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Histone acetylation: plants and fungi as model systems for the investigation of histone deacetylases

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Abstract. The basic element of chromatin is the nucleosome. Histones H4, H3, H2A and H2B form the core histone octamer by protein-protein interactions of their folded domains. The free, flexible N-terminal extensions of the histones protrude from the nuclesome; they contain conserved lysines undergoing posttranslational acetylation. Histone acetyltransferases (HATs) transfer the acetyl moiety of acetyl-coenzyme A to the ε-amino group; this reaction is reverted by histone deacetylases (HDACs). The dynamic equilibrium of the acetylation/deacetylation reaction varies throughout the genome; some regions in chromatin undergo rapid acetylation/deacetylation, whereas others are fixed in a certain acetylation state without significant changes. In general, chromatin regions engaged in transcription display dynamic acetylation, i.e. HATs and HDACs are recruited to these regions. Higher plants and fungi have considerably contributed to the unraveling of the multiplicity of HDACs; in particular, plants possess HDACs that have so far not been identified in animal cells.

Key words. Chromatin; histone acetylation; histone deacetylase; transcriptional regulation; cyclic tetrapeptides; trichostatin; plant-microbe interaction.

Introduction

After 30 years of research on histone acetylation, a *Tetrahymena* HAT was identified as a close homolog of the yeast transcription factor GCN5 [1]; soon after a mammalian homolog of another well-known regulatory yeast protein, RPD3, was found to be an HDAC [2]. Since then numerous transcriptional regulators and coregulators have been shown to act as HATs or HDACs.

Several possibilities for the biological effects of lysine acetylation on chromatin structure may be suggested. (i) Each acetylation reaction neutralizes a positive charge and therefore potentially weakens the interaction of the core histone octamer with the negatively charged DNA. This may result in destabilization or disintegration of nucleosomes, allowing transcriptional regulators to gain access to the DNA. (ii) Acetylation may interfere with the higher-order packing of chromatin and thus alter the accessibility of large chromatin areas for regulatory proteins. (iii) Acetylation may act as a highly specific signal that alters histone-protein interactions. This possibility has been suggested previously [3–7] and is supported by the finding that nonhistone proteins can also be acetylated and deacetylated by HATs and HDACs [8–11]. Among these proteins are structural proteins (HMG proteins), transcriptional activators (e.g. p53, c-myb, GATA-1, MyoD, E2F, HIV-Tat), nuclear receptor coactivators (ACTR, SRC-1, TIF2), general transcription factors (TFIIE, TFIIF) and importin-α7 (for review see [12]). The fact that the N-terminal extensions of core histones contain sites for different modifications (acetylation, phosphorylation, ubiquitination, ADP-ribosylation, methylation) has raised the question of how these modifications could cross-talk to each other in the sense of a histone-encoded language [7].

Experimental evidence suggests that one of the main functions of histone acetylation is a regulatory role in gene expression. Recently, chromatin immunoprecipitation (CHIP) assays have shown that acetylation of H3 and H4 within the promoter chromatin is associated with gene expression [13, 14]. In contrast, the use of antibodies recognizing acetylated isoforms of all core histones has

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shown that highly acetylated histones are not restricted to promoter regions in transcriptionally active genes [15, 16]. This indicates that acetylation of distinct histone species may have different functions and effects in different regions of chromatin.

Acetylation can either turn on [14] or turn off [17] transcription of a specific gene (IFN-β), depending on which protein is modified; if CBP/p300 acetylates histones H3 and H4 in the context of the IFN-β enhanceosome, gene expression is turned on; if CBP acetylates HMG-I(Y), this results in disruption of the IFN-β enhanceosome and in turning off gene expression. It might be speculated that acetylation of histones by HATs generally has a positive effect on transcription, whereas acetylation of nonhistone regulatory factors by HATs may have activating or repressing effects. Activation of gene expression by CBP/p300 does not necessarily involve direct acetylation. MyoD is not acetylated by p300 itself, but by PCAF; so p300 recruits PCAF to acetylate the proper sites on MyoD for transcriptional activation [10]. It should be considered that some putative HATs may acetylate transcription factors in vivo, but not at all histones, and this could modulate the binding of these factors to chromatin.

Structural rearrangements of chromatin are necessary for functional changes; one can assume that all processes occuring in chromatin, such as DNA replication, transcription, DNA repair, silencing and recombination involve posttranslational histone modifications. The identification of HATs and HDACs as modulators of transcription has largely focused the attention of molecular biologists on the regulation of gene expression. However, there are many more processes which depend on changes in histone acetylation. It has been recently shown that V(D)J recombination in T and B lymphocytes is regulated by histone acetylation [18]. In mammalian cells a HAT has been identified that associates with a replication origin recognition complex [19]. This HAT, HBO1, is a member of the MYST family of acetyltransferases,which includes a number of putative HATs (e.g. MOZ, Tip60, ESA1, SAS2 and 3), some of which are implicated in human disease [20]. Interestingly, yeast SAS2 is genetically linked to the function of the replication origin recognition complex [21]. It may be that HBO1 or analogous acetyltransferases in other systems mark histone H4 (in particular lysine 12) for chromatin assembly during S phase. It has been shown that H4 is acetylated on lysines 12 and 5 in a sequential manner by a cytoplasmic HAT B [22, 23]. On the other hand, origin recognition complex subunits also participate in silencing of the mating-type loci in yeast, indicating diverse roles of these proteins in the cell. In yeast, hypoacetylation seems to be required for the stable maintenance of silenced chromatin [24]. In mammalian cells, transcriptional regulators (Ikaros, Helios) are associated with A/T-rich satellite sequences and may recruit HDACs to centromeres to maintain this chromatin in a hypoacetylated state [25].

HATs have attracted considerable more research attention than HDACs during the past 10 years. However, the identification of yeast RPD3 and RPD3-related proteins as transcriptional corepressors and the fact that HDACs often exert their function as multisubunit complexes with a va-

Table 1. Histone deacetylases.

HDAC-type	Enzymes	Organism	Proteins associated with HD complexes (examples)
RPD3-like			Sin3, Rbap, SAP, MAD, MAX, NCoR, SMRT, Mi2, MTA2, MBD3, MeCP1, MeCP2, Ikaros, UME6, Ski, p53, HPV E7, PcG, YY1, LIM, Hunchback, Groucho, LAZ3, PLZF, BRCA1, HDAC4, HDAC5
	RPD3, HOS1-3	*S. cerevisiae*	
	dHDAC1-3	*Drosophila*	
	HDA1-3	*Caenorhabditis elegans*	
	HDm	*Xenopus laevis*	
	HDAC1-3		
	HDAC7, 8	chicken, mammals	
	RPD3/HD1-B	*Zea mays*	
HDA1-like			HDAC3, MEF2A, NCoR, SMRT
	HDA1	*S. cerevisiae*	
	dHDA2	*Drosophila*	
	mHDA1, 2	mouse	
	HDAC4-6	human	
HD2-like			homopolymer of HD2-p39 and phosphorylated forms
	HD2 plants		
SIR2-like NAD-dependent			
	SIR2	*S. cerevisiae*	SIR3, SIR4
	SIR2-homolog	mouse	

riety of regulatory protein partners (table 1), has now led to the conclusion that gene regulation is a complex interrelation of histone-acetylating and histone-deacetylating activities. Repression of transcription is often linked to the recruitment of multisubunit complexes containing one or multiple HDACs. Moreover, some of the known HDACs are part of chromatin-remodeling machines. More recently, evidence has accumulated that HDACs are implicated in human cancer [26, 27]. A great deal of research on functional aspects of histone acetylation has exploited the fact that inhibitors of HDACs have been available, whereas no inhibitors are known for HATs. Inhibitors used are butyrate and a panel of highly specific and potent natural compounds of diverse chemical composition, such as TSA (Trichostatin A) or members of the cyclic tetrapeptide family (HC-toxin, chlamydocin, trapoxin). The latter class plays a significant role in plant-microbe interactions [28]; the maize pathogen *Cochliobolus carbonum* colonizes maize, and the production of the host-specific HC toxin by *Cochliobolus* and its inhibitory effect on HDACs seems to be the main pathogenicity mechanism.

Posttranslational acetylation of plant histones

In plants, as in other eukaryotes, research was focused on acetylation of histone H4 and H3. Whereas H4 is the predominant target of acetylation in animal cells, fungi or protists, followed by H3 and to a lower extent H2B and H2A, in plants H3 was found to be the most extensively acetylated histone species [29]. Immunofluorescence studies of mitotic chromosomes from several plants (e. g. *Vicia, Gagea, Silene*) using antisera against specific acetylated H4 isoforms confirmed the staining pattern which is also seen in animal and insect cells, with highly acetylated H4 associated with heavily transcribed regions, such as the nucleolus organizer region (NOR), and underacetylation of H4 in heterochromatic areas of the genome. This distribution pattern could also be observed in heterochromatinized whole chromosomes such as the transcriptionally inert B chromosomes of *Brachycome dichromosomatica* [30] or even in whole inactive chromosome sets as was shown for the pentaploid endosperm of *Gagea lutea* [31], reflecting a direct relation between transcriptional activity and histone acetylation. Intriguingly, no difference in H4 acetylation patterns could be detected between the two X chromosomes in *Silene latifolia* homogametic cells, which is somewhat unexpected since the second X is supposed to be inactivated as a consequence of dosage compensation [32]. Recently, it was shown for *Vicia* and barley root tip cells that the pattern of histone acetylation, at least of lysine 5 of H4, is changing with progression of the cell cycle [33].

In contrast to nonplant species, H4 from *Medicago*, *Arabidopsis*, tobacco and carrot can be detected in five distinct acetylated isoforms (mono- to penta-acetylated at Lys 5, 8, 12, 16 and 20), thus establishing an obvious difference from other eukaryotic systems where H4 was found to be modified only up to the tetra-acetylated level [34]. However, as yet no HAT capable of modifying H4Lys20 has been identified.

Enzymes of histone acetylation in plants

A better understanding of the functional role of histone acetylation resulted from the characterization and molecular identification of the enzymes responsible for catalyzing the reactions of addition and removal, respectively, of acetyl groups on or from the respective lysine residues: HATs and HDACs. For more than 20 years these enzymes have been studied biochemically in a variety of organisms from yeast to mammalian cells. Among these, the monocot *Zea mays* represents a model organism regarding the detailed biochemical, enzymatic and molecular characterization of HAT and HDAC types, their substrate specificities and developmental regulation. In maize seedlings, at least three types of HATs and four distinct histone-deacetylating activities can be detected by chromatographic fractionation of cellular extracts [5, 35]. Aside from a predominantly cytoplasmic HATB, which has been purified and identified as a homolog to the yeast protein Hat1p [36, 37], two nuclear HATs (HATA1 and HATA2) could be distinguished in *Zea mays* [38, 39]. However, immunoblotting experiments with an antibody against a maize homolog to yeast Gcn5p revealed the presence of ZmGcn5 in both HATA fractions, suggesting the existence of complexes with distinct composition [R. Thompson, personal communication] reminiscent of the yeast SAGA and ADA complexes, respectively (for review see [12, 40]).

A clear analogy to the situation in *Saccharomyces cerevisiae* as well as in mammals can be observed for the major histone-acetylating activity in the cytoplasm of maize-germinating embryos, HATB. This enzyme was shown to be responsible for the acetylation of newly synthesized histone H4 in a highly specific manner, like the corresponding enzymes in yeast and mammals. It does not react with any other of the histones in vitro, and accepts only the nonacetylated H4 isoform for acetylation at lysines 5 and 12 [23]. A unique pattern of acetylation was demonstrated for pea HATB, which acetylates H4 up to the triacetylated state by incorporating an acetyl group also in lysine 16 [41].

Since chromatin assembly factor-1 (CAF-1) has been shown to be physically associated with acetylated H4, it is thought that acetylation might be required for the transport of H4 into the nucleus and/or assembly into the nucleosome [42]. Consequently, HATB activity would be required to provide free H4 with a tag for its subsequent

fate. However, deletion of Hat1p in yeast had no apparent mutant phenotype [43], and recently it was shown that histones need not to be acetylated in order to interact with CAF-1 or to be correctly assembled into chromatin (for review see [44]). Since H4 and H3 N-termini in yeast are functionally redundant with each other, it was assumed that acetylation of H3 N-termini by another, yet unidentified enzyme could complement the loss in H4 acetylation. Alternatively, or in addition, other acetylation-independent nucleosome assembly pathways may exist. Evidence for that came from the existence of replication independent assembly mechanisms that require less modified histones [44].

HDACs

Four biochemically distinct HDAC activities have been identified in maize (HD1A, HD1BI and II, HD2) and three in pea [35]. This classification is due to chromatographic behaviour, subcellular localization and enzymatic properties of the respective protein (complexes). Since the molecular identification of human HDAC1 as an ortholog to yeast RPD3, proteins from all kinds of eukaryotic species have been identified as HDACs basically belonging to two related families, the RPD3 family and the HDA1 family [45]. In maize, two RPD3-type HDACs (HD1BI, HD1BII) have been reported [38, 46, 47], and at least one additional member can be detected in the databases. Also, for the HDA1 family several EST clones from maize and *Arabidopsis* are available, though as yet none of them has been studied in detail. The maize HD1B enzymes have been characterized in terms of biochemical properties, but information on their physiological functions or target genes is still lacking. Immunological data indicated that maize HD1BI and -II cofractionate with a protein related to the tomato WD-repeat protein LeMSI and the human Rbap46/48 proteins [47], similar to what has been shown for human HDAC1. Rbap46/48-like proteins have been suggested to be responsible for targeting the enzymes to histones. This function seems likely, since Rbap-related proteins associate with B-type HATs as well as with chromatin assembly factor CAF-1. In maize, only the HD1B activity can deacetylate the characteristic diacetylation introduced by HATB on H4 [48]; since both enzymes may be associated with an Rbap related protein [37, 47], it could well be that Rbap proteins in this context are involved in proper substrate recognition.

Maize histone deacetylase HD1A is regulated by phosphorylation

Although its molecular structure still remains unidentified, HD1A from maize differs from HD1BI and -II by its degree of chromatin association apart from other biochemical properties. HD1A is only loosely associated with chromatin [49] and is subject to phosphorylation. Changes in phosphorylation status result in a significant activation of enzyme activity and a change in substrate specificity [48]. HD1A was purified to homogeneity and turned out to be present as a monomeric enzyme in crude as well as highly purified preparations [49]. HD1A differs from other HDACs in this respect since it is obviously not associated with other proteins in high molecular weight complexes.

Maize histone deacetylase HD2 defines a novel family of HDACs

In fact, there already exists an example of a plant HDAC that is not related to those identified in other eukaryotes: maize HD2. This enzyme form is tightly chromatin bound and can be isolated from chromatin extracts as a complex of ~ 400 kDa [50]. Extensive purification yielded three almost identical polypeptides (p39, p42, p45); cloning of a corresponding complementary DNA (cDNA) identified a protein with a calculated molecular mass of 32 kDa that displayed no significant homology to any other protein in the databases. Further characterization showed that it could be phosphorylated by casein kinase II in vitro and in contrast to HD1A whose activity is elevated upon dephosphorylation, removal of phosphate from HD2 drastically reduced its enzymatic activity [48]. Immunofluorescence studies revealed that HD2 is localized exclusively in the nucleolus of maize cells during the early embryo germination stages [51]. This subnuclear distribution pattern suggests a function for HD2 in the regulation of ribosomal chromatin, which is concentrated in the nucleolus. Recently, the characterization of the functional role of AtHD2A, a homolog from *Arabidopsis thaliana*, was reported [52]; AtHD2A was able to repress transcription when targeted to a reporter gene in vivo, and this property was linked to the N-terminal part of the protein, which was proposed to harbour residues critical for enzymatic activity [53]. The ability to repress transcription is in line with findings for the RPD3 type HDACs that have been shown to function as transcriptional corepressors in context with a great number of regulatory proteins. Antisense expression of AtHD2A resulted in aborted seed development in transgenic *Arabidopsis* plants. Hence, a role for AtHD2A in reproductive development was assumed [52]. However, no effect of silenced AtHD2A on expression of ribosomal RNA (rRNA) genes was observed, as might have been expected, considering the nucleolar localization of HD2 in maize. However, the subcellular localization of the HD2 homolog has not been checked in *Arabidopsis*; thus the possibility exists that this particular enzyme type does not reside in the nucleolus, or another HD2 homolog

could be responsible for regulation of rRNA genes. In *Arabidopsis* at least four close homologs of HD2 exist [54]. Alternatively, one could hypothesize that HD2's localization in the nucleolus is not linked to a role in the regulation of ribosomal chromatin but is a consequence of controlling subcellular concentration of HD2. Examples for this kind of regulation of a protein's function have become evident recently [55], e. g. for Mdm2, a regulator of p53, whose recruitment to the nucleolus results in stabilization of the p53 protein [56].

Although no straight homologs of HD2 can be detected in other eukaryotic species, this type of deacetylase forms multigene families of highly similar members within the plant kingdom. Analysis of available sequences in the expressed sequence tag (EST) databases revealed the existence of at least four orthologs in *Arabidopsis* and maize [54, 57]. Proteins with homology to HD2 type deacetylases have also been identified in insects, yeast and two parasitic apicomplexans [53]. The insect proteins belong to a family of FK506-binding proteins, that exhibit peptidyl-prolyl cis-trans isomerase (PPIase) activity. Their similarity to HD2 includes the conserved N-terminal part and the highly charged middle domain, but excludes the C-terminal region with the putative zinc finger motif which can be found in some but not all plant HD2 family members. The FKBPs in addition display a PPIase domain, suggesting that two enzyme activities might be included in one protein, whereas a homologous protein from *Trypanosoma* contains an additional RNA binding domain. Although so far no significant HDAC activity could be detected for any of the homologous proteins [G. Brosch and P. Loidl, unpublished results] the potential combination of HDAC and PPIase activity in one protein is intriguing considering that both activities might be involved in chromatin rearrangements, e. g. during chromatin assembly; apart from prolines in their folded domains, the histones H3, H2A, and especially H2B have prolines within their flexible amino-terminal extensions. Database searches revealed the existence of an *Arabidopsis* FKBP that shows homology to HD2 and displays the PPIase domain. This finding suggests that plants might have separated the two functions during evolution, resulting in two related enzyme families: one that only harbours the HDAC domain and one that might combine HDAC and PPIase activity within a single polypeptide chain.

HDACs of the SIR2 family: are they involved in gene silencing in plants?

Recently, another structurally unrelated family of HDACs with homology to yeast SIR2 (silent information regulator) was established. Yeast SIR2, 3 and 4 elicit silencing by forming repressive chromatin structures that are confined to specific chromosomal domains, such as telomeres [58], mating type loci [59] and rDNA [60]. Silencing of these regions requires the deacetylated state of particular lysines in the amino-terminal tails of H3 and H4, presumably to allow folding of the nucleosomes into a more compact higher-order structure. Therefore and because of the fact that overexpression of SIR2 in yeast cells led to global deacetylation of histones, SIR2 was suggested to be a deacetylase [61]. Very recently, this activity was actually demonstrated for yeast but also for mouse SIR2 [62]. Furthermore, SIR2 was shown to possess ADP-ribosyltransferase activity [63, 64], which is separable from HDAC activity, although deacetylation of histones is dependent on NAD [64, 65]. This NAD dependence might provide a link between cellular metabolism and chromatin structure and thus was suggested to be a possible mechanism to explain processes like aging of cells (for review see [66]). Unlike other families of HDACs, SIR2 deacetylase activity cannot be inhibited by known HDAC inhibitors, such as TSA. SIR2-like proteins are conserved among species from bacteria to human with apparent homologs also in plants [67], but so far little information is available concerning the functional role and the targets of these homologs. Altogether, these exciting new findings open up an interesting field for studies in plants, especially with respect to a potential role in silencing of transgenes.

HDACs in fungi

Yeast has a long-standing importance in biotechnological applications. For this reason and the relative ease with which the genome of *Saccharomyces cerevisiae* can be manipulated, this 'simple' unicellular eukaryote has been intensively studied and has become a useful model organism for analyzing mechanisms of gene regulation in eukaryotes. As a result, the yeast RPD3 was the founding member of the first HDAC family discovered [2, 68, 69]. In addition to RPD3, a related protein with HDAC activity – HDA1 – was identified (table 2). The HDA1 protein is part of a 350-kDa complex that is highly sensitive to the well-known HDAC inhibitor TSA. RPD3 is associated with a 600-kDa complex that was predicted to be less sensitive.

Disruption of either gene causes hyperacetylation and changes in transcription levels of specific gene products. Although increased histone acetylation in RPD3 and HDA1 mutant strains might also be expected to derepress telomeric loci, an increased repression of a reporter gene (URA3) integrated at the telomeric end of chromosome VR was found [69]. However, even an RPD3/HDA1 double mutant is viable and does not appear to increase the acetylation status more than either single mutant. Because of the presence of three further HDA1/RPD3 simi-

Table 2. RPD3/HDA1-like histone deacetylases in fungi.

Organisms	RPD3-like (class 1)			HDA1-like (class II)		
	Name	Acc. no.	Ref.	Name	Acc. no.	Ref.
S. cerevisiae	RPD3	S66438	[2, 68, 69]	HDA1	Z71297	[69]
	HOS1	Z49219	[69]			
	HOS2	Z72716	[69]			
	HOS3*	U43503	[69, 70]			
S. pompe	Hda1/Phd1	AL021046	[74, 73]	Clr3	AFo64207	[75, 76]
	Clr6	AF064206	[72]			
P. polycephalum	ppHDAC1	–	–			
U. maydis	umRpd3	–	[80]			
	umHda	AJ133752	–			
C. carbonum	HDC2	–	–	HDC3	AF307341	–
	HDC1	AF306507	–			
A. nidulans	RpdA	AF163862	[84]	HdaA	AF306859	–
	HosA	AF164342	[84]			

* HOS3 is added to the class I HDACs but does not correlate well with either HDAC class.

lar open reading frames in the yeast genome (HOS1, HOS2, and HOS3; HDA one similar; table 2) it has been proposed that these putative HDACs may be upregulated in the absence of RPD3/HDA1 and may (partly) counterbalance HDAC activity in the mutant strains. If so, this would demonstrate functional redundancy among transcription-associated deacetylase activities in yeast. Recently, HDAC activity has been demonstrated for HOS3 [70]. Moreover, a recombinant HOS3 protein shows intrinsic enzyme activity and strong resistance to TSA, which has not yet been shown for any other HDAC of that family. This intrinsic activity and the strong inhibitor resistance weaken the idea of a redundant enzyme that only substitutes the activity of RPD3 or HDA1, both of which are assumed to be enzymatically active only in association with other proteins and are inhibitor sensitive. The remarkable features of HOS3 suggest that each gene product plays a different biological role in budding yeast. However, like RPD3 and HDA1, HOS3 also seems to be a member of a larger protein complex where the associated partners in the complex may sequester HOS3 but are not required for its enzymatic activity towards acetylated substrates in vitro. The enzymatic activities of HOS1 and HOS2 are as yet unidentified, and their biological role is still unclear.

The phylogenetic position of another fungus, the fission yeast *Schizosaccharomyces pombe,* is distantly related to *S. cerevisiae.* As in budding yeast, the mechanism of epigenetically controlled patterns of gene repression, called silencing, has been extensively studied in *S. pombe* on several silenced domains, such as centromeres *(cen1,2,3),* mating-type donor loci *(mat2,3)* and telomeres [71]. It became clear that the heterochromatic properties of these domains are intimately associated with the modification of histones. Molecular analyses of some genetically defined silencing proteins provided a link between these trans-acting factors and genes encoding enzymes with biochemically defined HDAC activity [72–74]. Mutations of these factors not only derepress donor mating-type loci in *S. pombe* but also alleviate the repression of marker genes inserted within the silent domain. So far, three trans-acting factors with distinct similarities to members of the HDAC family were identified in *S. pombe* (table 2). The identical proteins Hda1 [74] and Phd1 (*S. pombe* histone deacetylase-related protein; [73]) share strong similarity with *S. cerevisiae* RPD3. Another protein, Clr6 (cryptic loci regulator 6; [72]), is a second member of this class. In contrast, Clr3, a third trans-acting factor, belongs to the *S. cerevisiae* HDA-type HDACs [72, 75, 76]. Interestingly, Clr6 acts in a partially redundant manner with Clr3 and the putative DNA-binding protein Clr1. Whereas Clr3 and the zinc-finger protein Clr1 seem to work together, either in the same pathway or as a complex [76], Clr6 may be part of another redundant histone deacetylation system [72]. Although TSA treatment of *S. pombe* cells generally causes an inhibition of the silencing mechanism, the distinct biological roles of Hda1, Clr6 and Clr3 on chromatin structure and silencing mechanisms in *S. pombe* remain unclear.

So far, only a few facts are known about histone acetylation and deacetylation processes in other fungi. HDAC activity has been reported in the lower eukaryote and myxomycete *Physarum polycephalum* [77, 78]. Due to the natural synchrony of the macroplasmodial cell cycle it is possible to investigate biochemical processes in regard to distinct stages of the cell cycle. Biochemical studies revealed the existence of at least two deacetylase activities in *Physarum*. Both HDAC activities fluctuate during the cell cycle; interestingly, they seem to have dif-

ferent histone specificities [77]. However, both activities are highly sensitive to TSA and other deacetylase inhibitors. Recently, a first putative HDAC gene of *P. polycephalum* was cloned and sequenced [T. Lechner et al., unpublished]. The predicted protein shows high similarity with HDACs of the RPD3 class. Pilot expression studies from different stages of the cell cycle confirmed an increase of expression of the enzyme in two distinct stages of the cell cycle. A possible explanation for the fluctuation of Rpd3 expression was that transcription is stopped at mitosis. In the following S phase, nucleosome assembly takes place where acetylation and deacetylation processes of histones may be important for proper assembly of the nucleosome [79].

Recently, a protein that acts as a transcriptional repressor of a specific set of genes and controls developmental transition during sporogenesis was identified in the filamentous fungus and causal agent of corn smut disease *Ustilago maydis.* This protein, Rum1 (regulator *U. maydis)*, binds in combination with other sequence-specific binding factors to selected promoters and contributes to the maintenance of a repressed state of these genes [80]. Interestingly, distinct conserved sequence motifs are present in Rum1. These motifs include a highly conserved DNA binding domain and two zinc-binding PHD-finger or LAP domains which were found in nuclear proteins and are thought to mediate protein-protein interactions. Recently, it was shown that such PHD domains are also essential for the interaction of proteins with HDAC1, the human Rpd3 ortholog [81]. Thus, the repressing activity of Rum1 in *U. maydis* could be explained by the recruitment of an HDAC; Rum1 would be a component of an HDAC complex and exerts its function via the alteration of chromatin structure. This hypothesis is supported by the finding that mutation of a gene encoding a protein with similarities to yeast RPD3 leads to a phenotype in *U. maydis* reminiscent of that obtained for a Rum1 mutation [80].

Another plant pathogen, the filamentous fungus *Cochliobolus carbonum,* causes leaf spot and ear rot of corn. The high virulence on specific maize lines is due to production of a cyclic tetrapeptide, the HC toxin with which the pathogen inhibits HDACs from maize and causes hyperacetylation of core histones in vivo in susceptible, but not resistant maize strains [28, 82]. HC toxin of *C. carbonum* either influences the expression of maize defense genes or probably inhibits protein synthesis by interfering with ribosomal gene replication or transcription through action on the nucleolar enzyme HD2 (see above). However, the fungus must have a mechanism to protect itself against its own toxin. Originally, it was proposed that resistance against HDAC inhibitors might be due to the export of produced toxin via a putative HC-toxin efflux carrier [83], but recently HDAC activity was purified from *C. carbonum* mycelia, which seems to be resistant to commonly known HDAC inhibitors, including the *Cochliobolus* toxin [G. Brosch and S. Graessle, unpublished]. At the same time, resistance was shown for the plant pathogen fungus *Diheterospora chlamydosporia* (= *Verticillium chlamydosporium*), which produces chlamydocin, another strong HDAC inhibitor [J. D. Walton, personal communication].

Our laboratory has identified three putative HDAC sequences *(rpdA, hosA* and *hdaA)* in the closely related filamentous fungus *Aspergillus nidulans* ([84] and unpublished data of our lab). Corresponding orthologs of these genes were also found in *C. carbonum* [J. D. Walton et al., unpublished]. Database searches revealed a close relationship of the *Aspergillus* and *Cochliobolus* sequences to the yeast *RPD3/HOS2* and the *HDA1* family, respectively. Since the identified enzymes of *C. carbonum* and *A. nidulans* are orthologs, but the latter does not possess a resistant HDAC activity comparable to that of *C. carbonum,* alternative explanations for the basis of resistance are conceivable. Either *C. carbonum* possesses a novel enzyme with an intrinsic resistance to inhibitors (as was shown for HOS3 in *S. cerevisiae*) or an additional extrinsic factor other than HDAC itself is responsible for the distinct feature of *C. carbonum*. Such an extrinsic factor could be a protein that interacts with and thereby somehow protects the HDAC; it may well be that such a factor neutralizes HDAC inhibitors. However, the elucidation of the mechanism of toxin resistance would give further insight into the structure of HDACs and could help to understand their catalytic center and mode of action.

Treatment with HDAC inhibitors was also used for investigations of gene regulation in the fungus *Neurospora crassa,* which offers an attractive system to investigate a possible correlation of DNA methylation and histone acetylation in silencing of specific genes. Although most of the *Neurospora* genome is unmethylated and methylation is nonessential [85, 86], it is clear that DNA methylation can control some genes in this organism. TSA treatment of *N. crassa* was found to reverse the effect attributable to methylation. Analysis of DNA methylation demonstrated that TSA can cause selective loss of demethylation in the fungus, implying that acetylation of histones or other proteins can somehow control DNA methylation [87]. In addition to this investigation, an HDAC complex from *Xenopus laevis* egg extracts was purified that consists of six subunits, including an RPD3-like deacetylase and a subunit that binds specifically to methylated DNA in vitro [88]. These data substantiate a mechanistic link between DNA methylation, histone deacetylation and transcriptional silencing.

Table 3. SIR2-like histone deacetylases in fungi.

Organisms	Name	Acc. no.	Ref.
S. cerevisiae	SIR2	X01419	[67]
	HST1	U39041	[67]
	HST2	U39063	[67]
	HST3	U39062	[67]
	HST4	Z48784	[67]
S. pombe	spHst4	AF173939	[89]
C. albicans	caSir2	AF045774	[90]
K. lactis	klSir2	X74569	[91]
A. nidlulans	SirA	AF306860	–

SIR2 orthologs in fungi

SIR2 was found to be highly conserved from yeast to human [67]. *S. cerevisiae* contains another four members of the SIR2 family, HST1-4 (homologues of SIR two), that function either directly or indirectly in silencing processes [67]. Furthermore, a gene related to *HST4* has recently been identified in fission yeast (table 3). This ortholog influences silencing at both telomeres and centromeres in *S. pombe* [89].

The human pathogen *Candida albicans* showed abnormally high levels of variant colony formation and a high frequency of karyotypic changes when both copies of a *SIR2*-related gene were disrupted. Moreover, overexpressed *C. albicans* SIR2 was able to partially restore mating of a *S. cerevisiae sir2* mutant strain [90].

A recent study reported the characterization of silencing of the cryptic a-locus from the budding yeast *Kluyveromyces lactis,* which is responsible for the α-mating-type information similar to *S. cerevisiae.* As in *Saccharomyces*, SIR proteins control the chromatin structure at *HML*α. In the case of SIR2, this protein appeared to control functions unrelated to mating type, telomeres, or ribosomal DNA (rDNA) [91].

Recently, a *SIR2* ortholog was also found in *Aspergillus nidulans*, which is the first member of this enzyme family analyzed from filamentous fungi.

Toxins as histone deacetylase inhibitors and plant pathogens

An important tool for the study of histone acetylation and the elucidation of the structure and precise function of enzymes involved in this histone modification is the use of specific inhibitors of HDACs (figs 1 and 2). In contrast to

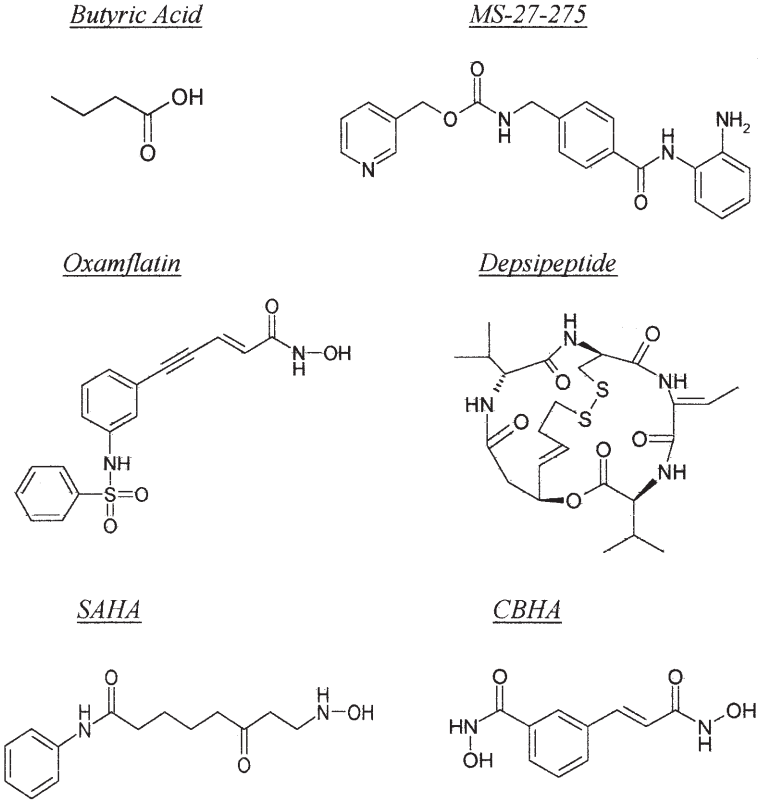

Figure 1. Nonfungal histone deacetylase inhibitors. SAHA, suberoylanilide hydroxamic acid; CBHA, *m*-carboxycinamic acid bishydroxamide.

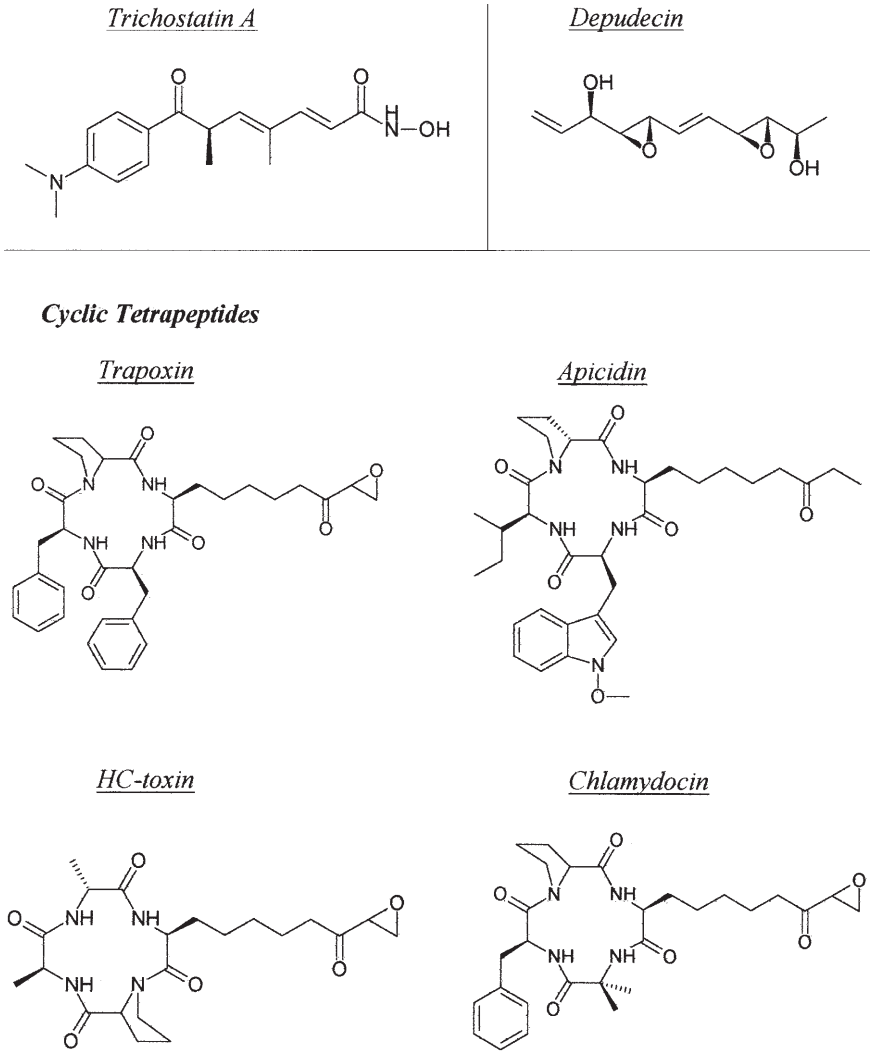

Figure 2. Fungal histone deacetylase inhibitors.

HATs, for which to date no potent inhibitors are known, there is a panel of structurally unrelated agents available that affect HDACs in a very selective and effective way and which were shown to have numerous effects on cancer cells.

Short-chain fatty acids

Short-chain fatty acids as butyrate [92] have for a long time been the only known inhibitors of HDACs. Butyrate is produced in the human colon by bacterial fermentation of carbohydrates such as dietary fibres. Butyrate exerts various biological effects such as G1-cell-cycle block in mammalian cells, induction of differentiation and modulation of transcriptional regulatory elements [92-95]. Additionally, studies were performed that demonstrate a therapeutic potential of butyrate in colon cancer [96].

FR901228 of *Chromobacterium violaceum*

Another group of HDAC inhibitors is produced by the bacterium *C. violaceum*. The bicyclic depsipeptide FR901228 was identified as a novel antitumor substance chemically unrelated to fungal toxins such as TSA or cyclic tetrapeptides [101]. Some of the effects observed by FR901228 are induction of morphological reversion of H-*ras*-transformed NIH3T3 cells [102], inhibition of proliferation of tumor cells in vitro [103] and suppression of tumor growth in mice [104].

Synthetic compounds

Finally, a series of synthetic compounds was shown to be highly specific inhibitors of HDACs. Oxamflatin, an aromatic sulfonamide derivative with a hydroxamic group [105], and the benzamide derivative MS-27-275 [106]

were shown to have antitumor activities in vivo and in vitro. Hybrid polar compounds as suberoylanilide hydroxamic acids (SAHA) and *m*-carboxycinamic acid bishydroxamide (CBHA) were reported to induce terminal differentiation and/or apoptosis in various transformed cells [107]; and finally, analogues of TSA and trapoxin, which inhibited maize HD2 in nanomolar range, induced differentiation in Friend leukemic cells [108, 109].

Fungal toxins

Fungal metabolites represent a group of potent and highly specific toxins. Among these are trichostatin A (TSA) [97], cyclic tetrapeptides such as apicidin [98], trapoxin [99], HC toxin and chlamydocin [28], and the structurally distinct agent depudecin [100].

Trichostatin A

The most prominent and the first to be identified among highly specific inhibitors of HDACs was the fungal metabolite TSA, which contains a hydroxamate group. TSA was originally isolated by Tsuji et al. [110] from *Streptomyces hygroscopicus* as an antifungal antibiotic active against *Trichophyton*. Some of the initially observed effects after treatment of various cell lines with TSA were the induction of differentiation of Friend murine erythroleukemia cells as well as the inhibition of cell proliferation of mammalian cells at very low concentrations of TSA [111–113]. The finding of highly aceytylated histones in TSA-treated cells finally led to the identification of HDAC as the target of TSA. In vitro experiments using partially purified HDAC from mouse mammary tumor cells confirmed this assumption, thereby inhibiting HDAC in nanomolar concentrations and in a noncompetitive fashion [97, 99].

Since its identification in 1976, multiple cellular effects of TSA have been observed. Treatment of cells thereby resulted in induction or suppression of a variety of genes responsible for differentiation, apoptosis and proliferation. One of these genes was shown to be gelsolin [114]. Gelsolin is an actin-binding protein that is regarded as a tumor supressor since expression of gelsolin is greatly decreased in many transformed cell lines and tumor tissues, whereas high gelsolin expression is consistently observed in differentiated and nonproliferative cells [115]. By studying TSA effects on the morphology and cell cycle of human carcinoma cell lines (T24, HeLa), a 7-fold or 12-fold increase in the intracellular levels of gelsolin was demonstrated [114]. Furthermore, in human breast cancer cells it was shown that epigenetic modifications and not point mutations in the coding region of the gelsolin gene are responsible for downregulation of gelsolin expression [116]. Treatment of MDA231, MCF7 and T47D human breast cancer cells with TSA resulted in increased expression of gelsolin and apoptotic cell death. Thus, an increased level of gelsolin might be the direct cause of TSA-induced morphological changes and cell cycle arrest [116].

p21waf1, responsible for inhibition of cyclin-dependent kinase activity in p53-mediated cell cycle arrest induced by DNA damage [117], is another gene induced by HDAC inhibitors. Treatment of human colon cancer cells [118, 119] and lung adenocarcinoma cells [120] as well as NIH3T3 cells with TSA or butyrate [121] led to upregulation of p21expression and therefore identified p21 as a target gene of HDACs. Furthermore, during differentiation of prostate epithelial cancer cells after treatment with TSA, the induction of a novel gene, designated carboxypeptidase A3 (CPA3), was reported [122]. Interestingly, the induction of CPA3 was inhibited by p21WAF/CIP1 antisense messenger RNA (mRNA), indicating that p21 transactivation might be required for the induction of CPA3 [122].

Proof that TSA might have potential as a topical treatment for epidermal malignancies was demonstrated by Saunders et al. [123]. Treatment of keratinocytes or squamous cells with TSA and butyrate thereby induced growth arrest and differentiation.

Besides the induction of genes as described above, TSA was also shown to specifically downregulate genes linked to proliferation, apoptosis and differentiation. In leukemia cell lines treatment with TSA and butyrate resulted in the abrogation of interleukin (IL)-2-mediated gene expression prior to induction of apoptosis in IL-2-dependent ILT-Mat and BAF-B03 transfectant F7 cells [124]. HDAC inhibitors therefore represent promising candidates for cancer therapy as effective inducers of apoptosis.

TSA has been presented as an effective treatment of acute promyelocytic leukemia (APL) [125]. In APL reciprocal chromosomal translocation of chromosomes 15 and 17 [t(15;17)] causes fusion of the retinoic acid receptor-α gene (RARα) and the pro-myelocytic leukemia gene, and expression of PML/RARα proteins in all leukemic cells [126–128]. In some cases, the promyelocytic leukemia zink finger (PLZF) is fused to RARα by t(11;17) translocation [129]. RARα fusion proteins are resistent to physiological levels of retinoic acid [129, 130] and interfere with the expression of RA-inducible genes that promote myeloid differentiation [130, 131]. In this context, TSA has been shown to restore RA responsiveness to PLZF/RARα and to allow leukemic cells expressing this fusion protein to differentiate in response to all-trans-retinoic acid [130, 132, 133].

Another chromosomal translocation [t(8;21)] creates the chimeric fusion protein AML1/ETO [134, 135]. The transcription factor AML (acute myeloid leukemia), which regulates the expression of a number of genes involved in

hematopoiesis [136, 137], is fused to a protein called eight-twenty-one (ETO) [138], which additionally was shown to recruit a transcription repression complex including the HDAC1 enzyme [139]. The AML1/ETO fusion protein blocks trans-activation of AML1-dependent target genes [140, 141]. Using transient transfection assays, TSA again was capable of relieving ETO-mediated repression and induced differentiation of AML1/ETO leukemia cells [142]. These data suggest that HDAC inhibitors, in particular TSA, could function as promising differentiation-enhancing agents in the therapy of AML or APL.

Cyclic tetrapeptides

Besides TSA, cyclic tetrapeptides represent another group of fungal toxins with potent and highly specific inhibitory activity to HDACs. After identification of trapoxin [143], several cyclic tetrapeptides with similar structures and properties have been reported. In general, these toxins inhibit HDACs in nanomolar or micromolar concentrations. Among these inhibitors are Aeo (2-amino-9,10-epoxy-8-oxodecanoic acid) containing the cyclic peptides trapoxin [143], HC toxin, and chlamydocin [28] and apicidin, which contains the 2-amino-8-oxodecanoic acid without the epoxide moiety [98].

Trapoxin

Trapoxin (TPX) was the first cyclic tetrapeptide to be identified with potent activity as HDAC inhibitor in vitro [143]. TPX was originally isolated from the culture broth of *Helicoma ambiens* RF-1023 exhibiting detransformation activities against v-sis oncogene-transformed NIH3T3 cells (sis/NIH3T3) as antitumor agent [144]. The structure was found to be cyclo(L-phenylalanyl-L-phenylalanyl-D-pipecolinyl-L-Aeo). TPX inhibited mammalian HDAC at nanomolar concentrations and, interestingly, the inhibition of TPX was found to be irreversible, in contrast to other inhibitors. Therefore, it is believed that TPX binds covalently to HDAC, leading to its deviating property [143]; in addition, it was shown that TPX causes the accumulation of highly acetylated histones in a variety of mammalian cells, and a specific G1- and G2-phase cell cycle arrest of rat fibroblasts and morphological and biochemical changes in the phenotypes of F9 teratocarcinoma cells were demonstrated, effects that had also been observed with TSA and butyrate.

In a further approach, TPX was shown to specifically alter the expression and activity of proteins involved in cell cycle regulation of different human tumor cell lines [145]. For example, TPX induced transcription and elevated protein levels of the cdk inhibitor p21waf1 without altering the protein levels of cdk2, cdk4 or cyclin B, resulting in G1 and G2 cell cycle arrest in H1299 human lung and MDA-MB-435 breast carcinoma cells, and apoptosis in A549 lung carcinoma cells. Thus, inhibition of HDAC is able to activate a specific subset of genes that control cell cycle arrest and apoptosis.

Apicidin

Another naturally ocurring cyclic tetrapeptide which is structurally related to trapoxin is the fungal metabolite apicidin. Apicidin with the structure cyclo(*N*-*O*-methyl-L-tryptophanyl-L-isoleucinyl-D-piecolinyl-L-2-amino-8-oxodecanoyl) was isolated from fermentations of *Fusarium* spp. as an antiprotozoal agent against Apicomplexan parasites such as *Plasmodium berghei* and *Cryptosporidium parvum*, which represent significant threats to human and animal health via malaria and cryptosporidiosis, respectively [98, 146]. It was shown that the antiparasitic activity of apicidin might be due to nanomolar inhibition of apicomplexan HDAC, causing hyperacetylation of histones. Other toxic effects include severe hemorrhage in internal organs [147]. In addition to the biological and toxic effects, Kim et al. described a possible involvement of HDAC in the ras-signaling pathway via inhibition by apicidin [148]. In that case, apicidin inhibited H-ras-induced malignant phenotypes in MCF10A human breast epithelial cells. Furthermore, they showed morphological reversal and growth inhibition of H-ras MCF10A cells induced by apicidin, similar to that observed with depudecin [149] and oxamflatin [150]. Thus, apicidin represents an attractive tool for the development of antimalarial agents, but the detransforming activities of apicidin and other inhibitors of HDACs support a potential use in the treatment of cancer.

HC toxin and Chlamydocin

Another cyclic tetrapeptide with specific and potent inhibitory effects on HDACs is the host-specific toxin of the filamentous fungus *C. carbonum*. *C. carbonum* (imperfect stage *Helminthosporium carbonum* or *Bipolaris zeicola*) causes leaf spot and ear rot of maize plants that are homozygous recessive at the Hm locus, whereas maize that is heterozygous or homozygous dominant at Hm is 100-fold less sensitive to HC toxin and develops only small, nonexpanding lesions when infected with Tox2+ isolates [151, 152]. The production of HC toxin [structure cyclo-(D-proline-L-alanine-D-alanine-L-Aeo)] is under the control of TOX2, a complex genetic locus that consists of at least three different duplicated genes that are unique to Tox2+ isolates [151], encoding a 570-kDa tetrapartite cyclic peptide synthetase [153], a putative membrane antibiotic efflux pump encoded by TOXA and a gene called TOXC that encodes a putative Aeo synthase [154]. The demonstration that HC toxin inhibits partially purified

HD1-A, HD1-B and HD2 of maize, but also HDACs of chicken and the myxomycete *P. polycephalum* led to the hypothesis that HDAC is the biologically significant site of action of HC toxin [28]. Furthermore, treatment of maize plants with HC toxin demonstrated that HDACs are also inhibited in vivo resulting in the accumulation of highly acetylated histone H3 and H4 subspecies in both maize embryos and tissue cultures [28, 82]. Although, it is not clear how HC toxin allows colonization of susceptible maize, it is speculated that inhibition of maize HDACs by HC toxin promotes infection of maize by *C. carbonum* and thereby causes the accumulation of hyperacetylated core histones and the development of a compatible disease interaction, e.g. by interfering with the induction of defense genes in maize [28].

A strong inhibitory effect on HDACs was also demonstrated for chlamydocin [28], a cyclic tetrapeptide containing the Aeo side chain with the structure cyclo (L-Aeo-α-aminobutyric acid-L-phenylalanyl-D-proline-L-Aeo). It is produced by the soil inhabiting fungus *Diheterospora chlamydosporia* [155] and was originally isolated as a cytostatic agent against mammalian cells [156].

Depudecin

Depudecin is a structurally distinct natural product whose putative molecular target was determined by Kwon et al. [100]. Depudecin was isolated from the fungus *Alternaria brassicicola* by screening for agents that morphologically detransform NIH3T3 cells transfected with v-ras and v-src oncogenes [149]. It also showed detransforming activity in v-raf-transformed cells and in human osteosarcoma cells [157]. Since depudecin shares the same binding site as trapoxin, induces histone hyperacetylation and inhibits HDAC activity in vitro, the morphological reversion of transformed NIH3T3 fibroblasts is suggested to occur via inhibition of HDAC [100]. Furthermore, depudecin was also tested for its antiangiogenic activity, since several epoxide-group-containing agents had been shown to be potent inhibitors of angiogenesis [158, 159]. Using an in vivo system, depudecin inhibited embryonic angiogenesis in a dose-dependent manner, and it also affected the growth of vascolar endothelial cells, indicating the impact of depudecin as an antiangiogenic agent [159].

Inhibitor resistance of HDACs

During the past years evidence has accumulated that alterations of chromatin structure by histone acetylation and deacetylation play a central role in the regulation of gene transcription; more recent studies imply that aberrant histone acetylation may be a critical mechanism in the development of cancer. As outlined, inhibition of HDACs in cancer cells by specific toxins can lead to diverse cellular effects. As a consequence, inhibition leads to transcriptional activation or silencing, cell cycle arrest, induction of apoptosis or differentiation in vitro and in vivo.

Specific HDAC inhibitors are useful tools for several reasons. The identification and analysis of new HDAC inhibitors is of paramount importance for the elucidation of the general role of histone acetylation for chromatin structure and function. Due to their differentiation- and apoptosis-inducing activities in neoplastic cells, HDAC inhibitors are regarded as promising agents for cancer therapy and chemoprevention [160, 161].

An interesting aspect in this context would be deacetylases that show greatly reduced sensitivity or resistance to well-known inhibitors. Resistance could be due either to structural differences in the catalytic region of the enzyme itself or to an external protective factor that masks the enzyme and counteracts binding of toxin(s). The existence of toxin-resistant HDACs may have implications for the clinical use of toxins, and it could furthermore help to analyze and clarify the correlation between certain neoplastic diseases and aberrant histone acetylation.

Some HDACs have been reported to be much less sensitive to inhibitors. A mammalian cell line that was selected for resistance to trichostatin is at least 20 times less sensitive than the parental line to both TSA and trapoxin, and HDAC partially purified from the resistant cell line is also ~20 times less sensitive as well [97, 143]. The yeast HDAC complexes HDA and HDB were also shown to differ in their sensitivity to TSA. Whereas HDA was completely sensitive to TSA, the K_i for TSA against RPD3 as part of the HDB complex was reported to be 10-fold greater than against yeast HDA1 (i.e. ~5 nM vs. 50 nM) [162].

Another HDAC in yeast that was identified by sequence similarity to HDA1 (designated HOS3) was even more resistant to high concentrations of TSA. Immunoprecipitated HOS3 protein was found to be less sensitive to TSA than HDA1 by two orders of magnitude, indicating a difference in the structure of the catalytic subunit of HOS3 [70].

Recently, yeast SIR2, a nicotinamide adenine dinucleotide dependent HDAC, was shown to be completely resistant to TSA. At 400 nM TSA, a concentration that completely inhibits other HDACs had no effect on purified recombinant yeast SIR2 [62].

The finding of inhibitor-resistant HDACs would be of additional importance, since such enzymes could also serve as targets for the development of novel antiparasitic agents. For example, the fungal metabolite apicidin, a cyclic tetrapeptide of *Fusarium pallidoroseum*, related to HC toxin and chlamydocin, was shown to exhibit strong antiprotozoal activity against apicomplexan parasites due

to nanomolar inhibition of HDACs [98]. Interestingly, apicidin was inactive against several of the flagellated protozoa. This could be due to a detoxifying system in these organisms or a specific and distinct HDAC structure. HC toxin of the maize pathogen *C. carbonum* was shown to have an in vitro and in vivo efficiency comparable to apicidin [98]. Since inhibition of HDACs might interfere with transcriptional control, cell proliferation and ribosomal gene replication/ transcription, HDACs could be targets for the development of antiparasitic agents.

The study of HDAC inhibitors, their mode of binding to HDACs and the mechanism of HDAC-inhibitor-mediated cell cycle arrest could support the design of new potent HDAC inhibitors with potential application in chemoprevention and chemotherapy of malignant disease such as combined therapy of acute myeloid leukemia, using HDAC inhibitors and retinoids.

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1 Brownell J. E., Zhou J., Ranalli T., Kobayashi R., Edmondson D. G., Roth S. Y. and Allis C. D. (1996) *Tetrahymena* histone acetyltransferase A: a homolog to yeast Gcn5p linking histone acetylation to gene activation. Cell **84:** 843–851
2 Taunton J., Hassig C. A. and Schreiber S. L. (1996) A mammalian histone deacetylase related to the yeast transcriptional regulator Rpd3p. Science **272:** 408–411
3 Loidl P. (1988) Towards an understanding of the biological function of histone acetylation. FEBS Lett. **227:** 91–95
4 López-Rodas G., Brosch G., Georgieva E. I., Sendra R., Franco L. and Loidl P. (1993) Histone deacetylase–a key enzyme for the binding of regulatory proteins to chromatin. FEBS Lett. **317:** 175–180
5 Loidl P. (1994) Histone acetylation: facts and questions. Chromosoma **103:** 441–449
6 Kouzarides T. (2000) Acetylation: a regulatory modification to rival phosphorylation? EMBO J. **19:**1176–1179
7 Strahl B. D. and Allis C. D. (2000) The language of covalent histone modifications. Nature **403:** 41–45
8 Gu W. and Roeder R. G. (1997) Activation of p53 sequence-specific DNA binding by acetylation of the p53 C-terminal domain. Cell **90:** 595–606
9 Herrera J. E., Sakaguchi K., Bergel M., Trieschmann L., Nakatani Y. and Bustin M. (1999) Specific acetylation of chromosomal protein HMG-17 by PCAF alters its interaction with nucleosomes. Mol. Cell. Biol. **19:** 3466–3473
10 Sartorelli V., Puri P. L., Hamamori Y., Ogryzko V., Chung G., Nakatani Y. et al. (1999) Acetylation of MyoD directed by PCAF is necessary for the execution of the muscle program. Mol. Cell **4:** 725–734
11 Martínez-Balbás M., Bauer U.-M., Nielsen S. J., Brehm A. and Kouzarides T. (2000) Regulation of E2F1 activity by acetylation. EMBO J. **19:** 662–671
12 Sterner D. E. and Berger S. L. (2000) Acetylation of histones and transcription-related factors. Microbiol. Mol. Biol. Rev. **64:** 435–459
13 Krebs J. E., Kuo M. H., Allis C. D. and Peterson C. L. (1999) Cell cycle regulated histone acetylation required for the expression of the yeast HO-gene. Genes Dev. **13:** 1412-1421
14 Parekh B. S. and Maniatis T. (1999) Virus infection leads to localized hyperacetylation of histone H3 and H4 at the IFN-beta promoter. Mol.Cell **3:** 125–129
15 Crane-Robinson C., Myers F. A., Hebbes T. R., Clayton A. L. and Thorne A. W. (1999) Chromatin immunoprecipitation assays in acetylation mapping of higher eukaryotes. Methods Enzymol. **304:** 533–547
16 Madisen L., Krumm A., Hebbes T. R. and Groudine M. (1998) The immunoglobulin heavy chain locus control region increases histone acetylation along linked c-myc genes. Mol. Cell. Biol. **18:** 6281–6292
17 Munshi N., Merika M., Yie J., Senger K., Chen G. and Thanos D. (1998) Acetylation of HMGI(Y) by CBP turns off IFN beta expression by disrupting the enhancosome. Mol. Cell **2:** 457–467
18 McMurry M. T. and Krangel M. S. (2000) A role for histone acetylation in the developmental regulation of VDJ recombination. Science **287:** 495–498
19 Iiuzuka M. and Stillman B. (1999) Histone acetyltransferase HBO1 interacts with the ORC1 subunit of the human initiator protein. J. Biol. Chem. **274:** 23027–23034
20 Jacobson S. and Pillus L. (1999) Modifying chromatin and concepts of cancer. Curr. Opin. Genet. Dev. **9:** 175–184
21 Ehrenhofer-Murray A. E., Rivier D. H. and Rine J. (1997) The role of Sas2, an acetyltransferase homologue of *Saccharomyces cerevisiae*, in silencing and ORC function. Genetics **145:** 923–934
22 Parthun M. R., Widom J. and Gottschling D. E. (1996) The major cytoplasmic histone acetyltransferase in yeast: links to chromatin replication and histone metabolism. Cell **87:** 85–94
23 Kölle D., Sarg B., Lindner H. and Loidl P. (1998) Substrate and sequential site-specificity of cytoplasmic histone acetyltransferases of maize and rat liver. FEBS Lett. **421:** 109-114
24 Ekwall K., Olsson T., Turner B. M., Cranston G. and Allshire R. C. (1997) Transient inhibition of histone deacetylation alters the structural and functional imprint at fission yeast centromeres. Cell **91:** 1021–1032
25 Cortes M., Wong E., Koipally J. and Georgopoulos K. (1999) Control of lymphocyte development by the Ikaros gene family. Curr. Opin. Immunol. **11:** 167–171
26 Cress W. D. and Seto E. (2000) Histone deacetylases, transcriptional control, and cancer. J. Cell. Physiol. **184:** 1–16
27 Timmermann S., Lehrmann H., Polesskaya A. and Harel-Belan A. (2001) Histone acetylation and disease. Cell. Mol. Life Sci., this issue.
28 Brosch G., Ransom R., Lechner T., Walton J. D. and Loidl P. (1995) Inhibition of maize histone deacetylases by HC toxin, the host-selective toxin of *Cochliobolus carbonum*. Plant Cell **7:** 1941–1950
29 Waterborg J. H., Harrington R. E. and Winicov I. (1990) Dynamic histone acetylation in alfalfa cells. Butyrate interference with acetate labeling. Biochim. Biophys. Acta **1049:** 324–330
30 Houben A., Belyaev N. D., Leach C. R. and Timmis J. N. (1997) Differences of histone H4 acetylation and replication timing between A and B chromosomes of brachycome dichromosomatica. Chromosome Res. **5:** 233–237
31 Buzek J., Riha K., Siroky J., Ebert I., Greilhuber J. and Vyskot B. (1998) Histone H4 underacetylation in plant facultative heterochromatin. J.Biol.Chem. **379:** 1235–1241
32 Vyskot B., Siroky J., Hladilova R., Belyaev N. D. and Turner B. M. (1999) Euchromatic domains in plant chromosomes as

revealed by H4 histone acetylation and early DNA replication. Genome **42:** 343–350
33 Jasencakova Z., Meister A., Walter J., Turner B. M. and Schubert I. (2000) Histone H4 acetylation of euchromatin and heterochromatin is cell cycle dependent and correlated with replication rather than with transcription. Plant Cell **12:** 2087–2100
34 Waterborg J. H. (1992) Identification of five sites of acetylation in alfalfa histone H4. Biochemistry **31:** 6211–6219
35 Lechner T., Lusser A., Brosch G., Eberharter A., Goralik-Schramel M. and Loidl P. (1996) A comparative study of histone deacetylases of plant, fungal and vertebrate cells. Biochim. Biophys. Acta **1296:** 181–188
36 Eberharter A., Lechner T., Goralik-Schramel M. and Loidl P. (1996) Purification and characterization of the cytoplasmic histone acetyltransferase B of maize embryos. FEBS Lett. **386:** 75–81
37 Lusser A., Eberharter A., Loidl A., Goralik-Schramel M., Horngacher M., Haas H. et al. (1999) Analysis of the histone acetyltransferase B complex of maize embryos. Nucleic Acids Res. **27:** 4427–4435
38 Lopez-Rodas G., Georgieva E. I., Sendra R. and Loidl P. (1991) Histone acetylation in *Zea mays*. I. Activities of histone acetyltransferases and histone deacetylases. J. Biol. Chem. **266:** 18745–18750
39 Georgieva E. I., López-Rodas G., Sendra R., Gröbner P. and Loidl P. (1991) Histone acetylation in *Zea mays*. II. Biological significance of posttranslational histone acetylation during embryo germination. J. Biol. Chem. **266:** 18751–18760
40 Ogryzko V. V. (2001) Mammalian histone acetyltransferases and their complexes. Cell. Mol. Life Sci., this issue
41 Mingarro I., Sendra R., Salvador M. L. and Franco L. (1993) Site specificity of pea histone acetyltransferase B in vitro. J. Biol. Chem. **268:** 13248–13252
42 Grunstein M. (1997) Histone acetylation in chromatin structure and transcription. Nature **389:** 349–352
43 Kleff S., Andrulis E. D., Anderson C. W. and Sternglanz R. (1995) Identification of a gene encoding a yeast histone H4 acetyltransferase. J. Biol. Chem. **270:** 24674–24677
44 Verreault A. (2000) De novo nucleosome assembly: new pieces in an old puzzle. Genes Dev. **14:** 1430–1438
45 Johnson C. A. and Turner B. M. (1999) Histone deacetylases: complex transducers of nuclear signals. Semin. Cell Dev. Biol. **10:** 179–188
46 Rossi V., Hartings H. and Motto M. (1998) Identification and characterisation of an RPD3 homologue from maize (*Zea mays* L.) that is able to complement an rpd3 null mutant of *Saccharomyces cerevisiae*. Mol. Gen. Genet. **258:** 288–296
47 Lechner T., Lusser A., Pipal A., Brosch G., Loidl A., Goralik-Schramel M. et al. (2000) RPD3-Type histone deacetylases in maize embryos. Biochemistry **39:** 1683–1692
48 Kölle D., Brosch G., Lechner T., Pipal A., Helliger W., Taplick J. et al. (1999) Different types of maize histone deacetylases are distinguished by a highly complex substrate and site specificity. Biochemistry **38:** 6769–6773
49 Brosch G., Goralik-Schramel M. and Loidl P. (1996) Purification of histone deacetylase HD1-A of germinating maize embryos. FEBS Lett. **393:** 287–291
50 Brosch G., Lusser A., Goralik-Schramel M. and Loidl P. (1996) Purification and characterization of a high molecular weight histone deacetylase complex (HD2) of maize embryos. Biochemistry **35:** 15907–15914
51 Lusser A., Brosch G., Loidl A., Haas H. and Loidl P. (1997) Identification of maize histone deacetylase HD2 as an acidic nucleolar phosphoprotein. Science **277:** 88–91
52 Wu K., Tian L., Malik K., Brown D. and Miki B. (2000) Functional analysis of HD2 histone deacetylase homologues in *Arabidopsis thaliana*. Plant J. **22:** 19–27
53 Aravind L. and Koonin E. V. (1998) Second family of histone deacetylases. Science **280:** 1167a
54 Dangl M., Brosch G., Haas H., Loidl P. and Lusser A. (2001) Comparative analysis of HD2 type histone deacetylases in higher plants. Planta, in press
55 Garcia S. N. and Pillus L. (1999) Net results of nucleolar dynamics. Cell **97:** 825–828
56 Lohrum M. A., Ashcroft M., Kubbutat M. H. and Vousden K. H. (2000) Identification of a cryptic nucleolar-localization signal in MDM2. Nature Cell Biol. **2:** 179–181
57 Dangl M., Lusser A., Brosch G., Loidl A., Haas H. and Loidl P. (1998) Second family of histone deacetylases: response. Science **280:** 1167a
58 Gottschling D. E., Aparicio O. M., Billington B. L. and Zakian V. A. (1990) Position effect at *S. cerevisiae* telomeres: reversible repression of Pol II transcription. Cell **63:** 751–762
59 Rine J. and Herskowitz I. (1987) Four genes responsible for a position effect on expression from HML and HMR in *Saccharomyces cerevisiae.* Genetics **116:** 9–22
60 Bryk M., Banerjee M., Murphy M., Knudsen K. E., Garfinkel D. J. and Curcio M. J. (1997) Transcriptional silencing of Ty1 elements in the RDN1 locus of yeast. Genes Dev. **11:** 255–269
61 Braunstein M., Rose A. B., Holmes S. G., Allis C. D. and Broach J. R. (1993) Transcriptional silencing in yeast is associated with reduced nucleosome acetylation. Genes Dev. **7:** 592–604
62 Imai S., Armstrong C. M., Kaeberlein M. and Guarente L. (2000) Transcriptional silencing and longevity protein Sir2 is an NAD-dependent histone deacetylase. Nature **403:** 795–800
63 Frye R. A. (1999) Characterization of five human cDNAs with homology to the yeast SIR2 gene: Sir2-like proteins (sirtuins) metabolize NAD and may have protein ADP-ribosyltransferase activity. Biochem. Biophys. Res. Commun. **260:** 273–279
64 Tanny J. C., Dowd G. J., Huang J., Hilz H. and Moazed D. (1999) An enzymatic activity in the yeast Sir2 protein that is essential for gene silencing. Cell **99:** 735–745
65 Landry J., Sutton A., Tafrov S. T., Heller R. C., Stebbins J., Pillus L. et al. (2000) The silencing protein SIR2 and its homologs are NAD-dependent protein deacetylases. Proc. Natl. Acad. Sci. USA **97:** 5807–5811
66 Guarente L. (2000) Sir2 links chromatin silencing, metabolism and aging. Genes Dev. **14:** 1021–1026
67 Brachmann C. B., Sherman J. M., Devine S. E., Cameron E. E., Pillus L. and Boeke J. D. (1995) The SIR2 gene family, conserved from bacteria to humans, functions in silencing, cell cycle progression and chromosome stability. Genes Dev. **9:** 2888–2902
68 Vidal M. and Gaber R. F. (1991) RPD3 encodes a second factor required to achieve maximum positive and negative transcriptional states in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **11:** 6317–6327
69 Rundlett S. E., Carmen A. A., Kobayashi R., Bavykin S., Turner B. M. and Grunstein M. (1996) HDA1 and RPD3 are members of distinct yeast histone deacetylase complexes that regulate silencing and transcription. Proc. Natl. Acad. Sci. USA **93:**14503–14508
70 Carmen A. A., Griffin P. R., Calaycay J. R., Rundlett S. E., Suka Y. and Grunstein M. (1999) Yeast HOS3 forms a novel trichostatin A-insensitive homodimer with intrinsic histone deacetylase activity. Proc. Natl. Acad. Sci. USA **96:** 12356–12361
71 Allshire R. C. (1995) Elements of chromosome structure and function in fission yeast. Semin. Cell Biol. **6:** 55–64
72 Grewal S. I., Bonaduce M. J. and Klar A. J. (1998) Histone deacetylase homologs regulate epigenetic inheritance of transcriptional silencing and chromosome segregation in fission yeast. Genetics **150:** 563–576

73 Kim Y. B., Honda A., Yoshida M. and Horinouchi S. (1998) phd1$^+$, a histone deacetylase gene of *Schizosaccharomyces pombe*, is required for the meiotic cell cycle and resistance to trichostatin A. FEBS Lett. **436:** 193–196

74 Olsson T. G. S., Ekwal K., Allshire R. C., Sunnerhagen P., Partridge J. F. and Richardson W. A. (1998) Genetic characterization of hda1$^+$, a putative fission yeast histone deacetylase gene. Nucleic Acids Res. **26:** 3247–3254

75 Ekwall K. and Ruusala T. (1994) Mutations in rik1, clr2, clr3 and clr4 genes asymmetrically derepress the silent mating-type loci in fission yeast. Genetics **136:** 53–64

76 Thon G., Cohen A. and Klar A. J. (1994) Three additional linkage groups that repress transcription and meiotic recombination in the mating-type region of *Schizosaccharomyces pombe*. Genetics **138:** 29–38

77 López-Rodas G., Brosch G., Golderer G., Lindner H., Gröbner P. and Loidl P. (1992) Enzymes involved in the dynamic equilibrium of core histone acetylation of *Physarum polycephalum*. FEBS Lett **296:** 82–86

78 Lusser A., Brosch G., López-Rodas G. and Loidl P. (1997) Histone acetyltransferases during the cell cycle and differentiation of *Physarum polycephalum*. Eur. J. Cell Biol. **74:** 102–110

79 Loidl P. and Gröbner P. (1987) Postsynthetic acetylation of histones during the cell cycle: a general function for the displacement of histones during chromatin rearrangements. Nucleic Acids Res. **15:** 8351–8366

80 Quadbeck-Seeger C., Wanner G., Huber S., Kahmann R. and Kamper J. (2000) A protein with similarity to the human retinoblastoma binding protein 2 acts specifically as a repressor for genes regulated by the b mating type locus in *Ustilago maydis*. Mol. Microbiol. **38:** 154–166

81 Zhang Y., LeRoy G., Seelig H. P., Lane W. S. and Reinberg D. (1998) The dermatomyositis-specific auto-antigen Mi2 is a component of a complex containing histone deacetylase and nucleosome remodeling activities. Cell **95:** 279–289

82 Ransom, R. F. and Walton, J. D. (1997) Histone hyperacetylation in maize in response to treatment with HC toxin or infection by the filamentous fungus *Cochliobolus carbonum*. Plant Physiol. **115:** 1021–1027

83 Pitkin J. W., Panaccione D. G. and Walton J. D. (1996) A putative cyclic peptide efflux pump encoded by the TOXA gene of the plant-pathogenic fungus *Cochliobolus carbonum*. Microbiology **142:**1557–1565

84 Graessle S., Dangl M., Haas H., Mair K., Trojer P., Brandtner E. et al. (2000) Characterization of two putative histone deacetylase genes from *Aspergillus nidulans*. Biochim. Biophys. Acta **1492:** 120–126

85 Foss H. M., Roberts C. J., Claeys K. M. and Selker E. U. (1993) Abnormal chromosome behavior in *Neurospora* mutants defective in DNA methylation. Science **262:** 1737–1741

86 Foss H. M., Roberts C. J., Claeys K. M. and Selker E. U. (1995) Abnormal chromosome behavior in *Neurospora* mutants defective in DNA methylation (Erratum). Science **267:** 316

87 Selker E. U. (1998) Trichostatin A causes selective loss of DNA methylation in *Neurospora.* Proc. Natl. Acad. Sci. USA **95:** 9430–9435

88 Wade P. A., Gegonne A., Jones P. L., Ballestar E., Aubry F. and Wolffe A. P. (1999) Mi-2 complex couples DNA methylation to chromatin remodelling and histone deacetylation. Nat. Genet. **23:** 62–66

89 Freeman-Cook L. L., Sherman J M., Brachman B. B., Allshire R. C., Boeke J. D. and Pillus L. (1999) The *Schizosaccharomyces pombe* hst4$^+$ gene is a SIR2 homologue with silencing and centromeric functions. Mol. Biol. Cell **10:** 3171–3186

90 Perez-Martin J., Uria J. A. and Johnson A. D. (1999) Phenotypic switching in *Candida albicans* is controlled by a SIR2 gene. EMBO J. **18:** 2580–2592

91 Astrom S. U., Kegel A., Sjostrand J. O. and Rine J. (2000) *Kluyveromyces lactis* Sir2p regulates cation sensitivity and maintains a specialized chromatin structure at the cryptic alpha-locus. Genetics **158:** 81–91

92 Kruh J. (1982) Effects of sodium butyrate, a new pharmacological agent, on cells in culture. Mol. Cell. Biochem. **42:** 65–82

93 Collins S. J., Ruscetti F. W., Gallagher R. E. and Gallo R. C. (1978) Terminal differentiation of human promyelocytic leukemia cells induced by dimethyl sulfoxide and other polar compounds. Proc. Natl. Acad. Sci. USA **75:** 2458–2462

94 Leder A. and Leder P. (1975) Butyric acid, a potent inducer of erythroid differentiation in cultured erythroleukemic cells. Cell **5:** 319–322

95 Tang D. C. and Taylor M. W. (1990) Transcriptional activation of the adenine phosphoribosyltransferase promoter by an upstream butyrate-induced Moloney murine sarcoma virus enhancer-promoter element. J. Virol. **64:** 2907–2911

96 Hassig C. A., Tong J. K. and Schreiber S. L. (1997) Fiber-derived butyrate and the prevention of colon cancer. Chem. Biol. **4:** 783–789

97 Yoshida M., Kijima M., Akita M. and Beppu T. (1990) Potent and specific inhibition of mammalian histone deacetylase both in vivo and in vitro by trichostatin A. J. Biol. Chem. **265:** 17174–17179

98 Darkin-Rattray S. J., Gurnett A. M., Myers R. W., Dulski P. M., Crumley T. M., Allocco J. J. et al. (1996) Apicidin: a novel antiprotozoal agent that inhibits parasite histone deacetylase. Proc. Natl. Acad. Sci. USA **93:** 13143–13147

99 Yoshida M., Horinouchi S. and Beppu T. (1995) Trichostatin A and trapoxin**:** novel chemical probes for the role of histone acetylation in chromatin structure and function. Bioessays **17:** 423–430

100 Kwon H. J., Owa T., Hassig C. A., Shimada J. and Schreiber S. L. (1998) Depudecin induces morphological reversion of transformed fibroblasts via the inhibition of histone deacetylase. Proc. Natl. Acad. Sci. USA **95:** 3356–3361

101 Nakajima H., Kim Y. B., Terano H., Yoshida M. and Horinouchi S. (1998) FR901228, a potent antitumor antibiotic, is a novel histone deacetylase inhibitor. Exp. Cell. Res. **241:** 126–133

102 Ueda H., Nakajima H., Hori Y., Fujita T., Nishimura M., Goto T. et al. (1994) FR901228, a novel antitumor bicyclic depsipeptide produced by *Chromobacterium violaceum* No. 968. I. Taxonomy, fermentation, isolation, physico-chemical and biological properties, and antitumor activity. J. Antibiot. **47:** 301–310

103 Ueda H., Nakajima H., Hori Y., Goto T. and Okuhara M. (1994) Action of FR901228, a novel antitumor bicyclic depsipeptide produced by *Chromobacterium violaceum* no. 968, on Ha-ras transformed NIH3T3 cells. Biosci. Biotechnol. Biochem. **58:** 1579–1583

104 Ueda H., Manda T., Matsumoto S., Mukumoto S., Nishigaki F., Kawamura I. et al. (1994) FR901228, a novel antitumor bicyclic depsipeptide produced by *Chromobacterium violaceum* No. 968. III. Antitumor activities on experimental tumors in mice. J. Antibiot. **47:** 315–323

105 Kim Y. B., Lee K. H., Sugita K., Yoshida M. and Horinouchi S. (1999) Oxamflatin is a novel antitumor compound that inhibits mammalian histone deacetylase. Oncogene **18:** 2461–2470

106 Saito A., Yamashita T., Mariko Y., Nosaka Y., Tsuchiya K., Ando T. et al. (1999) A synthetic inhibitor of histone deacetylase, MS-27-275, with marked in vivo antitumor activity against human tumors. Proc. Natl. Acad. Sci. USA **96:** 4592–4597

107 Richon V. M., Emiliani S., Verdin E., Webb Y., Breslow R., Rifkind R. A. et al. (1998) A class of hybrid polar inducers of transformed cell differentiation inhibits histone deacetylases. Proc. Natl. Acad. Sci. USA **95:** 3003–3007

108 Jung M., Hoffmann, K., Brosch, G., and Loidl, P. (1997) Analogues of Trichostatin A and Trapoxin B as histone deacetylase inhibitors. Bioorg. Med. Chem. Lett. **7:** 1655–1658

109 Jung M., Brosch G., Kölle D., Scherf H., Gerhauser C. and Loidl P. (1999) Amide analogues of trichostatin A as inhibitors of histone deacetylase and inducers of terminal cell differentiation. J. Med. Chem. **42:** 4669–4679

110 Tsuij N., Kobayashi M., Nagashima K., Wakisaka Y., and Koizumi K. (1976) A new antifungal antibiotic, trichostatin. J. Antibiot. **29:** 1–6

111 Yoshida M., Nomura S. and Beppu T. (1987) Effects of trichostatins on differentiation of murine erythroleukemia cells. Cancer Res. **47:** 3688–3691

112 Yoshida M. and Beppu T. (1988) Reversible arrest of proliferation of rat 3Y1 fibroblasts in both the G1 and G2 phases by trichostatin A. Exp. Cell. Res. **177:** 122–131

113 Morioka H., Ishihara, M., Takezawa, M., Shibai, H., and Komoda, Y. (1988) Trichostatin A, a potent differentiation inducer of Friend leukemia cells. Agric. Biol. Chem. **52:** 251–253

114 Hoshikawa Y., Kwon H. J., Yoshida M., Horinouchi S. and Beppu T. (1994) Trichostatin A induces morphological changes and gelsolin expression by inhibiting histone deacetylase in human carcinoma cell lines. Exp. Cell. Res. **214:** 189–197

115 Kwiatkowski D. J. (1999) Functions of gelsolin: motility, signaling, apoptosis, cancer. Curr. Opin. Cell Biol. **11:** 103–108

116 Mielnicki L. M., Ying A. M., Head K. L., Asch H. L. and Asch B. B. (1999) Epigenetic regulation of gelsolin expression in human breast cancer cells. Exp. Cell. Res. **249:** 161–176

117 el-Deiry W. S., Tokino T., Velculescu V. E., Levy D. B., Parsons R., Trent J. M. et al. (1993) WAF1, a potential mediator of p53 tumor suppression. Cell **75:** 817–825

118 Archer S. Y., Meng S., Shei A. and Hodin R. A. (1998) p21(WAF1) is required for butyrate-mediated growth inhibition of human colon cancer cells. Proc. Natl. Acad. Sci. USA **95:** 6791–6796

119 Nakano K., Mizuno T., Sowa Y., Orita T., Yoshino T., Okuyama Y. et al. (1997) Butyrate activates the WAF1/Cip1 gene promoter through Sp1 sites in a p53- negative human colon cancer cell line. J. Biol. Chem. **272:** 22199–22206

120 Eickhoff B., Ruller S., Laue T., Kohler G., Stahl C., Schlaak M. et al. (2000) Trichostatin A modulates expression of p21waf1/cip1, Bcl-xL, ID1, ID2, ID3, CRAB2, GATA-2, hsp86 and TFIID/TAFII31 mRNA in human lung adenocarcinoma cells. Biol. Chem. **381:** 107–112

121 Xiao H., Hasegawa T., Miyaishi O., Ohkusu K. and Isobe K. (1997) Sodium butyrate induces NIH3T3 cells to senescence-like state and enhances promoter activity of p21WAF/CIP1 in p53-independent manner. Biochem. Biophys. Res. Commun. **237:** 457–460

122 Huang H., Reed C. P., Zhang J. S., Shridhar V., Wang L. and Smith D. I. (1999) Carboxypeptidase A3 (CPA3): a novel gene highly induced by histone deacetylase inhibitors during differentiation of prostate epithelial cancer cells. Cancer Res. **59:** 2981–2988

123 Saunders N., Dicker A., Popa C., Jones S. and Dahler A. (1999) Histone deacetylase inhibitors as potential anti-skin cancer agents. Cancer Res. **59:** 399–404

124 Koyama Y., Adachi M., Sekiya M., Takekawa M. and Imai K. (2000) Histone deacetylase inhibitors suppress IL-2-mediated gene expression prior to induction of apoptosis. Blood **96:** 1490–1495

125 Fenrick R. and Hiebert S. W. (1998) Role of histone deacetylases in acute leukemia. J. Cell. Biochem. Suppl. **31:** 194–202

126 Goddard A. D., Borrow J., Freemont P. S. and Solomon E. (1991) Characterization of a zinc finger gene disrupted by the t(15;17) in acute promyelocytic leukemia. Science **254:** 1371–1374

127 Kakizuka A., Miller W. H. Jr, Umesono K., Warrell R. P. Jr, Frankel S. R., Murty V. V. et al. (1991) Chromosomal translocation t(15;17) in human acute promyelocytic leukemia fuses RAR alpha with a novel putative transcription factor, PML. Cell **66:** 663–674

128 de The H., Lavau C., Marchio A., Chomienne C., Degos L. and Dejean A. (1991) The PML-RAR alpha fusion mRNA generated by the t(15;17) translocation in acute promyelocytic leukemia encodes a functionally altered RAR. Cell **66:** 675–684

129 Kalantry S., Delva L., Gaboli M., Gandini D., Giorgio M., Hawe N. et al. (1997) Gene rearrangements in the molecular pathogenesis of acute promyelocytic leukemia. J. Cell. Physiol. **173:** 288–296

130 He L. Z., Guidez F., Tribioli C., Peruzzi D., Ruthardt M., Zelent A. et al. (1998) Distinct interactions of PML-RARalpha and PLZF-RARalpha with co-repressors determine differential responses to RA in APL. Nat. Genet. **18:** 126–135

131 Grimwade D. and Solomon E. (1997) Characterisation of the PML/RAR alpha rearrangement associated with t(15;17) acute promyelocytic leukaemia. Curr. Top. Microbiol. Immunol. **220:** 81–112

132 Lin R. J., Nagy L., Inoue S., Shao W., Miller W. H. Jr and Evans R. M. (1998) Role of the histone deacetylase complex in acute promyelocytic leukaemia. Nature **391:** 811–814

133 Grignani F., De Matteis S., Nervi C., Tomassoni L., Gelmetti V., Cioce M. et al. (1998) Fusion proteins of the retinoic acid receptor-alpha recruit histone deacetylase in promyelocytic leukaemia. Nature **391:** 815–818

134 Erickson P., Gao J., Chang K. S., Look T., Whisenant E., Raimondi S. et al. (1992) Identification of breakpoints in t(8;21) acute myelogenous leukemia and isolation of a fusion transcript, AML1/ETO, with similarity to *Drosophila* segmentation gene, runt. Blood **80:** 1825–1831

135 Miyoshi H., Shimizu K., Kozu T., Maseki N., Kaneko Y. and Ohki M. (1991) t(8;21) breakpoints on chromosome 21 in acute myeloid leukemia are clustered within a limited region of a single gene, AML1. Proc. Natl. Acad. Sci. USA **88:** 10431–10434

136 Tanaka T., Tanaka K., Ogawa S., Kurokawa M., Mitani K., Nishida J. et al. (1995) An acute myeloid leukemia gene, AML1, regulates hemopoietic myeloid cell differentiation and transcriptional activation antagonistically by two alternative spliced forms. EMBO J. **14:** 341–350

137 Okuda T., van Deursen J., Hiebert S. W., Grosveld G. and Downing J. R. (1996) AML1, the target of multiple chromosomal translocations in human leukemia, is essential for normal fetal liver hematopoiesis. Cell **84:** 321–330

138 Lenny N., Westendorf J. J. and Hiebert S. W. (1997) Transcriptional regulation during myelopoiesis. Mol. Biol. Rep. **24:** 157–168

139 Wang J., Hoshino T., Redner R. L., Kajigaya S. and Liu J. M. (1998) ETO, fusion partner in t(8;21) acute myeloid leukemia, represses transcription by interaction with the human N-CoR/mSin3/HDAC1 complex. Proc. Natl. Acad. Sci. USA **95:** 10860–10865

140 Yergeau D. A., Hetherington C. J., Wang Q., Zhang P., Sharpe A. H., Binder M. et al. (1997) Embryonic lethality and impairment of haematopoiesis in mice heterozygous for an AML1-ETO fusion gene. Nat. Genet. **15:** 303–306

141 Meyers S., Lenny N. and Hiebert S. W. (1995) The t(8;21) fusion protein interferes with AML-1B-dependent transcriptional activation. Mol. Cell. Biol. **15:** 1974–1982

142 Wang J., Saunthararajah Y., Redner R. L. and Liu J. M. (1999) Inhibitors of histone deacetylase relieve ETO-mediated repression and induce differentiation of AML1-ETO leukemia cells. Cancer Res. **59:** 2766–2769

143 Kijima M., Yoshida M., Sugita K., Horinouchi S. and Beppu T. (1993) Trapoxin, an antitumor cyclic tetrapeptide, is an irreversible inhibitor of mammalian histone deacetylase. J. Biol. Chem. **268:** 22429–22435

144 Itazaki H., Nagashima K., Sugita K., Yoshida H., Kawamura Y., Yasuda Y. et al. (1990) Isolation and structural elucidation of new cyclotetrapeptides, trapoxins A and B, having detransformation activities as antitumor agents. J. Antibiot. **43:** 1524–1532
145 Sambucetti L. C., Fischer D. D., Zabludoff S., Kwon P. O., Chamberlin H., Trogani N. et al. (1999) Histone deacetylase inhibition selectively alters the activity and expression of cell cycle proteins leading to specific chromatin acetylation and anti-proliferative effects. J. Biol. Chem. **274:** 34940–34947
146 Andrews K. T., Walduck A., Kelso M. J., Fairlie D. P., Saul A. and Parsons P. G. (2000) Anti-malarial effect of histone deacetylation inhibitors and mammalian tumour cytodifferentiating agents. Int. J. Parasitol. **30:** 761–768
147 Park J. S., Lee K. R., Kim J. C., Lim S. H., Seo J. A. and Lee Y. W. (1999) A hemorrhagic factor (Apicidin) produced by toxic *Fusarium* isolates from soybean seeds. Appl. Environ. Microbiol. **65:** 126–130
148 Kim M., Son M., Kim W., In Park Y. and Moon A. (2000) Apicidin, an inhibitor of histone deacetylase, prevents H-ras-induced invasive phenotype. Cancer Lett. **157:** 23–30
149 Sugita K., Yoshida H., Matsumoto M. and Matsutani S. (1992) A novel compound, depudecin, induces production of transformation to the flat phenotype of NIH3T3 cells transformed by ras-oncogene. Biochem. Biophys. Res. Commun. **182:** 379–387
150 Sonoda H., Nishida K., Yoshioka T., Ohtani M. and Sugita K. (1996) Oxamflatin: a novel compound which reverses malignant phenotype to normal one via induction of JunD. Oncogene **13:** 143–149
151 Walton J. D., Ransom, R. and Pitkin, J.W. (1997) Northern corn leaf spot of maize: chemistry, enzymology, and molecular genetics of a host-selective phytotoxin. In: Plant-Microbe Interactions, vol. 3, Stacey G. and Keen N. T., (eds), pp. 94–123, Chapman and Hall, New York
152 Scheffer R. P., Nelson, R.R., and Ullstrup, A.J. (1967) Inheritance of toxin production and pathogenicity in *Cochliobolus carbonum* and *Cochliobolus victoriae.* Phytopathology **57:** 1288–1291
153 Scott-Craig J. S., Panaccione D. G., Pocard J. A. and Walton J. D. (1992) The cyclic peptide synthetase catalyzing HC-toxin production in the filamentous fungus *Cochliobolus carbonum* is encoded by a 15.7-kilobase open reading frame. J. Biol. Chem. **267:** 26044–26049
154 Walton J. D., Akimitsu, K., Ahn, J. H. and Pitkin, J. W. (1994) Towards an understanding of the TOX2 gene of *Cochliobolus carbonum*. In: Host-Specific Toxin: Biosynthesis, Receptor and Molecular Biology, pp. 227–237, Kohmoto K. and Yoder O. C. (eds) Tottori University Press, Tottori Japan
155 Walton J. D., Earle E. D., Stahelin H., Grieder A., Hirota A. and Suzuki A. (1985) Reciprocal biological activities of the cyclic tetrapeptides chlamydocin and HC-toxin. Experientia **41:** 348–350
156 Closse A. and Huguenin R. (1974) [Isolation and structural clarification of chlamydocin]. Helv. Chim. Acta **57:** 533–545
157 Shimada J., Kwon H. J., Sawamura M. and Schreiber S. L. (1995) Synthesis and cellular characterization of the detransformation agent, (–)-depudecin. Chem. Biol. **2:** 517–525
158 Oikawa T., Hasegawa M., Shimamura M., Ashino H., Murota S. and Morita I. (1991) Eponemycin, a novel antibiotic, is a highly powerful angiogenesis inhibitor. Biochem. Biophys. Res. Commun. **181:** 1070–1076
159 Oikawa T., Onozawa C., Inose M. and Sasaki M. (1995) Depudecin, a microbial metabolite containing two epoxide groups, exhibits anti-angiogenic activity in vivo. Biol. Pharm. Bull. **18:** 1305–1307
160 Archer S. Y. and Hodin R. A. (1999) Histone acetylation and cancer. Curr. Opin. Genet. Dev. **9:** 171–174
161 Marks P. A., Richon V. M. and Rifkind R. A. (2000) Histone deacetylase inhibitors: inducers of differentiation or apoptosis of transformed cells. J. Natl. Cancer Inst. **92:** 1210–1216
162 Carmen A. A., Rundlett S. E. and Grunstein M. (1996) HDA1 and HDA3 are components of a yeast histone deacetylase (HDA) complex. J. Biol. Chem. **271:** 15837–15844