains
1	17	 EEE53729, EEE61986, EEC69795, BAF03709, NP_001041795, BAH00519, CBD20685, CBD23005, CBD25483, CBD19699, CBD20674, CBD22994, CBD25474, CBD19688, BAD44865 EEC78333, AAO72669	2 PAH 1 HDAC
2	4	 BAF18390, NP_001056476, EAY99233, EEE64875	2 PAH 1 HDAC

**Table 3.** *A. thaliana* HDAC clusters and their pfam structure.

S/N	Number of similar structures	Domain structures and accession no.	Number and name of domains
1	45	 CBD20673, CBD22993, CBD25471, CBD19687, CAC01821, CBD20672, CBD22992, CBD25470, CBD19686, Q9SRH9, NP_186781, NP_197006, Q9LFQ3, CBD20681, CBD23001, CBD25479, CBD19695, AAF03494, O48686, CBD31870, CBD20669, CBD22989, CBD25466, CBD19683.1, NP_173829, CBD20676, CBD22996, CBD25476, CBD19690, AAC00578, ABR13973, AAB61107, CBD20675, CBD22995, CBD25475, CBD19689, CBD20671, CBD22991, CBD25469, CBD19685, O04539, NP_177163, BAF03709, NP_001041795, BAC43533	3 PAH 1 HDAC INTERACT
2	16	 CBD20683, CBD23003, CBD25481, CBD19697, AAD39565, CBD20670, CBD22990, CBD25468, CBD19684, Q9XIE1, NP_176197, CBD20682, CBD23002, CBD25480, CBD19696, AAD39330	2 PAH 1 HDAC INTERACT
3	13	 NP_001078550, NP_568207, NP_850795, NP_974753, NP_850796, NP_850797, NP_974754, BAB02925, NP_189069, NP_189529, BAB02130, NP_200387, AAX92703	1 SIR2
4	8	 BAF18390, NP_001056476, NP_172515, CBD20668, CBD22988, CBD25467, CBD19682, Q9XIK6	1 PAH 1 HDAC INTERACT



**Figure 1.** Classification of HDAC families of *O. sativa*.

Acetyltransferases (HAT) and histone deacetylases (HDAC). Most of the HDAC genes are responsive to drought or salt stresses. Down-regulation of most of HDAC genes may be important for stress-inducible gene expression in rice. These HDACs may repress stress

responsive gene expression in the absence of stress signals. Therefore, histone acetylation / deacetylation switch is suggested to be an important mechanism of short-term gene regulation in plants (Zhou, 2009), may be largely involved in stress responses in rice. It has been

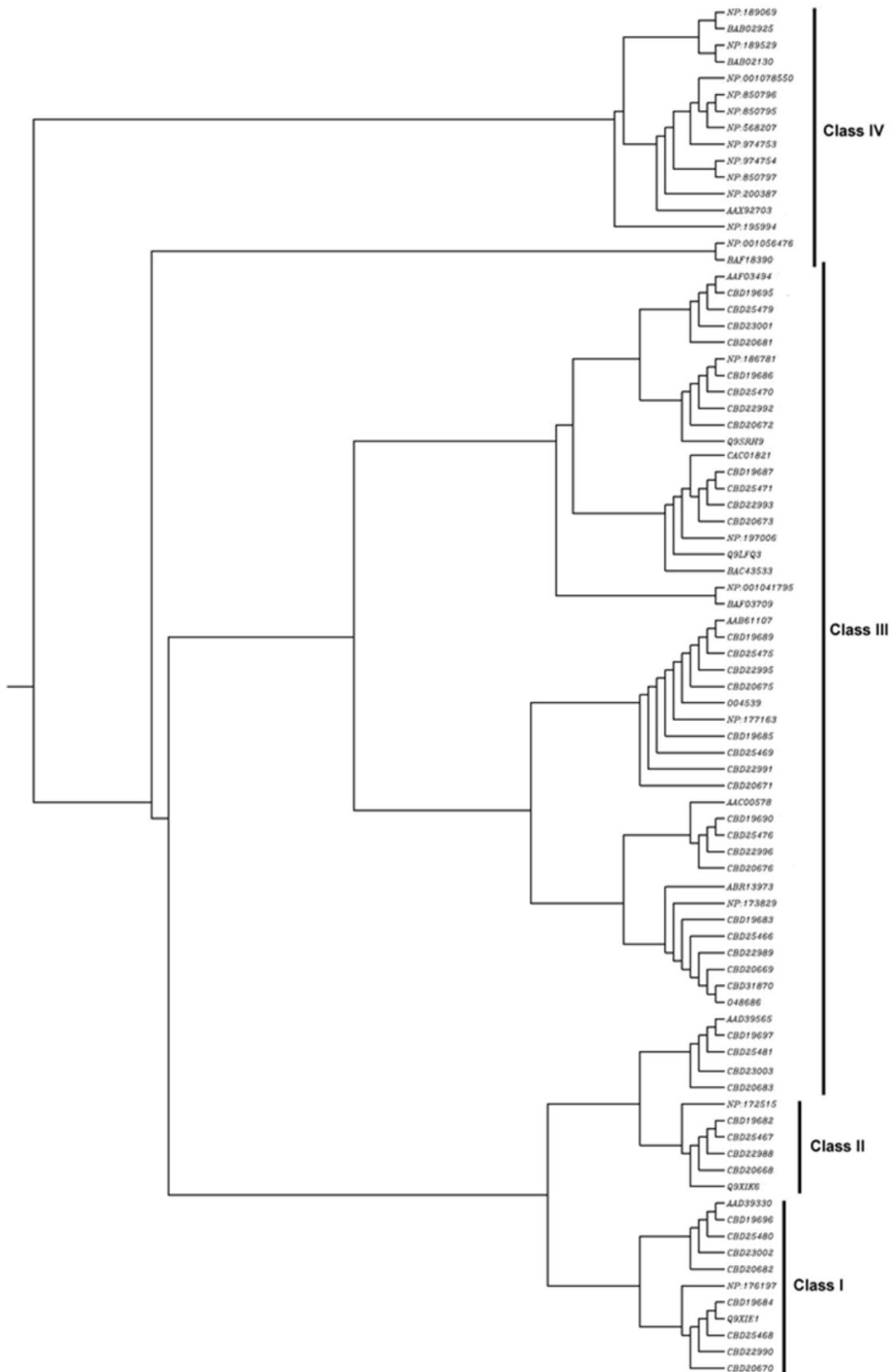


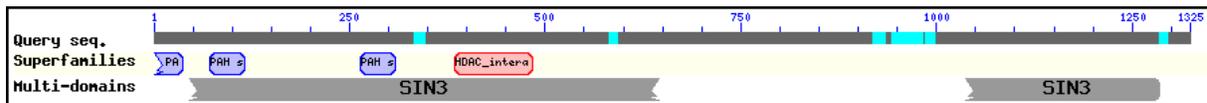
Figure 2. Classification of HDAC family of *A. thaliana*.

```

CBD19699          CTPSYRLLPKHYPMPPAGNKTELGASVLNDHWVSVTSGSEDYSFKHMRKN 437
CBD25483.1       CTPSYRLLPKHYPMPPAGNKTELGASVLNDHWVSVTSGSEDYSFKHMRKN 437
CBD23005.1       CTPSYRLLPKHYPMPPAGNKTELGASVLNDHWVSVTSGSEDYSFKHMRKN 437
CBD20685.1       CTPSYRLLPKHYPMPPAGNKTELGASVLNDHWVSVTSGSEDYSFKHMRKN 437
BAH00519.1       CTPSYRLLPKHYPMPPAGNKTELGASVLNDHWVSVTSGSEDYSFKHMRKN 437
BAF03709.1       CTPSYRLLPKHYPMPPAGNKTELGASVLNDHWVSVTSGSEDYSFKHMRKN 438
NP_001041795     CTPSYRLLPKHYPMPPAGNKTELGASVLNDHWVSVTSGSEDYSFKHMRKN 438
BAD44865.1       CTPSYRLLPKHYPMPPAGNKTELGASVLNDHWVSVTSGSEDYSFKHMRKN 355
CBD20674.1       CTPSYRLLPKHYPMPPAGNKTELGASVLNDHWVSVTSGSEDYSFKHMRKN 355
CBD22994.1       CTPSYRLLPKHYPMPPAGNKTELGASVLNDHWVSVTSGSEDYSFKHMRKN 355
CBD25474.1       CTPSYRLLPKHYPMPPAGNKTELGASVLNDHWVSVTSGSEDYSFKHMRKN 355
CBD19688.1       CTPSYRLLPKHYPMPPAGNKTELGASVLNDHWVSVTSGSEDYSFKHMRKN 355
EEE53729.1       CTPSYRLLPKHYPMPPAGNKTELGASVLNDHWVSVTSGSEDYSFKHMRKN 530
EEC69795.1       CTPSYRLLPKHYPMPPAGNKTELGASVLNDHWVSVTSGSEDYSFKHMRKN 472
AAO72669.1       CTPSYRLLPKHYPMPPAGNKTELGASVLNDHWVSVTSGSEDYSFKHMRKN 136
EEE61986.1       CTPSYRLLPKNYPMPPASCRTDLGASVLNDLWVSVTSGSEDYSFKHMRKN 513
EEC78333.1       CTPSYRLLPKNYPMPPASCRTDLGASVLNDLWVSVTSGSEDYSFKHMRKN 482
CTPSYCLLPKNCVTLQSSYQTELGRSILNDSLVSVTSGRED-CYKFRTKN 313
EEE64875.1       CTPSYCLLPKNCVTLQSSYQTELGRSILNDSLVSVTSGRED-CYKFRTKN 313
EAY99233.1       CTPSYCLLPKNCVTLQSSYQTELGRSILNDSLVSVTSGRED-CYKFRTKN 313
NP_001056476     CTPSYCLLPKNCVTLQSSYQTELGRSILNDSLVSVTSGRED-CYKFRTKN 341
BAF18390.1       CTPSYCLLPKNCVTLQSSYQTELGRSILNDSLVSVTSGRED-CYKFRTKN 341
*****  *****:      :.  :*:** *:**** .***** ** .:*.  **

```

**Figure 3.** Multiple sequence alignment of HDACs in *O. sativa*. Multiple sequence alignment of Histone deacetylases was generated using ClustalW. HDAC interact Domains CTPSYCLLPK and SVTSGRED were found (shown in red).



**Figure 4.** Shows conserved domain of a HDAC protein sequence of *O. sativa*.

shown that over-expression of OsHDAC1 (HDA702) leads to increased growth rate and altered architecture in transgenic rice (Jang et al., 2003). HDAC induces DNA fragmentation, cell death, and lesions mimicking plant hypersensitive responses induced by pathogen attacks. In this work, the HDACs of *A. thaliana* and *O. sativa* have been identified and their conserved domains are analyzed. Phylogenetic analysis of histone deacetylases of *A. thaliana* and *O. sativa* is done and classified.

## REFERENCES

- Ahringer J (2000). NuRD and SIN3. Trends Genet. 16:351-356.
- Alinsug MV, Yu CW, Wu K (2009). Phylogenetic analysis, subcellular localization, and expression patterns of RPD3/HDA1 family histone deacetylases in plants. BMC Plant Biol. 9:37 doi:10.1186/1471-2229-9-37.
- Aquea F, Timmermann T, Arce-Johnson P (2010). Analysis of histone acetyltransferase and deacetylase families of *Vitis vinifera*. Plant Physiol. Biochem. 48:194-199.
- Grant PA, Berger SL (1999). Histone acetyltransferase complexes. Semin. Cell Dev. Biol. 10:169-177.
- Grunstein M (1997) Histone acetylation in chromatin structure and transcription. Nature 389:349-352.
- Jang IC, Pahk YM, Song SI, Kwon HJ, Nahm BH, Kim JK. (2003). Structure and expression of the rice class-I type histone deacetylase genes OsHDAC1-3: OsHDAC1 over expression in transgenic plants leads to increased growth rate and altered architecture. Plant J. 33:531-41.
- Johnson CA, Turner BM (1999). Histone deacetylases: Complex transducers of nuclear signals. Semin. Cell Dev. Biol. 10:179-188.
- Kadonaga JT (1998) Eukaryotic transcription: an interlaced network of transcription factors and chromatin - modifying machines. Cell 92:307-313.
- Knoepfler PS, Eisenman RN (1999). Sin meets NuRD and other tails of repression. Cell 99:447-450.
- Kölle D, Brosch G, Lechner T, Pipal A, Helliger W, Taplick J, Loidl P (1999). Different types of maize histone deacetylases are distinguished by a highly complex substrate and site specificity. Biochemistry 38:6769-6773.
- Kornberg RD, Lorch Y (1999) Twenty - five years of the nucleosome, fundamental particle of the eukaryote chromosome. Cell 98:285-294.
- Lechner T, Lusser A, Pipal A, Brosch G, Loidl A, Goralik- Schramel M, Sendra R, Wegener S, Walton, JD, Loidl P (2000). RPD3-type histone deacetylases in maize embryos. Biochemistry 39:1683-1692.
- Lusser A, Brosch G, Loidl, A, Haas H, Loidl P (1997). Identification of maize histone deacetylase HD2 as an acidic nucleolar phosphoprotein. Science 277:88-91.
- Meyer P (2000). Transcriptional transgene silencing and chromatin components. Plant Mol. Biol. 43:221-234.
- Ng HH, Bird A (2000). Histone deacetylases: Silencers for hire. Trends Biochem. Sci. 25:121-126.
- Strahl BD, Allis CD (2000). The language of covalent histone modifications. Nature 403:41-45.
- Struhl K, Kadosh D, Keaveney M, Kuras L, Moqtaderi Z (1998). Activation and repression mechanisms in yeast. Cold Spring Harb. Symp. Quant. Biol. 63:413-421.

- Wu K, Tian L, Malik K, Brown D, Miki B (2000). Functional analysis of HD2 histone deacetylase homologues in *Arabidopsis thaliana*. *Plant J.* 22:19-27.
- Zhou DX (2009). Regulatory mechanism of histone epigenetic modifications in plants. *Epigenetics* 4:15-18.