Biochemical Journal Immediate Publication. Published on 12 Nov 2002 as manuscript BJ20021321

HISTONE DEACETYLASES: CHARACTERISATION OF THE CLASSICAL HDAC FAMILY

Annemieke J.M. de Ruijter, Albert H. van Gennip, Huib N. Caron, Stephan Kemp, André B.P. van Kuilenburg*

Academic Medical Centre, University of Amsterdam, Laboratory Genetic Metabolic Diseases, Department of Paediatrics/Emma Children's Hospital and Clinical Chemistry, PO Box 22700, 1100 DE Amsterdam, The Netherlands

* Corresponding author: Dr. A.B.P. van Kuilenburg

Academic Medical Centre

Lab. Genetic Metabolic Diseases, F0-224

Meibergdreef 9, 1105 AZ Amsterdam, The Netherlands

Tel: +31 20 5665958

Fax: +31 20 6962596

e-mail: a.b.vankuilenburg@amc.uva.nl

Abbreviations list

14-3-3	phosphodependent signalling chaperone
AML1	Acute myelocytic leukaemia protein 1
BCoR	BCL-6 interacting Co-Repressor
BRCA1	Breast cancer associated susceptibility protein 1
CHAPs	cyclic hydroxamic acid containing tetrapeptides
Co-repressor	Protein that is involved in HDAC complex, helping to repress gene expression
CRM1	Cellular export factor for proteins with a leucine-rich NES
CtBP	C-terminal Binding Protein
CtBP	C-terminal binding protein, transcriptional coactivator
DLT	Dose Limiting Toxicity
DNMT1	DNA Methyl Transferase 1
E2F	Transcription factor with crucial function in cell cycle
ER	Estrogen Receptor, estrogen dependent DNA binding transcription factor, nuclear receptor
GATA-2	Zinc-finger transcription factor 2
GR	Glucocorticoid Receptor, glucocorticoid dependent DNA binding transcription factor, nuclear receptor
HDAC	Histone deacetylase
HDACi	Histone deacetylases Inhibitor
HDRP	HDAC related protein
Ikaros	Zinc-finger DNA binding protein
LHR	Luteinizing Hormone Receptor
MBD3	Methyl-CpG-binding domain containing protein 3
MeCP-1	Methylated CpG binding protein-1
MEF2	Myocyte enhancer factor 2, transcription factor
Mi-2	ATP-dependent nucleosome remodeling factor
MITR	MEF2 interacting transcriptional repressor
Miz-1	Myc Interacting zinc-finger protein 1
mSin3a	mammalian Sin3a
MTA2	Metastasis associated protein 2
Myc	transcription factor, part of MAD/MAX network regulating transcription
N-CoR[4]	nuclear receptor co-repressor
NES	Nuclear export signal
NLS	Nuclear localisation signal
NuRD	Nucleosome remodelling histone deacetylase
PML	Promyelocytic leukaemia
RAR	Retinoic acid Receptor (α, β), retinoic acid dependent DNA binding transcription factor, nuclear receptor
Rb[1]	Retinoblastoma protein, cell cycle protein, well known oncogene
RBP-1	Rb binding protein-1
Rbp46 [48]	Rb binding protein 46 [48]
SAGE	Serial Analysis of Gene Expression
SAP 18 / 30	Sin3 associated proteins 18 / 30
SMRT[4]	silencing mediator for retinoic acid and thyroid hormone receptors
Sp-1[3]	Zinc-finger containing DNA binding transcription factor
Suv39H1	histone methyl transferase
TBL1	Transducin β–like protein 1
TFII-I	Transcription factor
TPX	Trapoxin, HDACi
TR	Thyroid hormone Receptor
TSA	Trichostatin A, HDACi
YY1[2]	Ying-Yang 1, cellular nuclear matrix regulatory protein (alias δ, NF-E1, UCRBP)

Biochemical Journal Immediate Publication. Published on 12 Nov 2002 as manuscript BJ20021321

Abstract

Transcriptional regulation in eukaryotes occurs within a chromatin setting and is strongly influenced by posttranslational modification of histones, the building blocks of chromatin, such as methylation, phosphorylation and acetylation. Acetylation is probably the best understood type of these modifications where hyper-acetylation leads to an increase in expression of particular genes and hypo-acetylation results in the opposite effect. Many studies have identified several large, multisubunit enzyme complexes responsible for the targeted deacetylation of histones. The aim of this review is to give a comprehensive overview on the structure, function and tissue distribution of the classical HDAC family members in order to gain insight in the regulation of gene expression through HDAC activity. SAGE data show that HDACs are generally expressed in almost all tissues investigated. Surprisingly, no major differences were observed between the expression pattern in normal tissues and malignant tissues. However, significant intra-variation in HDAC expression was observed within tissue types. HDAC inhibitors have been shown to induce specific changes in gene expression and a variety of other processes including growth arrest, differentiation, cytotoxicity and induction of apoptosis. This challenging field has generated many fascinating results which will ultimately lead to a better understanding of the mechanism of gene transcription as a whole.

Keywords: gene expression, tissue distribution, co-repressor, histone deacetylase inhibitor, gene-expression.

Biochemical Journal Immediate Publication. Published on 12 Nov 2002 as manuscript BJ20021321

Introduction

Transcription in eukaryotic cells is influenced by the manner in which DNA is packaged (1). In resting cells, DNA is tightly compacted to prevent accessibility of transcription factors. DNA is packaged into chromatin, a highly organised and dynamic protein-DNA complex. The fundamental subunit of chromatin, the nucleosome is composed of an octomer of four core histones, an H3-H4 tetramer and two H2A-H2B dimers, surrounded by 146 bp of DNA (fig. 1) (2,3). Local chromatin architecture is now generally recognised as an important factor in the regulation of gene expression. During activation of gene transcription, this compact inaccessible DNA is made available to DNA-binding proteins via modification of the nucleosome (2). This architecture of chromatin is strongly influenced by post-translational modifications of the histones. Compared to methylation and phosphorylation, the acetylation of core histones is probably the best understood type of these modifications. Histone acetylation occurs at the epsilon amino groups of evolutionarily conserved lysine (K) amino acids located at the N- termini. All core histones are acetylated *in vivo*: modifications of histones H3 and H4 are, however, much more extensively characterised than H2A and H2B (1). Important positions for acetylation on H3 are K9 and K14 and on H4 positions K5, K8, K12, K16 (fig. 1) (4). Steady-state levels of acetylation of the core histones result from the balance between the opposing activities of histone acetyl-transferases (HAT) and histone deacetylases (HDAC) (1). In general, increased levels of histone acetylation (hyperacetylation) are associated with increased transcriptional activity whereas decreased acetylation levels (hypoacetylation) are associated with repression of gene expression (fig.1) (1,2,5) .

The fact that acetylation is a key component in the regulation of gene expression has stimulated research in studying HDACs in combination with the aberrant gene expression often observed in cancer. Although no direct alteration in the expression of HDACs has yet been demonstrated in human onco-genesis, it is now understood that HDACs associate with a number of well-characterised cellular oncogenes and tumour-supressor genes (e.g. MAD and Rb[1]) leading to an aberrant recruitment of HDAC activity which in turn results in changes in gene expression (6,7). In acute promyelocytic leukaemia (APL) for example, the onco-protein, produced by the fusion of the PML (promyelocytic leukaemia) gene and retinoic acid receptor α (RARα) gene, appears to suppress transcription of specific genes through recruitment of HDACs. In this manner, the cancer cell is unable to undergo differentiation, leading to excessive proliferation (8-10). Similar phenomena have been described for RARα-PLZF fusion, AML1-ETO fusion and also in the MYC/MAD/MAX signalling pathway involved in solid malignancies (11-14). From the previous section it is clear that HDAC enzymes seldom operate alone. Many proteins, with various functions like recruitment, co-repression or chromatin remodelling, are involved in forming a complex that results in the repressor complex shown in fig. 1. The most important signal involved in the initiation process of repression is situated in the DNA itself. Methyl groups bound to the cytosine residues situated 5' to guanosines in DNA, in so called CpG islands, are directly responsible for the recruitment of the HDAC complex via proteins like Methylated-CpG-binding proteins (MeCpG) and Methyl-CpG-binding domain containing proteins (MBD) or via the enzymes that methylate the CpG islands, the DNA methyltransferases (DNMT). The methyl groups provide the basis for epigenetic gene silencing like imprinting and X chromosome inactivation, since a high degree of methylated CpG will result in a loss of expression of the gene encoded by this stretch of DNA. Although it seems that HDAC could be solely

responsible for the repression of gene transcription via the recruitment to methylated CpG, this is not the case. When HDAC activity is inhibited, the transcription of the gene under study is not always (completely) restored (15-21).

In addition to deacetylation of histones, other proteins can also be deacetylated by HDACs including p53[1], E2F[1], α-tubuline and MyoD illustrating the complex function of HDACs in many processes in the cell (22,23).

Over the years many different types of HDAC inhibitors have been developed, ranging from complicated structures of bacterial or fungal origin (Trichostatin A, Trapoxin) to the very simple butyrate. HDACi are capable of inhibiting HDACs with varying efficiency (from nM to mM range). Inhibition of HDAC can result in a general hyperacetylation of histones followed by transcriptional activation of certain genes through relaxation of DNA conformation (24). Generally, HDACi are known to be able to induce growth arrest, differentiation or apoptosis of cancer cells *in vitro* and *in vivo* (2,10,24-33). DNA micro-arrays using malignant cell lines cultured in the presence of a HDACi, showed a specific small number of genes (1-7%) of which the expression was altered (10,27,34,35). Thus, the effect of HDACi on gene expression is believed not to be a general one but rather the alteration of the transcription of a specific subset of genes (18,20,21).

The aim of this review is to give a comprehensive overview on the structure, function and tissue distribution of the classical HDAC family members in order to gain insight in the regulation of gene expression through HDAC activity. Knowledge about the regulation of HDAC activity, the expression level and tissue distribution is crucial in order to come to a better understanding of changes in gene expression for example during development or in malignancy. In this review we made an assessment of the

tissue distribution of HDACs by using SAGE databases on the internet. In addition, a brief overview is given on HDAC inhibitors (HDACi) and on the genes currently known to respond to HDACi treatment.

General overview

Classification

There are two protein families with histone deacetylase (HDAC) activity: the recently discovered SIR2 family of NAD-dependent HDACs (this family will not be subject of discussion in this review) and the classical HDAC family. The classical HDAC family members fall into two different phylogenetic classes, namely class I and II (fig. 2) (4,36). The class I HDACs (HDAC1, 2, 3, and 8) are most closely related to the yeast (Saccharomyces *cerevisiae*) transcriptional regulator RPD3. Class II HDACs (HDAC4, 5, 6, 7, 9, and 10) share domains with similarity to HDA1, another deacetylase found in yeast (4). Recently a new member of the HDAC family was identified, HDAC11. The protein does contain all the necessary features to be designated as HDAC and although it is tempting to conclude from figure 2 and 3 that HDAC11 is most related to the class I HDACs, no classification of HDAC11 to class I / II or SIR2 family could be made since the overall homology was to limited (37). Currently, it is thought that HDACs of class I are expressed in most cell types, whereas the expression pattern of class II HDACs is more restricted (table 3), suggesting that they might be involved in cellular differentiation and developmental processes (38,39).

Localisation

To exert their function HDACs need to be in the nucleus where the predominant substrate is found. Nuclear localisation of HDACs is arranged via a nuclear localisation signal (NLS) or via co-localisation together with other proteins/HDACs. Most HDACs contain a NLS but some HDACs can be cytosolic as well: this depends on other regulatory domains. Class I HDACs are found almost exclusively in the cell nucleus (figure 4). HDAC1 and 2 localisation is nuclear resulting from a lack of nuclear export signals (NES) (19). HDAC3, however, has both a nuclear import and an export signal suggesting that HDAC3 can also localise to the cytoplasm. Interestingly, HDAC3 is nearly always localised in the nucleus in the experiments described so far which in part might by explained by the recruitment of the HDAC3 complex by HDAC4, 5 and 7 when they are bound to the DNA via co-repressors (table 1) (36,40,41). This possibly indicates a cell type specific domination of one signal over the other (41). After over-expression of HDAC8, due to its low abundance, it could be demonstrated that the protein was localised in the nucleus (42).

Class II HDACs are able to shuttle in and out of the nucleus upon certain cellular signals as can be seen in panel B and C from figure 4. Panel B shows that the predominant localisation of HDAC6 is in the cytoplasm (22,43). HDAC11 resides in the nucleus but in activity assays, however, HDAC11 co-precipitates with the cytoplasmic localised HDAC6 (37,43). HDAC10 can be localised in both the nucleus and the cytoplasm although the function of the localisation in both compartments has not been clarified yet (44,45). The subcellular localisation of HDAC9 can be cytosolic

as well as nuclear depending on splice variant (43,46). Panel C of figure 4 shows that HDAC4, 5 and 7 localisation is a carefully regulated process. The shuttling of HDAC4, 5 and 7 between the cytosol and the nucleus has been extensively studied in differentiating muscle cells resulting is a clear model (36,40,47). Due to a (pre-) differentiating signal HDAC4 is phosphorylated by CaMK resulting in export of HDAC4 together with CRM1. 14-3-3 (cytosolic 'anchor' protein) binds the phosphorylated form of HDAC4 and thereby achieves retention of HDAC4 in the cytosol. After fusion of muscle cells, terminal differentiation (post-differentiation) has been reached and as a result HDAC4 will be released from 14-3-3 due to a decrease in phosphorylation status. HDAC4 will consequently shuttle back to the nucleus. HDAC5 resides in the nucleus during proliferation of muscle cells (pre-differentiation) and is triggered to go from the nucleus to the cytoplasm during differentiation. A mediator involved in this compartmental change of HDAC5 is believed to be CaMK (the 14-3-3 consensus binding domain is present) although since HDAC5 also has a NES domain it could not be confirmed that CaMK is solely responsible for the transport of HDAC5 out of the nucleus. In summary the shuttling of HDAC5 takes place in opposite direction and on a different time schedule when compared to that of HDAC4. Both HDAC4 and 5 initially reside in the same compartment but end up in the cytosol and the nucleus respectively. HDAC7 has a very high degree of homology with HDAC5 except HDAC7 does not have a NES domain. Like HDAC5, HDAC7 is able to shuttle from the nucleus to the cytoplasm during muscle cell differentiation indicating that calcium signalling (14-3-3 binding) might also be involved here. In other cell types than the muscle cells we used as a model the regulation of the localisation of HDAC5 and 7 is less clear. In general, the localisation of HDAC7 seems to depend on the presence of CaMK and 14-3-3 (other unknown proteins might be needed as well). When CaMK and 14-3-3 are present HDAC7

resides in the cytoplasm, if both CaMK and 14-3-3 are not expressed HDAC7 is found in the nucleus. However, even though there is a high amount of resemblance in function and localisation between HDAC5 and 7, in different cell lines both enzymes can be found in the nucleus (CV-1, MCF 7) while in other cell lines HDAC5 is located in the nucleus and HDAC7 is localised in the cytosol (HepG2). The presence of the NES domain on HDAC5 might have a dominant role in determining the localisation of HDAC5 in these cell lines but clearly more research is needed to clarify these contradicting findings (43,48,49). The chromosomal localisation of the HDACs is shown in table 2.

Mechanism of action

The mechanism of action of the histone deacetylase enzyme exists of removing the acetyl-group from the histones composing the nucleosome. Hypoacetylation results in a decrease in the space between the nucleosome and the DNA that is wrapped around it (fig. 1). The tighter wrapping of the DNA diminishes the accessibility for transcription factors, consequently leading to transcriptional repression (fig. 1) (1,3,50). The catalytic domain of HDAC is formed by a stretch of ~390 amino acids consisting of a set of conserved amino acids. The active site consists of a lightly curved tubular pocket with a wider bottom (51). The removal of the acetyl-group occurs via a charge-relay system consisting of two adjacent histidine amino acids, two aspartic amino acids, located approximately 30 amino acids from the histidine amino acids and spaced by approximately 6 amino acids, and one tyrosine amino acid, approximately 123 amino acids downstream from the aspartic amino acids (38,51). An essential component of the charge-relay system is the presence of a Zn^{2+}

atom. This atom is bound to the zinc-binding site on the bottom of the pocket. Before HDAC activity can occur, however, other co-factors are needed (table 1): most recombinantly expressed enzymes are found to be inactive. Inhibitors of HDAC activity function by displacing the zinc atom and thereby rendering the charge-relay system dysfunctional. Trichostatin A (TSA) with its hydroxamic acid group and its five-carbon atom linker to the phenyl group has the optimal conformation to fit in the active site (51). TSA is currently the most potent reversible HDACi, with an IC_{50} in low nM range (52). All HDACs are currently thought to be approximately equally sensitive to inhibition by TSA (19,32,50,53).

The class I HDACs

HDAC1 and HDAC2

HDAC1 and HDAC2 are highly homologous enzymes, with an overall protein identity of ~82 % (fig. 2). The catalytic domain on the N-terminus forms the major part of the protein (fig. 3) (1,6,54,55). HDAC1 and HDAC2 are inactive when produced with recombinant techniques implicating that cofactors are necessary for HDAC activity to occur. *In vivo* HDAC1 and 2 only display activity within a complex of proteins. These complexes consist of proteins necessary for modulating their deacetylase activity and for binding DNA together with proteins mediating the recruitment of HDACs to the promoters of genes (56). Three protein complexes have been characterised that contain both HDAC1 and HDAC2: Sin3, NuRD and Co-REST (table 1). Both the Sin3 (named after its characteristic element mSin3A) and the NuRD (Nucleosome Remodelling and Deacetylating) complex consist of a core complex containing HDAC1, HDAC2, RbAp48 (binds H4 directly) and RbAp46. The core complex alone

does not possess maximal HDAC activity, additional cofactors are needed (table 1) (39,56-59). Besides functioning through these complexes, HDAC1 and HDAC2 can also bind directly to DNA binding proteins such as YY1[2], RBP-1 and Sp1[3] (2,50,54,55,60-64). In addition to the regulation of HDAC1 and 2 activity by the availability of co-repressors, a second way of regulating activity is via posttranslational modification. Both activity and complex formation are regulated by phosphorylation. HDAC1 and 2 are phosphorylated at a low steady-state level in resting cells. Hyperphosphorylation of HDAC1 and HDAC2 leads to a slight but significant increase in deacetylase activity but at the same time to disruption of complex formation between HDAC1 and HDAC2 and between HDAC1 and mSin3A / YY1[2]. When hypophosphorylation of HDAC1 and 2 occurs the activity of HDAC1 and 2 decreases but complex formation is increased. The apparent contra productive consequences of phosphorylation maintains the HDAC activity at a certain optimal level. Mutational analysis for HDAC1 shows that Ser^{421} and Ser^{423} are crucial phosphorylation sites, when mutated, complex formation is hampered and HDAC activity reduced (39,47).

HDAC3

HDAC3 is evolutionary most related to HDAC8 with 34% overall identity and HDAC3 has the same domain structure as all the class I HDACs (fig. 2, 3). In HDAC1 and 2, the regions that correspond to amino acids 181-333 of HDAC3, are very similar to each other (93% identity). However, the corresponding region in HDAC3 only has 68% identity to HDAC1 and 2. Surprisingly, the non-conserved C-terminal region of HDAC3 is required for both deacetylase activity and transcriptional repression. Besides the nuclear localisation signal (NLS) that other class I HDACs possess, a

Nuclear Export Signal (NES) is also present in HDAC3 (amino acids 180-313). The balance between both these counteracting signals is probably dependent on cell type and on environmental conditions (41). HDAC3 shares structural and functional features with other class I HDACs but it exists in multi-subunit complexes different from other known HDAC complexes. This could implicate that individual HDACs have distinct functions due to their complex specificity. For HDAC3 activity, SMRT[4] and N-CoR[4] are found to be necessary factors (table 1). Both SMRT and N-CoR have a conserved DAD (deacetylase activating domain) for HDAC3 activation (65). HDAC3 is able to form oligomers *in vitro* and *in vivo* with other HDACs (41). Using over-expression coupled to immuno-precipitation, it was shown that HDAC3 can co-precipitate with HDAC4, 5, and 7 through the complex formation with SMRT and N-CoR (40,41). Endogenous HDAC3 mostly associates with itself and only a small fraction of HDAC3 interacts with HDAC4. HDAC3 can be found in complex with HDRP as well (see HDAC9) (36,55,66,67). HDAC3 shares the ability of HDAC1 to mediate Rb[1]-RbAp48 interaction suggesting a mediating role of these HDACs in the cell cycle process. HDAC3, however, does not interfere with Rb[1]-E2F activity in contrast to HDAC1 and 2 (table 1) (57,68).

HDAC8

Of all the class I HDACs, HDAC8 is most similar to HDAC3 (34% identity, fig. 2). HDAC8 consists largely of the catalytic domain with an NLS in the centre (fig.3) (32,38,43,50). Due to its very recent discovery it is not known yet whether HDAC8 function is regulated by a co-repressing complex of proteins. Two different transcripts of 2.0 Kb and 2.4 Kb have been found. Discrepancies with respect to the chromosomal localisation existed as the gene was mapped to Xq21.2-Xq21.3 and

Xq13 although the latter is considered to be correct (table 2). Northern analysis, after lengthy incubation, revealed a varying range of HDAC8 expression in several tissue types suggesting a very low abundance of HDAC8 mRNA (38,69).

The class II HDACs

HDAC4, HDAC5, and HDAC7

HDAC4, 5, 7 are found in the same region of the phylogenetic tree and represent a subgroup within the class II HDACs. HDAC4 and 5 are most similar to each other (overall similarity 70 %) but HDAC7 is also close related (~58 and ~57 % overall similarity respectively) (fig. 2). All three HDACs have the catalytic domain on the C-terminal half of the protein and the NLS is closely situated to their N-terminus (fig. 3). Binding domains for CtBP, MEF2 and 14-3-3 are conserved in all three HDACs on the N-terminus (1,43). Furthermore HDAC5 has a NES within the catalytic domain suggesting nuclear-cytoplasmic trafficking. HDAC4, 5 and 7 are able to interact with SMRT/N-CoR, co-repressors BCoR and CtBP (table 1).

The N-termini of HDAC4, 5 and 7 specifically interact with and repress the myogenic transcription factor MEF2. MEF2 plays an essential role, as a DNA binding transcription factor, in muscle differentiation (70). When MEF2 is associated with HDAC4, 5, or 7 the function of MEF2 as a transcription factor is inhibited, thus blocking muscle cell differentiation. Ca2+/calmodulin dependent kinase (CaMK) activity overcomes this inhibition by dissociating MEF2-HDAC due to phosphorylation of HDAC4/5/7. Subsequently, transport (with CRM1) of the HDAC out of the nucleus can occur illustrating another type of regulation of HDAC activity (65,71,72).

Interestingly, HDAC4, 5, and 7 associate with HDAC3 *in vivo* via co-recruitment to the SMRT/N-CoR factors in the nucleus (table 1) with absence of HDAC3 leading to inactivity. This suggests that HDAC4, 5, and 7 function as a link between DNA binding recruiters and the HDAC3 containing HDAC complex (36,40,41).

The subcellular localisation of HDAC5 and 7 is different to that of HDAC4 at the different stages of muscle cell differentiation (fig.4). They might compliment each other in order to arrange the differential regulation of gene expression during the various stages of differentiation in muscle cells. These three HDACs are able to fine tune the repression of gene expression due to the need for co-repressors for their activity but even more intriguing via their ability to change localisation upon a certain signal thus providing a carefully regulated sequence of changes in gene expression during differentiation (32,36,36,40,43,46,48-50,54,63,72-74).

HDAC6

The phylogenetic tree shows that HDAC6 is evolutionary most related to HDAC10 (fig. 2). In general though the homology of HDAC6 with the other human deacetylases is low, with some resemblance to the yeast HDA1 (Saccharomyces *cerevisiae*) indicating an early separation from the other HDACs in evolution. HDAC6 is a rather unique enzyme within the classical family of HDACs because it contains two catalytic domains arranged in tandem (fig. 3) (32,50). Another unique feature of HDAC6 is the presence of a HUB (HDAC6-, USP3-, and Brap2-related zinc finger motif) domain on the C-terminus. This domain is a signal for ubiquitination suggesting that this HDAC is particularly prone to degradation (43). The catalytic domains of HDAC6 are most similar to the catalytic domain of HDAC9. HDAC6 functions as a

tubulin deacetylase regulating micro-tubule-dependent cell motility (22) and although residing predominantly in the cytoplasm (NES) to exert its function, HDAC6 is also found in the nucleus in a complex together with HDAC11. The function of HDAC6 has hardly been studied in comparison to the other HDACs while HDAC 6 seems to have so many special and interesting features (32,42,43,50,75).

HDAC9

The phylogenetic tree in figure 3 shows that HDAC9 splice variants are clustered as a separate group related to HDAC4/5/7 within class II of the classical HDAC family. HDAC9 catalytic domain is located on the N-terminus like the class I HDACs. There are 3 known splice variants: HDAC9a, 9b and HDRP/ 9c but more variants are suspected (fig. 2, 3) (46). HDAC9c/HDRP lacks the catalytic domain and is 50% similar to the N terminus of HDAC4 and 5. In analogy with HDAC4, 5 and 7 HDRP is able to recruit HDAC3, thus circumventing the lack of a catalytic domain. In addition to that HDAC9 is also able to interact with MEF2 (CaMK / 14-3-3) indicating that HDAC9 may have an important function in muscle differentiation. The alternative splicing might also represent another way of fine tuning HDAC activity. Certain cell types might express one type of isoform while others express the other (43,46,67).

HDAC10

HDAC10 is the most recently discovered member of the class II HDACs. Two mRNA species, with a slight difference in length, have been found suggesting the existence of two splice variants of HDAC10 (44). Analysis of protein sequence homology shows that HDAC10 is mostly related (37% overall similarity) to HDAC6 (fig. 2) (75).

HDAC10 has a catalytic domain on its N-terminus and a NES and a putative second catalytic domain on the C-terminus. Also two putative Rb[1] binding domains have been found on HDAC10 suggesting a role in the regulation of the cell cycle. Furthermore, HDAC10 is found to interact with HDAC1, 2 and 3 (and/or SMRT) and HDAC4, 5 and 7 but not HDAC6 although some contradicting results are presented in literature. The fact that HDAC10 is able to associate with many other HDACs indicates that HDAC10 might function as a recruiter rather than deacetylate itself. However, when expressed by recombination HDAC10 alone does show deacetylating activity (44,45,75,76).

HDAC11

From the phylogenetic analysis it appears that HDAC11 is most closely related to HDAC3 and 8 suggesting that HDAC11 might be more related to the class I HDACs than to the class II HDACs (fig. 2). The classification of HDAC11, however, has not yet been determined since the overall sequence homology to the other HDACs is limited. HDAC11 contains a catalytic domain situated at the N-terminus (fig. 3), with proven HDAC activity that could be inhibited by TPX (a TSA analogue). HDAC11 was found not to reside in any of the known HDAC-complexes (Sin3, N-CoR/SMRT) possibly indicating a biochemical distinct function of HDAC11 (37).

Tissue distribution of HDACs

We used SAGE data from AMC Human transcriptome Map, available from the internet, to generate a first indication on the tissue distribution of HDACs in normal and tumour tissue (77). In table 3 a general overview of HDAC expression specified

per tissue type is given. The numbers indicate the amount of mRNA molecules that were found standardised to 100 000 tags. To enhance the reliability of the data it will be necessary to verify these expression profiles by Northern analysis. HDAC11 expression was not included in the table since no unigene cluster number or SAGE tag (table 2) are available yet.

The SAGE data on HDAC1, 2, 3, 5, 6, 7, and 10 suggests that they are more or less generally expressed in all the tissues examined. HDAC8 and 9 seem to be expressed more in tumour tissues than in normal tissues, this feature is even more striking for HDAC4. The lack of HDAC4 expression in normal somatic tissue could suggest that HDAC4 is not essential. The role of HDAC4 in muscle differentiation could, however, also suggest that HDAC4 is only expressed in embryonic muscle tissue which are lacking from the databases. Unlike HDAC4, HDAC5 is expressed in heart tissue together with expression of HDAC7 and HDAC9, which is in agreement with their proposed function in muscle tissue. Surprisingly HDAC expression in tumour tissue was only slightly higher than in normal tissue (37 increased, 13 decreased and 7 remained equal). Using Northern analysis it has been shown that nearly all HDACs have been found in many more other tissue types as well (for reference see individual chapters). Northern-analysis is a much more sensitive technique than SAGE indicating that the expression of HDACs is generally rather low. Rare transcripts have a slight chance of not being present in a SAGE database due to a limited amount of tags sequenced. The SAGE data show that HDAC expression is low and rather uniform and that HDACs are almost equally well expressed in normal tissue and tumour tissue. A comparison between nine cell lines/primary tumour SAGE databases of neuroblastoma showed a large variation in

HDAC expression within this tumour category ranging from no detectable HDAC expression to 31 tags / 100 000 HDAC2 (data not shown).

It has also been reported that HDAC expression can change under the influence of HDACi and cell density (78,79). Furthermore since HDAC6 and HDAC8 are located on chromosome X, a chromosome known for its ability to undergo X-inactivation via deacetylation, it might be interesting to find out whether these HDACs are subjected to or directly involved in X-inactivation (80). Studies using overexpression and deletion of RPD3 and HDA1 (class I and class II representatives) in yeast showed differences in the specificity concerning the deacetylation of lysine positions of histone H3 and H4. This might indicate that through differences in the expression of the HDACs (class I and II) in various cell types, a different acetylation pattern could occur resulting in different transcriptional regulation (81,82).

HDAC inhibitors (HDACi)

It is currently thought that many HDACi function via blocking access to the active site (reversible or irreversible) of HDAC. There are many inhibitors known today but the most potent one discovered so far is Trichostatin A (TSA). TSA is a fermentation product of Streptomyces. Originally TSA was used as an anti-fungal agent but later it was discovered to have potent proliferation inhibiting properties on cancer cells. TSA belongs to the group of hydroxamic acids and is effective at nM concentrations in *in vitro* experiments (see table 4). The ability of TSA to inhibit HDAC has been reported in 1990 for the first time by Yoshida *et al.* 1990 (52). Because the production of TSA is costly and highly inefficient (20 steps resulting in a 2% yield) the search for alternative HDACi is ongoing and of high importance (83,84).

Today TSA is used mainly as a reference substance in research for newly developed HDACi. Many related compounds belonging to the hydroxamic acid group have been developed but Oxamflatin is the only compound in this group with similar *in vitro* potency as compared to TSA as can be seen in table 4.

Another well-known group of HDACi are short-chain fatty acids like butyrate (NaBu), phenylbutyrate (PBA) and valproic acid. These compounds are far less efficient in their HDAC inhibiting capability when compared to TSA (mM versus nM range). Cyclic tetrapeptide antibiotics are the third group of inhibitors and these compounds are characterised by their complicated structure and their high HDAC inhibitory potential. Most of these compounds are products of bacteria or fungi but Apicidine and Depsipeptide are composed of a chemically engineered combination of hydroxamic acids and cyclic tetrapeptides, as is Oxamflatin. Benzamides including CI-994 (N-acetyl-dinaline) and MS-275 is the last group of established HDACi. Although the result of these compounds in inhibiting HDAC activity is considered to be the same, there are two exceptions where the mechanism of inhibition is concerned. All the HDACi inhibit HDAC in a reversible fashion except for TPX and Depudesin, they inhibit the enzyme irreversibly through a different mechanism namely via covalent binding to the epoxyketone group (32,83,85). The current vision on HDACi is that almost all HDACs are approximately equally sensitive to the different HDAC inhibitors. Exceptions are HDAC6 and HDRP because of their ‘abnormal’ amount of catalytic domains. It has been reported that HDAC6, comprised of a double catalytic domain, is sensitive to one (TSA, reversible) but resistant to inhibition by another inhibitor (TPX, irreversible) (83). Furthermore, it was discovered that class II HDACs are five times less susceptible to inhibition by valproic acid than class I HDACs and also HDAC4 was reported to be less sensitive to inhibition by butyrate. The development of new HDACi targeted at a specific HDAC, together with

the tissue distribution of the subtypes, might make a tailored use of HDACi possible (26,43).

Some of the compounds mentioned above have been entered into clinical trails (butyrate, phenylbutyrate, Depsipeptide, Pyroxamide, SAHA, valproic acid, CI-994). In many studies an increase of histone acetylation occurred which resulted in a partial response in some patients but also in few patients a complete response was achieved. The proposed mechanism consists of the re-expression of silenced genes and / or silencing of downstream genes due to regained availability of those promoters to other modulating factors (8,9,31,32,85-93). The intriguing results achieved in these clinical trails might contribute greatly in enhancing our understanding of the function of HDACs although little is known yet about the specific function of the individual HDACs and the specific consequences of using HDACi on normal cells. Therefore, studies investigating the mechanism of action of all the HDACs plus the global effects of using HDACi are warrented.

Responsive Genes

By using HDACi in *in vitro* model systems effects on the expression of many genes have been documented. The general effect often seen in cell lines is cytotoxicity, differentiation, inhibition of proliferation and induction of apoptosis. The mechanism via which gene expression changes through HDAC inhibition are thought to be taking place is via an increased acetylation resulting in the increased recruitment of DNA binding transcription factors which in turn leads to the increase of expression of particular genes and the decrease in expression of other (downstream) genes. It has been described extensively that the amount of acetylated histones (H4 or H3)

increased in the presence of HDACi as can be seen with some other acetylated proteins (e.g. p53, YY1[2]). For example the increased expression of p21 and LHR is realised only partly by a general increase in acetylation, Sp1[3] sites in the promoter seem obligatory for reaching full induction of expression after HDACi treatment. Possibly because HDAC1/2 can directly bind to Sp1 but also because Sp1 sites are able to protect against DNA methylation resulting in a more transcription-ready state of the promoter (16,94,95). On the other hand, the possibility of a direct response via other (unknown) DNA binding proteins that would use HDACi as a ligand could not be ruled out yet (table 5). The expression, either increased or decreased, of the genes mentioned in table 5 are the result of inhibition of HDACs. It is clear that by studying the effect of HDACi on the expression of different genes and their regulatory pathways, a more detailed picture will emerge as to how the inhibition of HDACs, combines with the HDAC expression profile of that cell, and ultimately determines the fate of the cell. Since it is not always entirely clear yet which sequence of events cumulated in the remission of disease in one patient and not in others the results of *in vitro* studies will be indispensable. They will help in developing a better understanding of the mechanisms involved (10,24-30,34,51,59,79,83,93,96-112).

Concluding remarks

HDACs appear to be key enzymes in the regulation of gene expression. HDAC function seems to be regulated by its intrinsic features, its abundance, cellular compartmentalisation and association with cofactors. Every cell type requires their specific gene-expression pattern, thus pre-scribing a certain need of HDAC expression. Surprisingly, cellular transformation and onco-genesis did not result in

major changes in HDAC expression. In the tumour tissues we examined, at least one HDAC proved to be present. The intra-tissue variation in HDAC expression might underlie the great variation in response noted so far in *in vitro* studies and in clinical trials with HDACi. A wide variety of processes are associated with inhibition of HDAC such as apoptosis, necrosis, differentiation, inhibition of proliferation and cytostasis. Also drug resistance can be overcome and restoration of expression of silenced genes is known to occur. Although little studies have been performed with normal healthy cells, animal experiments and clinical trials report little or no side effects within the therapeutic range of the tested HDACi (8,31,98,101-103). Last but not least the efficiency of HDACi is much dependent on their *in vivo* stability. Most HDACi are not very stable due to their innate structure but also they can be readily degraded by first pass liver metabolism (50). Currently many efforts are being made to expand our knowledge on the HDACs and to develop potent and stable HDACi (113). In future this might give rise to a tailored use of HDAC specific HDACi in order to dissect the complex function of HDACs in a cell type specific manor.

Footnotes

[1] Retinoblastoma protein (Rb) is a protein that is able to negatively regulate cell growth, it controls apoptosis during development. Rb is regarded as a tumour supressor gene since loss of Rb function leads to deregulated growth and apoptosis resulting in malignancies although in cancer often more of the players in this pathway are affected. Other major players in this pathway are E2F, p53, Bax and p21 (114,115).

[2] YY1 (also known as δ, NF-E1 and UCRBP) is a sequence specific DNA-binding transcription factor that is involved in activation or repression of many genes during cell growth and differentiation, and is required for normal development of mammalian embryos (61).

[3] Sp1 was the first mammalian transcription factor to be cloned. It binds CG-boxes, CACCC-boxes and basic transcription elements in promoters of genes. Studies indicate that Sp1 is responsible for recruiting TATA-binding protein and fixing the transcriptional start site at TATA-less promoters. The fact that Sp1 sites are also found in promoters of many housekeeping genes, led to the widely supported view that Sp1 acts as a basal transcription factor that could result in constitutive expression. Sp1 is subject to extensive posttranslational modifications like glycosylation and phosphorylation (116).

[4] N-CoR (nuclear receptor co-repressor) and SMRT (silencing mediator for retinoic acid and thyroid hormone receptors) are two distinct, but highly related proteins that share similar domain structure and function. Both act as co-repressors. For more see: (43,54).

Acknowledgements

This study was supported by funds granted by the Dutch Cancer Society.

References

1. Wade PA (2001) Transcriptional control at regulatory checkpoints by histone deacetylases: molecular connections between cancer and chromatin. *Hum.Mol.Genet.* **10**: 693-8.

2. Ito K, Barnes PJ, Adcock IM (2000) Glucocorticoid receptor recruitment of histone deacetylase 2 inhibits interleukin-1beta-induced histone H4 acetylation on lysines 8 and 12. *Mol.Cell Biol.* **20**: 6891-903.

3. Strahl BD, Allis CD (2000) The language of covalent histone modifications. *Nature* **403**: 41-5.

4. Bjerling P, Silverstein RA, Thon G, Caudy A, Grewal S, Ekwall K (2002) Functional divergence between histone deacetylases in fission yeast by distinct cellular localization and in vivo specificity. *Mol.Cell Biol.* **22**: 2170-81.

5. Forsberg EC, Bresnick EH (2001) Histone acetylation beyond promoters: long-range acetylation patterns in the chromatin world. *Bioessays* **23**: 820-30.

6. Cress WD, Seto E (2000) Histone deacetylases, transcriptional control, and cancer. *J.Cell Physiol.* **184**: 1-16.

7. Timmermann S, Lehrmann H, Polesskaya A, Harel-Bellan A (2001) Histone acetylation and disease. *Cell Mol.Life Sci.* **58**: 728-36.

8. He LZ, Tolentino T, Grayson P *et al.* (2001) Histone deacetylase inhibitors induce remission in transgenic models of therapy-resistant acute promyelocytic leukemia. *J.Clin.Invest.* **108**: 1321-30.

9. Zhou DC, Kim SH, Ding W, Schultz C, Warrell RPJ, Gallagher RE (2002) Frequent mutations in the ligand-binding domain of PML-RARalpha after multiple relapses of acute promyelocytic leukemia: analysis for functional relationship to response to all-trans retinoic acid and histone deacetylase inhibitors in vitro and in vivo. *Blood* **99**: 1356-63.

10. Munster PN, Troso-Sandoval T, Rosen N, Rifkind R, Marks PA, Richon VM (2001) The histone deacetylase inhibitor suberoylanilide hydroxamic acid induces differentiation of human breast cancer cells. *Cancer Res.* **61**: 8492-7.

11. Minucci S, Nervi C, Lo CF, Pelicci PG (2001) Histone deacetylases: a common molecular target for differentiation treatment of acute myeloid leukemias? *Oncogene* **20**: 3110-5.

12. Ferrara FF, Fazi F, Bianchini A *et al.* (2001) Histone deacetylase-targeted treatment restores retinoic acid signaling and differentiation in acute myeloid leukemia. *Cancer Res.* **61**: 2-7.

13. Kitamura K, Hoshi S, Koike M, Kiyoi H, Saito H, Naoe T (2000) Histone deacetylase inhibitor but not arsenic trioxide differentiates acute promyelocytic leukaemia cells with t(11;17) in combination with all-trans retinoic acid. *Br.J.Haematol.* **108**: 696-702.

14. David G, Alland L, Hong SH, Wong CW, DePinho RA, Dejean A (1998) Histone deacetylase associated with mSin3A mediates repression by the acute promyelocytic leukemia-associated PLZF protein. *Oncogene* **16**: 2549-56.

15. Jones PA, Baylin SB (2002) The fundamental role of epigenetic events in cancer. *Nat.Rev.Genet.* **3**: 415-28.

16. Baylin SB, Herman JG (2000) DNA hypermethylation in tumorigenesis: epigenetics joins genetics. *Trends Genet.* **16**: 168-74.

17. Cameron EE, Bachman KE, Myohanen S, Herman JG, Baylin SB (1999) Synergy of demethylation and histone deacetylase inhibition in the re-expression of genes silenced in cancer. *Nat.Genet.* **21**: 103-7.

18. Yang X, Phillips DL, Ferguson AT, Nelson WG, Herman JG, Davidson NE (2001) Synergistic activation of functional estrogen receptor (er)-alpha by dna methyltransferase and histone deacetylase inhibition in human er-alpha-negative breast cancer cells. *Cancer Res.* **61**: 7025-9.

19. Johnstone R.W. (2002) Histone-deacetylase inhibitors: novel drugs for the treatment of cancer. *Nature reviews drug discovery* **vol 1**: 287-99.

20. Yan L, Yang X, Davidson NE (2001) Role of DNA methylation and histone acetylation in steroid receptor expression in breast cancer. *J.Mammary.Gland.Biol.Neoplasia.* **6**: 183-92.

21. Zhu WG, Lakshmanan RR, Beal MD, Otterson GA (2001) DNA methyltransferase inhibition enhances apoptosis induced by histone deacetylase inhibitors. *Cancer Res.* **61**: 1327-33.

22. Hubbert C, Guardiola A, Shao R *et al.* (2002) HDAC6 is a microtubule-associated deacetylase. *Nature* **417**: 455-8.

23. Juan LJ, Shia WJ, Chen MH *et al.* (2000) Histone deacetylases specifically down-regulate p53-dependent gene activation. *J.Biol.Chem.* **275**: 20436-43.

24. Suenaga M, Soda H, Oka M *et al.* (2002) Histone deacetylase inhibitors suppress telomerase reverse transcriptase mRNA expression in prostate cancer cells. *Int.J.Cancer* **97**: 621-5.

25. Pelidis MA, Carducci MA, Simons JW (1998) Cytotoxic effects of sodium phenylbutyrate on human neuroblastoma cell lines. *Int.J.Oncol.* **12**: 889-93.

26. Gottlicher M, Minucci S, Zhu P *et al.* (2001) Valproic acid defines a novel class of HDAC inhibitors inducing differentiation of transformed cells. *EMBO J.* **20**: 6969-78.

27. Della RF, Criniti V, Della P, V *et al.* (2001) Genes modulated by histone acetylation as new effectors of butyrate activity. *FEBS Lett.* **499**: 199-204.

28. Remiszewski SW, Sambucetti LC, Atadja P *et al.* (2002) Inhibitors of human histone deacetylase: synthesis and enzyme and cellular activity of straight chain hydroxamates. *J.Med.Chem.* **45**: 753-7.

29. Lavoie R, Bouchain G, Frechette S *et al.* (2001) Design and synthesis of a novel class of histone deacetylase inhibitors. *Bioorg.Med.Chem.Lett.* **11**: 2847-50.

30. Kim MS, Son MW, Kim WB, In PY, Moon A (2000) Apicidin, an inhibitor of histone deacetylase, prevents H-ras-induced invasive phenotype. *Cancer Lett.* **157**: 23-30.

31. Sandor V, Bakke S, Robey RW *et al.* (2002) Phase I Trial of the Histone Deacetylase Inhibitor, Depsipeptide (FR901228, NSC 630176), in Patients with Refractory Neoplasms. *Clin.Cancer Res.* **8**: 718-28.

32. Marks P, Rifkind RA, Richon VM, Breslow R, Miller T, Kelly WK (2001) Histone deacetylases and cancer: causes and therapies. *Nature Rev.Cancer* **1**: 194-202.

33. Kijima M, Yoshida M, Sugita K, Horinouchi S, Beppu T (1993) Trapoxin, an antitumor cyclic tetrapeptide, is an irreversible inhibitor of mammalian histone deacetylase. *J.Biol.Chem.* **268**: 22429-35.

34. Van Lint C, Emiliani S, Verdin E (1996) The expression of a small fraction of cellular genes is changed in response to histone hyperacetylation. *Gene Expr.* **5**: 245-53.

35. Mariadason JM, Corner GA, Augenlicht LH (2000) Genetic reprogramming in pathways of colonic cell maturation induced by short chain fatty acids: comparison with trichostatin A, sulindac, and curcumin and implications for chemoprevention of colon cancer. *Cancer Res.* **60**: 4561-72.

36. Fischle W, Dequiedt F, Hendzel MJ *et al.* (2002) Enzymatic activity associated with class II HDACs is dependent on a multiprotein complex containing HDAC3 and SMRT/N-CoR. *Mol.Cell* **9**: 45-57.

37. Gao L, Cueto MA, Asselbergs F, Atadja P (2002) Cloning and functional characterization of HDAC11, a novel member of the human histone deacetylase family. *J.Biol.Chem.*

38. Buggy JJ, Sideris ML, Mak P, Lorimer DD, McIntosh B, Clark JM (2000) Cloning and characterization of a novel human histone deacetylase, HDAC8. *Biochem.J.* **350 Pt 1**: 199-205.

39. Galasinski SC, Resing KA, Goodrich JA, Ahn NG (2002) Phosphatase inhibition leads to histone deacetylase 1/2 phosphorylation and disruption of co-repressor interactions. *J.Biol.Chem.*

40. Fischle W, Dequiedt F, Fillion M, Hendzel MJ, Voelter W, Verdin E (2001) Human HDAC7 histone deacetylase activity is associated with HDAC3 in vivo. *J.Biol.Chem.* **276**: 35826-35.

41. Yang WM, Tsai SC, Wen YD, Fejer G, Seto E (2002) Functional domains of histone deacetylase-3. *J.Biol.Chem.* **277**: 9447-54.

42. Van den Wyngaert I, de Vries W, Kremer A *et al.* (2000) Cloning and characterization of human histone deacetylase 8. *FEBS Lett.* **478**: 77-83.

43. Bertos NR, Wang AH, Yang XJ (2001) Class II histone deacetylases: structure, function, and regulation. *Biochem.Cell Biol.* **79**: 243-52.

44. Fischer DD, Cai R, Bhatia U *et al.* (2002) Isolation and characterization of a novel class II histone deacetylase, HDAC10. *J.Biol.Chem.* **277**: 6656-66.

45. Kao HY, Lee CH, Komarov A, Han CC, Evans RM (2002) Isolation and characterization of mammalian HDAC10, a novel histone deacetylase. *J.Biol.Chem.* **277**: 187-93.

46. Zhou X, Marks PA, Rifkind RA, Richon VM (2001) Cloning and characterization of a histone deacetylase, HDAC9. *Proc.Natl.Acad.Sci.U.S.A.* **98**: 10572-7.

47. Pflum MK, Tong JK, Lane WS, Schreiber SL (2001) Histone deacetylase 1 phosphorylation promotes enzymatic activity and complex formation. *J.Biol.Chem.* **276**: 47733-41.

48. Kao HY, Verdel A, Tsai CC, Simon C, Juguilon H, Khochbin S (2001) Mechanism for nucleocytoplasmic shuttling of histone deacetylase 7. *J.Biol.Chem.* **276**: 47496-507.

49. Dressel U, Bailey PJ, Wang SC, Downes M, Evans RM, Muscat GE (2001) A dynamic role for HDAC7 in MEF2-mediated muscle differentiation. *J.Biol.Chem.* **276**: 17007-13.

50. Yoshida M, Furumai R, Nishiyama M, Komatsu Y, Nishino N, Horinouchi S (2001) Histone deacetylase as a new target for cancer chemotherapy. *Cancer Chemother.Pharmacol.* **48 Suppl 1**: S20-S26.

51. Finnin MS, Donigian JR, Cohen A *et al.* (1999) Structures of a histone deacetylase homologue bound to the TSA and SAHA inhibitors. *Nature* **401**: 188-93.

52. Yoshida M, Kijima M, Akita M, Beppu T (1990) Potent and specific inhibition of mammalian histone deacetylase both in vivo and in vitro by trichostatin A. *J.Biol.Chem.* **265**: 17174-9.

53. Marmorstein R (2001) Structure of histone deacetylases: insights into substrate recognition and catalysis. *Structure.(Camb.)* **9**: 1127-33.

54. Kao HY, Downes M, Ordentlich P, Evans RM (2000) Isolation of a novel histone deacetylase reveals that class I and class II deacetylases promote SMRT-mediated repression. *Genes Dev.* **14**: 55-66.

55. Li J, Lin Q, Wang W, Wade P, Wong J (2002) Specific targeting and constitutive association of histone deacetylase complexes during transcriptional repression. *Genes Dev.* **16**: 687-92.

56. Zhang Y, Ng HH, Erdjument-Bromage H, Tempst P, Bird A, Reinberg D (1999) Analysis of the NuRD subunits reveals a histone deacetylase core complex and a connection with DNA methylation. *Genes Dev.* **13**: 1924-35.

57. Brehm A, Miska EA, McCance DJ, Reid JL, Bannister AJ, Kouzarides T (1998) Retinoblastoma protein recruits histone deacetylase to repress transcription. *Nature* **391**: 597-601.

58. Heinzel T, Lavinsky RM, Mullen TM *et al.* (1997) A complex containing N-CoR, mSin3 and histone deacetylase mediates transcriptional repression. *Nature* **387**: 43-8.

59. Ashburner BP, Westerheide SD, Baldwin ASJ (2001) The p65 (RelA) subunit of NF-kappaB interacts with the histone deacetylase (HDAC) corepressors HDAC1 and HDAC2 to negatively regulate gene expression. *Mol.Cell Biol.* **21**: 7065-77.

60. Taplick J, Kurtev V, Kroboth K, Posch M, Lechner T, Seiser C (2001) Homo-oligomerisation and nuclear localisation of mouse histone deacetylase 1. *J.Mol.Biol.* **308**: 27-38.

61. Yao YL, Yang WM, Seto E (2001) Regulation of transcription factor YY1 by acetylation and deacetylation. *Mol.Cell Biol.* **21**: 5979-91.

62. Koipally J, Renold A, Kim J, Georgopoulos K (1999) Repression by Ikaros and Aiolos is mediated through histone deacetylase complexes. *EMBO J.* **18**: 3090-100.

63. Wu X, Li H, Park EJ, Chen JD (2001) SMRTE inhibits MEF2C transcriptional activation by targeting HDAC4 and 5 to nuclear domains. *J.Biol.Chem.* **276**: 24177-85.

64. Nair AR, Boersma LJ, Schiltz L, Chaudry A, Muschel RJ (2001) Paradoxical effects of trichostatin A: inhibition of NF-Y-associated histone acetyltransferase activity, phosphorylation of hGCN5 and downregulation of cyclin A and B1 mRNA. *Cancer Lett.* **166**: 55-64.

65. Guenther MG, Barak O, Lazar MA (2001) The SMRT and N-CoR corepressors are activating cofactors for histone deacetylase 3. *Mol.Cell Biol.* **21**: 6091-101.

66. Ozawa Y, Towatari M, Tsuzuki S *et al.* (2001) Histone deacetylase 3 associates with and represses the transcription factor GATA-2. *Blood* **98**: 2116-23.

67. Zhou X, Richon VM, Rifkind RA, Marks PA (2000) Identification of a transcriptional repressor related to the noncatalytic domain of histone deacetylases 4 and 5. *Proc.Natl.Acad.Sci.U.S.A* **97**: 1056-61.

68. Nicolas E, Ait-Si-Ali S, Trouche D (2001) The histone deacetylase HDAC3 targets RbAp48 to the retinoblastoma protein. *Nucleic.Acids.Res.* **29**: 3131-6.

69. Hu E, Chen Z, Fredrickson T *et al.* (2000) Cloning and characterization of a novel human class I histone deacetylase that functions as a transcription repressor. *J.Biol.Chem.* **275**: 15254-64.

70. McKinsey TA, Zhang CL, Olson EN (2001) Control of muscle development by dueling HATs and HDACs. *Curr.Opin.Genet.Dev.* **11**: 497-504.

71. Andreucci JJ, Grant D, Cox DM *et al.* (2002) Composition and function of AP-1 transcription complexes during muscle cell differentiation. *J.Biol.Chem.*

72. McKinsey TA, Zhang CL, Lu J, Olson EN (2000) Signal-dependent nuclear export of a histone deacetylase regulates muscle differentiation. *Nature* **408**: 106-11.

73. Miska EA, Langley E, Wolf D, Karlsson C, Pines J, Kouzarides T (2001) Differential localization of HDAC4 orchestrates muscle differentiation. *Nucleic.Acids.Res.* **29**: 3439-47.

74. Choi SJ, Park SY, Han TH (2001) 14-3-3tau associates with and activates the MEF2D transcription factor during muscle cell differentiation. *Nucleic.Acids.Res.* **29**: 2836-42.

75. Guardiola AR, Yao TP (2002) Molecular cloning and characterization of a novel histone deacetylase HDAC10. *J.Biol.Chem.* **277**: 3350-6.

76. Tong JJ, Liu J, Bertos NR, Yang XJ (2002) Identification of HDAC10, a novel class II human histone deacetylase containing a leucine-rich domain. *Nucleic.Acids.Res.* **30**: 1114-23.

77. Caron H, van Schaik B, van der MM *et al.* (2001) The human transcriptome map: clustering of highly expressed genes in chromosomal domains. *Science* **291**: 1289-92.

78. Dangond F, Henriksson M, Zardo G, Caiafa P, Ekstrom TJ, Gray SG (2001) Differential expression of class I HDACs: roles of cell density and cell cycle. *Int.J.Oncol.* **19**: 773-7.

79. Dangond F, Gullans SR (1998) Differential expression of human histone deacetylase mRNAs in response to immune cell apoptosis induction by trichostatin A and butyrate. *Biochem.Biophys.Res.Commun.* **247**: 833-7.

80. Gray SG, Ekstrom TJ (2001) The human histone deacetylase family. *Exp.Cell Res.* **262**: 75-83.

81. Chang KT, Min KT (2002) Regulation of lifespan by histone deacetylase. *Ageing Res.Rev.* **1**: 313-26.

82. Robyr D, Suka Y, Xenarios I *et al.* (2002) Microarray deacetylation maps determine genome-wide functions for yeast histone deacetylases. *Cell* **109**: 437-46.

83. Furumai R, Komatsu Y, Nishino N, Khochbin S, Yoshida M, Horinouchi S (2001) Potent histone deacetylase inhibitors built from trichostatin A and cyclic tetrapeptide antibiotics including trapoxin. *Proc.Natl.Acad.Sci.U.S.A* **98**: 87-92.

84. Kim YB, Lee KH, Sugita K, Yoshida M, Horinouchi S (1999) Oxamflatin is a novel antitumor compound that inhibits mammalian histone deacetylase. *Oncogene* **18**: 2461-70.

85. Kramer OH, Gottlicher M, Heinzel T (2001) Histone deacetylase as a therapeutic target. *Trends.Endocrinol.Metab.* **12**: 294-300.

86. Marks PA, Richon VM, Breslow R, Rifkind RA (2001) Histone deacetylase inhibitors as new cancer drugs. *Curr.Opin.Oncol.* **13**: 477-83.

87. Prakash S, Foster BJ, Meyer M *et al.* (2001) Chronic oral administration of CI-994: a phase 1 study. *Invest.New Drugs* **19**: 1-11.

88. Gilbert J, Baker SD, Bowling MK *et al.* (2001) A phase I dose escalation and bioavailability study of oral sodium phenylbutyrate in patients with refractory solid tumor malignancies. *Clin.Cancer Res.* **7**: 2292-300.

89. Gore SD, Weng LJ, Figg WD *et al.* (2002) Impact of prolonged infusions of the putative differentiating agent sodium phenylbutyrate on myelodysplastic syndromes and acute myeloid leukemia. *Clin.Cancer Res.* **8**: 963-70.

90. Warrell RP, Jr., He LZ, Richon V, Calleja E, Pandolfi PP (1998) Therapeutic targeting of transcription in acute promyelocytic leukemia by use of an inhibitor of histone deacetylase. *J.Natl.Cancer Inst.* **90**: 1621-5.

91. Grandori C, Cowley SM, James LP, Eisenman RN (2000) The Myc/Max/Mad network and the transcriptional control of cell behavior. *Annu.Rev.Cell Dev.Biol.* **16**: 653-99.

92. Piekarz RL, Robey R, Sandor V *et al.* (2001) Inhibitor of histone deacetylation, depsipeptide (FR901228), in the treatment of peripheral and cutaneous T-cell lymphoma: a case report. *Blood* **98**: 2865-8.

93. Richon VM, O'Brien JP (2002) Histone Deacetylase Inhibitors: A New Class of Potential Therapeutic Agents for Cancer Treatment : Commentary re: V. Sandor et al., Phase I Trial of the Histone Deacetylase Inhibitor, Depsipeptide (FR901228, NSC 630176), in Patients with Refractory Neoplasms. Clin. Cancer Res., 8: 718-728, 2002. *Clin.Cancer Res.* **8**: 662-4.

94. Han JW, Ahn SH, Kim YK *et al.* (2001) Activation of p21{superWAF1/Cip1} transcription through Sp1 sites by histone deacetylase inhibitor apicidin: Involvement of protein kinase C. *J.Biol.Chem.*

95. Zhang CL, McKinsey TA, Olson EN (2002) Association of Class II Histone Deacetylases with Heterochromatin Protein 1: Potential Role for Histone Methylation in Control of Muscle Differentiation. *Mol.Cell Biol.* **22**: 7302-12.

96. Su GH, Sohn TA, Ryu B, Kern SE (2000) A novel histone deacetylase inhibitor identified by high-throughput transcriptional screening of a compound library. *Cancer Res.* **60**: 3137-42.

97. Yu X, Guo ZS, Marcu MG *et al.* (2002) Modulation of p53, ErbB1, ErbB2, and Raf-1 Expression in Lung Cancer Cells by Depsipeptide FR901228. *J.Natl.Cancer Inst.* **94**: 504-13.

98. Butler LM, Webb Y, Agus DB *et al.* (2001) Inhibition of transformed cell growth and induction of cellular differentiation by pyroxamide, an inhibitor of histone deacetylase. *Clin.Cancer Res.* **7**: 962-70.

99. Glick RD, Swendeman SL, Coffey DC *et al.* (1999) Hybrid polar histone deacetylase inhibitor induces apoptosis and CD95/CD95 ligand expression in human neuroblastoma. *Cancer Res.* **59**: 4392-9.

100. Han JW, Ahn SH, Park SH *et al.* (2000) Apicidin, a histone deacetylase inhibitor, inhibits proliferation of tumor cells via induction of p21WAF1/Cip1 and gelsolin. *Cancer Res.* **60**: 6068-74.

101. Vigushin DM, Ali S, Pace PE *et al.* (2001) Trichostatin A is a histone deacetylase inhibitor with potent antitumor activity against breast cancer in vivo. *Clin.Cancer Res.* **7**: 971-6.

102. Nervi C, Borello U, Fazi F, Buffa V, Pelicci PG, Cossu G (2001) Inhibition of histone deacetylase activity by trichostatin A modulates gene expression during mouse embryogenesis without apparent toxicity. *Cancer Res.* **61**: 1247-9.

103. Coffey DC, Kutko MC, Glick RD *et al.* (2001) The histone deacetylase inhibitor, CBHA, inhibits growth of human neuroblastoma xenografts in vivo, alone and synergistically with all-trans retinoic acid. *Cancer Res.* **61**: 3591-4.

104. Siavoshian S, Segain JP, Kornprobst M *et al.* (2000) Butyrate and trichostatin A effects on the proliferation/differentiation of human intestinal epithelial cells: induction of cyclin D3 and p21 expression. *Gut* **46**: 507-14.

105. Coffey DC, Kutko MC, Glick RD *et al.* (2000) Histone deacetylase inhibitors and retinoic acids inhibit growth of human neuroblastoma in vitro. *Med.Pediatr.Oncol.* **35**: 577-81.

106. Sun H, Taneja R (2000) Stra13 expression is associated with growth arrest and represses transcription through histone deacetylase (HDAC)-dependent and HDAC-independent mechanisms. *Proc.Natl.Acad.Sci.U.S.A.* **97**: 4058-63.

107. Koyama Y, Adachi M, Sekiya M, Takekawa M, Imai K (2000) Histone deacetylase inhibitors suppress IL-2-mediated gene expression prior to induction of apoptosis. *Blood* **96**: 1490-5.

108. Hou M, Wang X, Popov N *et al.* (2002) The histone deacetylase inhibitor trichostatin A derepresses the telomerase reverse transcriptase (hTERT) gene in human cells. *Exp.Cell Res.* **274**: 25-34.

109. Lavelle D, Chen YH, Hankewych M, DeSimone J (2001) Histone deacetylase inhibitors increase p21(WAF1) and induce apoptosis of human myeloma cell lines independent of decreased IL-6 receptor expression. *Am.J.Hematol.* **68**: 170-8.

110. Saito A, Yamashita T, Mariko Y *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.U.S.A.* **96**: 4592-7.

111. Kwon HJ, Owa T, Hassig CA, Shimada J, Schreiber SL (1998) Depudecin induces morphological reversion of transformed fibroblasts via the inhibition of histone deacetylase. *Proc.Natl.Acad.Sci.U.S.A.* **95**: 3356-61.

112. Marks PA, Richon VM, Rifkind RA (2000) Histone deacetylase inhibitors: inducers of differentiation or apoptosis of transformed cells. *J.Natl.Cancer Inst.* **92**: 1210-6.

113. Woo SH, Frechette S, Khalil EA *et al.* (2002) Structurally simple trichostatin a-like straight chain hydroxamates as potent histone deacetylase inhibitors. *J.Med.Chem.* **45**: 2877-85.

114. Hickman ES, Moroni MC, Helin K (2002) The role of p53 and pRB in apoptosis and cancer. *Curr.Opin.Genet.Dev.* **12**: 60-6.

115. Cooper S, Shayman JA (2001) Revisiting retinoblastoma protein phosphorylation during the mammalian cell cycle. *Cell Mol.Life Sci.* **58**: 580-95.

116. Black AR, Black JD, Azizkhan-Clifford J (2001) Sp1 and kruppel-like factor family of transcription factors in cell growth regulation and cancer. *J.Cell Physiol* **188**: 143-60.

117. Vaute O, Nicolas E, Vandel L, Trouche D (2002) Functional and physical interaction between the histone methyl transferase Suv39H1 and histone deacetylases. *Nucleic.Acids.Res.* **30**: 475-81.

118. Downes M, Ordentlich P, Kao HY, Alvarez JG, Evans RM (2000) Identification of a nuclear domain with deacetylase activity. *Proc.Natl.Acad.Sci.U.S.A* **97**: 10330-5.

119. Yang J, Kawai Y, Hanson RW, Arinze IJ (2001) Sodium butyrate induces transcription from the G alpha(i2) gene promoter through multiple Sp1 sites in the promoter and by activating the MEK-ERK signal transduction pathway. *J.Biol.Chem.* **276**: 25742-52.

120. Pili R, Kruszewski MP, Hager BW, Lantz J, Carducci MA (2001) Combination of phenylbutyrate and 13-cis retinoic acid inhibits prostate tumor growth and angiogenesis. *Cancer Res.* **61**: 1477-85.

121. Stahelin H, Trippmacher A (1974) Cytostatic activity of chlamydocin, a rapidly inactivated cyclic tetrapeptide. *Eur.J.Cancer* **10**: 801-8.

122. Kitazono M, Rao VK, Robey R *et al.* (2002) Histone deacetylase inhibitor FR901228 enhances adenovirus infection of hematopoietic cells. *Blood* **99**: 2248-51.

123. Kwon HJ, Kim MS, Kim MJ, Nakajima H, Kim KW (2002) Histone deacetylase inhibitor FK228 inhibits tumor angiogenesis. *Int.J.Cancer* **97**: 290-6.

124. Seelig MH, Berger MR (1996) Efficacy of dinaline and its methyl and acetyl derivatives against colorectal cancer in vivo and in vitro. *Eur.J.Cancer* **32A**: 1968-76.

125. Burgess AJ, Pavey S, Warrener R *et al.* (2001) Up-regulation of p21(WAF1/CIP1) by histone deacetylase inhibitors reduces their cytotoxicity. *Mol.Pharmacol.* **60**: 828-37.

126. Richon VM, Sandhoff TW, Rifkind RA, Marks PA (2000) Histone deacetylase inhibitor selectively induces p21WAF1 expression and gene-associated histone acetylation. *Proc.Natl.Acad.Sci.U.S.A* **97**: 10014-9.

127. Huang H, Reed CP, Zhang JS, Shridhar V, Wang L, Smith DI (1999) Carboxypeptidase A3 (CPA3): a novel gene highly induced by histone deacetylase inhibitors during differentiation of prostate epithelial cancer cells. *Cancer Res.* **59**: 2981-8.

128. Khochbin S, Verdel A, Lemercier C, Seigneurin-Berny D (2001) Functional significance of histone deacetylase diversity. *Curr.Opin.Genet.Dev.* **11**: 162-6.

Table 1 Co-repressors of HDACs. The Co-repressors needed for function of HDACs grouped in different functional classes. Some of the co-repressors appear in more than one class due to their dual function. Several co-repressors are not in all events necessary that depends on tissue type and transcriptional need. Of the HDACs not shown in this table to little is known concerning their possible need for co-repressors.

Table 2 Chromosomal localisation of HDACs and reliable tags used to determine the expression. This data is obtained from NCBI SAGE database in combination with the Human transcriptome Map, freely available on the Internet. Grey sequence tags have a lesser certainty due to their appearance in more than one unigene cluster, or because no hits were found in either database. The numbers shown in table 3 are generated on the basis of these tags. http://www.ncbi.nlm.nih.gov/UniGene/ and http://www.amc.uva.nl/ under research, Human Transcriptome Map.

Table 3 General HDAC expression per 100 000 tags per tissue type. HDAC expression in different normal and cancerous cells. The numbers shown are an calculated average from the sites (http://www.amc.uva.nl under research, Human Transcriptome Map). The expression data in this table is based on the SAGE tags given in table 2.

Table 4 HDAC inhibitors. Features of four groups of HDACi including *in vitro* efficiency of the members and general structural make up.

Table 5 HDAC responsive genes. Genes whose expression are affected by HDACi. No discrimination between increase or decrease of expression has been made.

Biochemical Journal Immediate Publication. Published on 12 Nov 2002 as manuscript BJ20021321

TABLE 1

Complex	HDAC1 & HDAC2 (16-19,39,47,56-59,95,117,118) Sin3	NuRD	Co-REST	HDAC3 (36,41,50,55,65-67)	HDAC4 HDAC5 (32,36,36,46,50,66,73)		HDAC7 (32,40,48-50,54)	HDAC9 (43,46,67)
Histone binding	RbAp48	RbAp48		RbAp48				
Recruiters** (bind DNA, sequence specific, methylation specific or are intermediates between DNA and HDAC complex)	Ikaros	MBD3		HDAC4, HDAC5, HDAC7, HDRP, YY1[2], Rb[1], GATA-2, HDAC9, HDRP, HDAC10	MEF2	MEF2, GATA-2	MEF2	MEF2
	YY1[2], RBP-1, Sp1, BRCA1, Rb[1] , HDRP heterochromatin protein-1, NF-κB, MeCP2, HDAC10							
Nuclear hormone receptor* binding	mSin3A, N-CoR, SMRT***			SMRT, N-CoR	SMRT, N-CoR	SMRT, N-CoR	SMRT, N-CoR	SMRT, N-CoR
Remodelling (helicase/ ATP-ase family members)		Mi-2						
Necessary/ modulates HDAC activity		MTA2, p70, p32,	Co-REST	SMRT/ N-CoR	SMRT/ N-CoR, HDAC3	SMRT/ N-CoR, HDAC3	SMRT/ N-CoR, HDAC3	
Unknown	RbAp46, SAP18, SAP30	RbAp46		TBL1				
Association as intermediate	MAD/MAX, Mxi/MAX, MeCP-1, ER, GR, TR, RPX homeodomain proteins, c-Ski, Sno, Aiolos, p53, NY-F, REST, Suv39H1, PML-RARα, DNMT			CRM1, HDAC3	ERK1/2, CtBP, HDAC10	CtBP, HDAC10	CRM1, CtBP, HDAC10	HDAC1, HDAC3, SMRT, N-CoR,

* Nuclear hormone receptors (e.g. ER, GR, TR and RAR) function as transcriptional activators, directly binding the respective responsive element, when ligand is present. In the case of SMRT / N-CoR binding and function no ligand is bound to the receptors, thus functioning as transcriptional repressors.
** From the group of recruiters only one is usually present, all the other groups represent necessary co-repressors for HDAC activity.
*** SMRT does not interact directly with HDAC1

Biochemical Journal Immediate Publication. Published on 12 Nov 2002 as manuscript BJ20021321

Table 2

enzyme Unigene cluster # (Gi #)	*Chromosomal location SAGE tags**
HDAC1 Hs.88556 (12653070)	1p34, TCCAAAGTAA
HDAC2 Hs.3352 (4557640)	6q21, CTTTATGTGA
HDAC3 Hs.279789 (13128861)	5q31** ACAATGACAA
HDAC4 Hs.91400 (13259519) UTR?	2q37 CAACCTCCAG CACGCCTGGG
HDAC5 Hs.9028 (13259520)	17q21 GAGCAGGAGC
HDAC6 Hs.6764 (15079316)	Xp11 GCAAGGTTGC TGTCCTCCCA
HDAC7 Hs.275438 (13259521)	12q13, a and b isoform 7a: TTTTTGTAAA 7b is unclear
HDAC8 Hs.112272 (EST) (8923768)	Xq13 TGGTCTAGTT
HDAC9 Hs.116753 (9a: 17158038 9b: 17158040 9c: 7662279 = HDRP: 3882208)	7p15-p21, 3 isoforms TGGCTGAGCA
HDAC10 Hs.26593 (16903565)	22q13, 2 splice variants CAACCCACGC
HDAC11 Hs. ? (10438543)	3p25

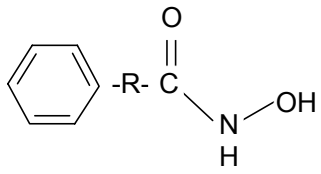
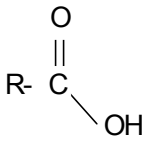
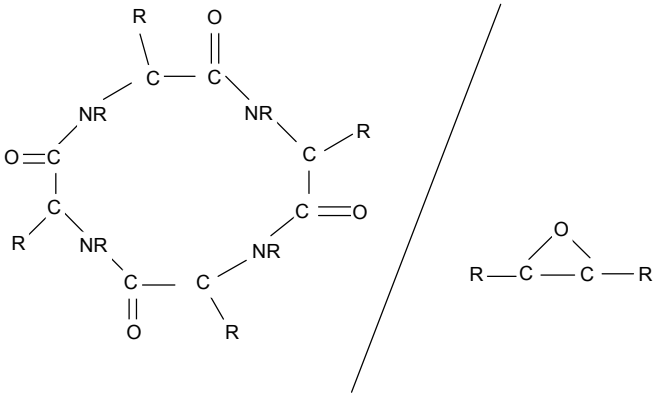
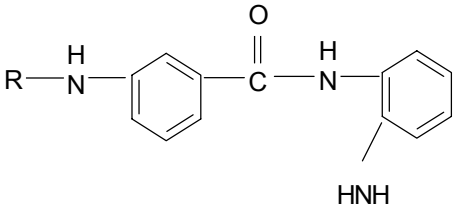
* Tags shown are unique for the gene and are considered reliable. ** from blast searching and looking at unigene clusters it seems HDAC3 has high homology with parts on chromosome 11, possibly another HDAC-like gene.

Table 3

		CLASS I				CLASS II					
	HDAC	1	2	3	8	4*	5*	6	7	9	10
Normal Tissue	**All Normal****	1.4	3.0	1.85	0.2	0.01	0.42	4.5	2.2	0.55	1.15
	brain	0.3	1.6	0.8	-	-	7.2	4.5	0.8	0.8	2.38
	breast	-	-	4.3	-	-	4.0	8.6	2.9	-	3.3
	colon	7	5.0	-	-	-	4.6	4.0	-	-	2.0
	kidney	1.8	11.9	2.7	0.9	-	-	-	-	-	4.6
	ovary	4.1	3.1	5.2	-	-	-	4.1	9.3	2.1	2.9
	pancreas	0.7	1.5	3.0	0.7	-	-	2.2	1.5	-	-
	prostate	1.3	3.3	3.3	-	-	3.3	5.3	2.7	-	5.2
	heart*	-	2.3	-	-	-	9.5	2.3	2.3	1.1	-
Tumour Tissue	**All Tumour****	1.75	4.1	1.95	0.55	0.19	3.4	5.5	2.15	1.0	1.3
	brain	1.9	3.8	2.5	0.9	6.1	6.1	7.2	1.5	1.7	3.1
	breast	0.2	3.0	1.1	-	-	-	8.9	3.2	-	4.3
	colon	1.8	2.3	1.5	0.6	1.6	3.7	2.0	1.5	2.6	2.5
	neuroblastoma	0.5	9.1	2.0	0.5	-	8.5	1.5	1.0	1.5	ND
	ovary	2.6	6.1	2.6	1.0	3.1	-	4.2	2.9	1.0	2.6
	pancreas	2.3	2.3	3.0	1.5	2.9	7.95	3.0	1.5	0.8	2.8
	prostate	2.1	3.2	1.9	0.2	2.4	3.8	2.6	1.6	-	2.95
	heart	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

- : no expression found. * From NCBI Unigene, no hits in Human Transcriptome Map. ** the overall average of all normal or tumour tissues found in the Human Transcriptome Map. ND: no data available

Table 4

Group	Compounds	*in vitro* IC_{50} range
Hydroxamic acids 	Trichostatin A (8,24,26,27,32,35,64,79,85,86,101,102, 107,108, 112,119) Suberoyl anilide bishydroxamide (SAHA) (8,10,32,85,86,112) M-carboxy-cinnamic acid bishydroxamide (CBHA) (32,103,105) Scriptaid (32,85,86) Pyroxamide (32,98) Oxamflatin* (32,84-86,112)	nM µM µM µM µM nM
short-chain fatty acids 	Butyrate (27,30,32,35,79,85,86,102,107,112,119) Phenylbutyrate (8,9,25,85,88-90,120) Valproic acid (26,32)	mM mM mM
Cyclic tetrapeptides/ epoxides** 	Trapoxin (TPX) (32,33,85,86,112) HC-toxin (119) Chlamydocin (121) Depudesin (32,85,86,111,112) Apicidine* (30,32,85,86,100,112) Depsipeptide (FK228)* (31,32,85,86,92,112,122,123)	nM nM nM µM nM-µM nM
benzamides 	N-acetyldinaline (CI-994) (32,85-87,112,124) MS-275 (32,85,86,110,112)	µM µM

* are CHAPS: are hybrids between hydroxamic acids and cyclic tetrapeptides grouped after their major feature (83)

** This is a heterogeneous group of compounds with a high degree of overlap indicating that many of the members have both features

Biochemical Journal Immediate Publication. Published on 12 Nov 2002 as manuscript BJ20021321

Table 5

Cell cycle	Apoptosis	Transcription factors	Other
cyclin A (64), cyclin E (19), cyclin B1(64), cyclin D1 and D3 (19,104), $p21^{WAF1/Cip1}$ (27,64,98,109,125, 126) p53 (79,97)	CD95/CD95 (99), gelsolin (35,100), Bax (100,109), Bcl2 (19)	GATA-2 (27), c-myc (NMYC analogue) (107), RARα and β (102)	erbB1(97), erbB2 (97), Raf-1 (97), MMP-2 (30), tob-1 (27), p55 Cdc (27), PKCδ (27), HM89 (27),HSP70 (27), ICAM-1 (27), STRA13 (related to HES genes) (106), hTERT (24,108), IL-6 receptor (109), IL-2, 8 and 10 (19) VEGF (123), Notch (102) CPA3 (127)

Figure 1 Various aspects of the transcription process and its regulation by histone modification. Panel A shows a schematic representation of a nucleosome. Yellow represents the histones. Dark red depicts the histone tail that can be modified to loosen the DNA (blue) winding. The dark red circle represents a tail without an acetyl group. The dark red banana shape represents a histone tail with an acetyl group relieving the tight packaging of the DNA. Panel B shows transcriptional repression and activation in chromatin. Yellow circles represent core histone octamers, in the upper panel acetylated histone tails (dark red) are depicted emerging from the octamer. DNA is in blue and the solid black arrow represents complex movement. Histone acetyl-transferase (HAT: activation) and histone deacetylase (HDAC: repression) enzymes need several cofactors (for DNA binding, for recruitment of the complex, for remodelling of the DNA helix to reduce the accessibility for transcription factors) for their activity (purple and pink) (1,3,50).

Figure 2 Schematic depiction of the evolutionary relation between the HDACs. the actual distance can be longer than shown. The class I HDACs are related to the yeast RPD3 and the class II HDACs are related to the yeast HDA1 enzyme (Saccharomyces cerevisiae). RPD3 is most related to HDAC 1 and HDAC 2. HDA1 is most closely related to HDAC 6 (4,42,69). From the phylogenetic tree it can be concluded that the HDAC 9a, 9b, and 9c/HDRP are a distinct group within class II seeming less related to other members of class II. HDAC11 has not enough homology to be classified to either class I or II. (www.ebi.ac.uk, under tools ClustalW, default settings.)

Figure 3 Schematic depiction of the different isoforms of HDAC. Bars depict the length of the protein. Striped domains represent the catalytic domain. Note that

HDRP does not posses any deacetylase activity. Black depicts a nuclear localisation signal (NLS). N = N-terminus and C = C-terminus. (1,38,40,41,43,46,48-50,54,60,67,72,73,76,128).

Figure 4 Schematic representation of localisation of HDACs. Grey depicts the nucleus. Grey half circle depicts the 14-3-3 protein that can retain HDAC4/5 or 7 in the cytoplasm when they are phosphorylated. Small black oval depicts the protein that takes care of nuclear export via the Calcium/ Calmodulin signalling pathway (CaMK). Panel A and B shows the subcellular localisation of class I and class II HDACs. Panel C shows the shuttling of HDAC4, 5 and 7 during muscle differentiation.

Biochemical Journal Immediate Publication. Published on 12 Nov 2002 as manuscript BJ20021321

Figure 1

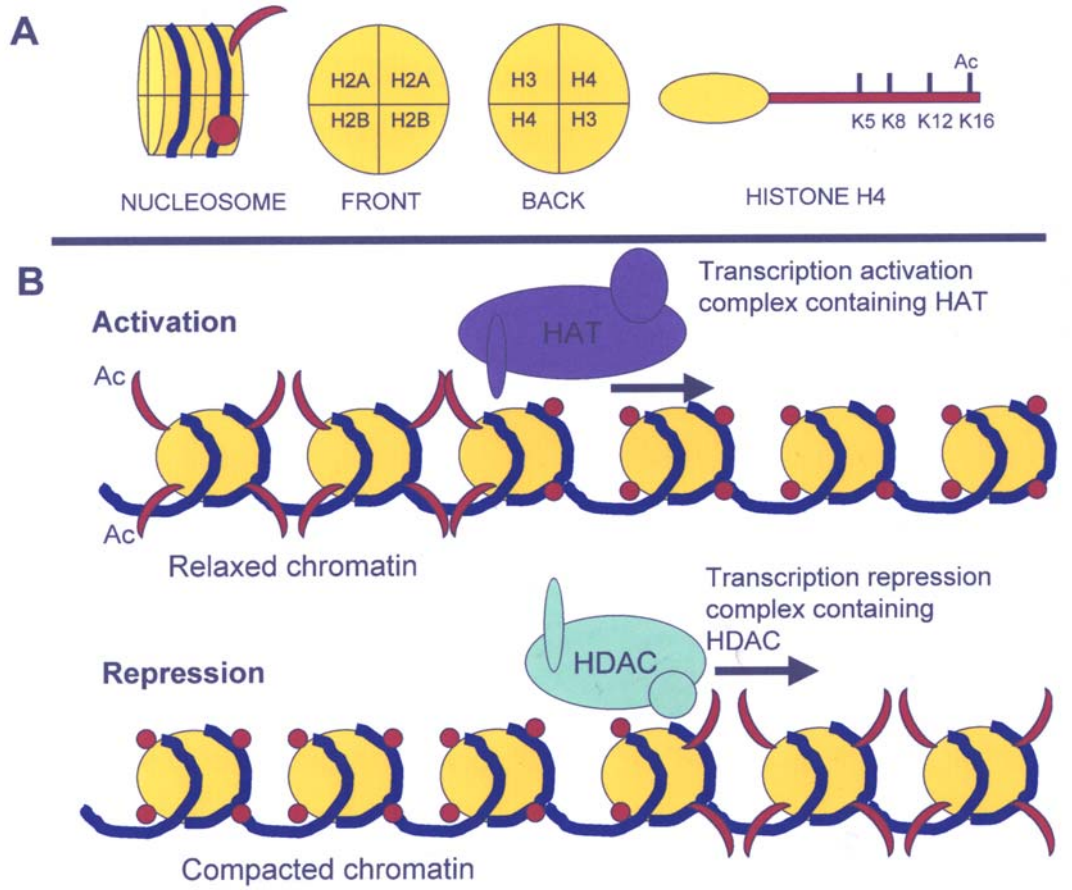
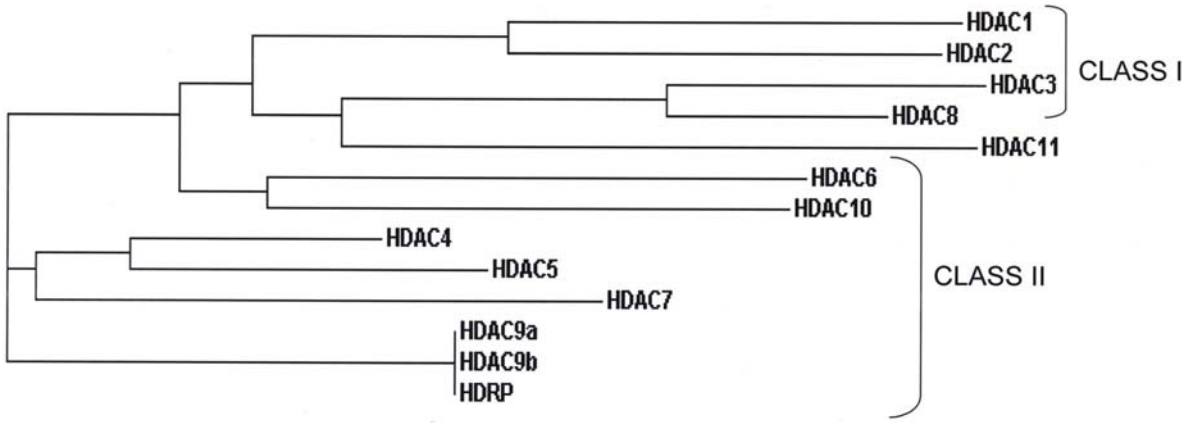


Figure 2



Biochemical Journal Immediate Publication. Published on 12 Nov 2002 as manuscript BJ20021321

Figure 3

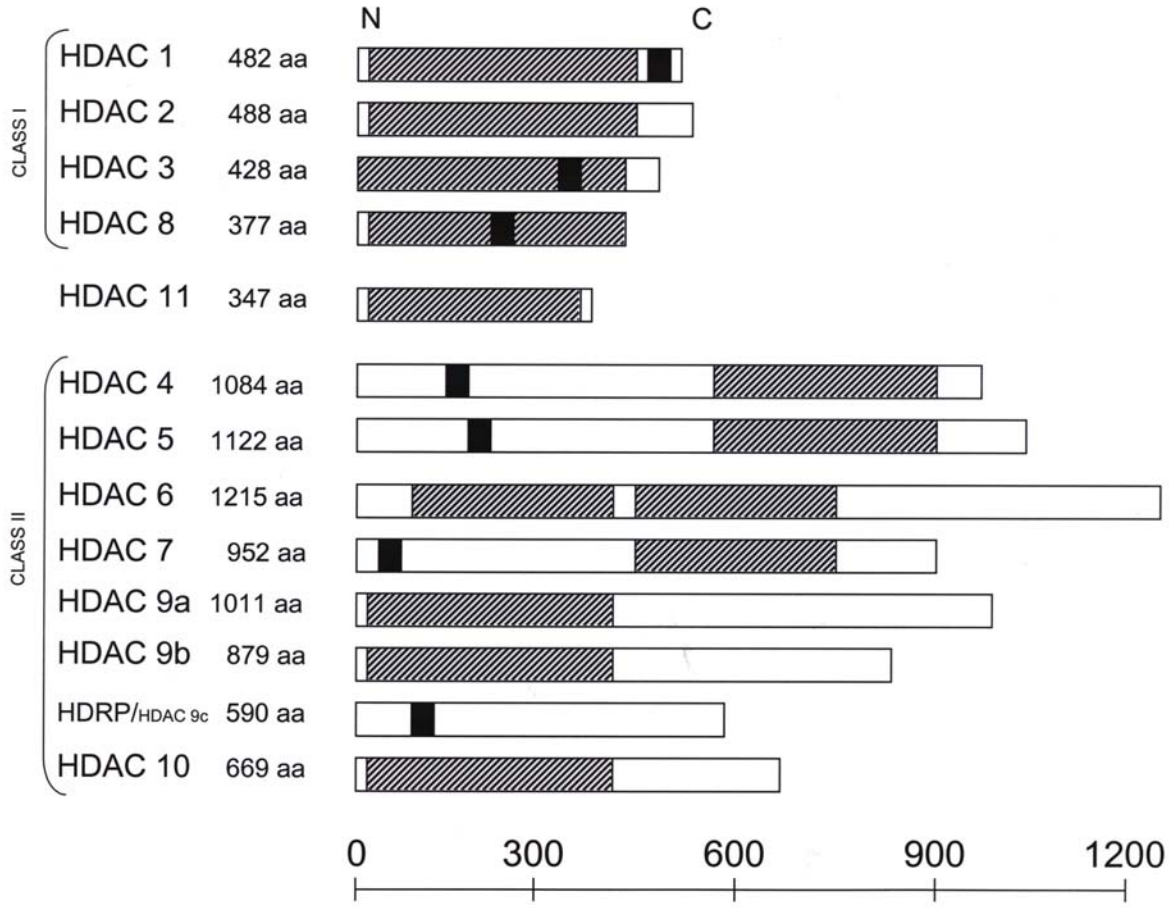


Figure 4