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# Histone deacetylase 1 and 2-controlled embryonic development and cell differentiation

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**ABSTRACT During development from the fertilized egg to a multicellular organism, cell fate decisions have to be taken and cell lineage or tissue-specific gene expression patterns are created and maintained. These alterations in gene expression occur in the context of chromatin structure and are controlled by chromatin modifying enzymes. Gene disruption studies in different genetic systems have shown an essential role of various histone deacetylases (HDACs) during early development and cellular differentiation. In this review, we focus on the functions of the class I enzymes HDAC1 and HDAC2 during development in different organisms and summarise the current knowledge about their involvement in neurogenesis, myogenesis, haematopoiesis and epithelial cell differentiation.**

KEY WORDS: *HDAC1, HDAC2, chromatin, differentiation, development*

## Histone deacetylases

Posttranslational modifications of histones cause changes in the accessibility of DNA, thereby regulating many important cellular processes including transcription. Acetylation of core histones leads to a change in the net positive charge of histone tails and local opening of chromatin structure, a feature of transcriptionally active genes. On the other hand, deacetylated histone tails interact more closely with DNA and lead to a repressive state. Histone deacetylases (HDACs) catalyse the removal of acetyl groups from histone tails and are therefore considered as transcriptional corepressors. In addition to histones, HDACs also deacetylate non-histone proteins, such as the cytoskeletal protein tubulin (Hubbert *et al.*, 2002) or transcription factors including p53 (Luo *et al.*, 2000), E2F1 (Martinez-Balbas *et al.*, 2000) and YY1 (Yao *et al.*, 2001). In fact, phylogenetic analysis suggests that the enzymatic activity was initially directed against non-histone proteins in a common ancestor devoid of histones (Gregoretti *et al.*, 2004). In mammals, 18 deacetylases have been identified so far. These enzymes have been divided into 4 classes based on sequence similarity: Classic HDACs comprise class I (*Saccharomyces cerevisiae* Rpd3-like), class II (*Saccharomyces cerevisiae* Hda1-like) and class IV (HDAC11-like) enzymes. Class III consists of NAD-dependent, functionally unrelated Sir2-like deacetylases named "sirtuins". Class I, II and IV HDACs are members of an ancient enzyme family, highly conserved throughout eukaryotic and prokaryotic evolution and are found in animals, plants, fungi, archaebacteria and eubacteria (Gregoretti *et al.*, 2004).

### *Class I histone deacetylases*

Phylogenetic analysis revealed that class I genes in animals can be grouped into *HDAC1/HDAC2*, *HDAC3* and *HDAC8*-like genes. Members of each subclass have been identified in protostomia (Oger *et al.*, 2008) and deuterostomia. Species analysed so far carry orthologs (i.e. genes of the same origin, but different as a result of species divergence) of *HDAC1/HDAC2* and *HDAC3*; the *HDAC8* gene has been lost during evolution in some species (e.g. in *Drosophila melanogaster* and *Caenorhabditis elegans*). The ancestral *HDAC1/HDAC2*-like gene has been subjected to independent duplication events in several, but not all lineages (e.g. *Caenorhabditis elegans* and the sea urchin *Strongylocentrotus purpuratus* possess two *HDAC1/HDAC2*-like genes, while *Drosophila melanogaster* harbours only one). Importantly, it seems that the pair of genes referred to as *HDAC1* and *HDAC2* originate from a gene

*Abbreviations used in this paper:* HAT, histone acetyltransferase; HDA, HDAC, histone deacetylase; HDI, HDAC inhibitor; NuRD, nucleosome remodelling and deacetylase complex; Rpd3, reduced potassium dependency 3.

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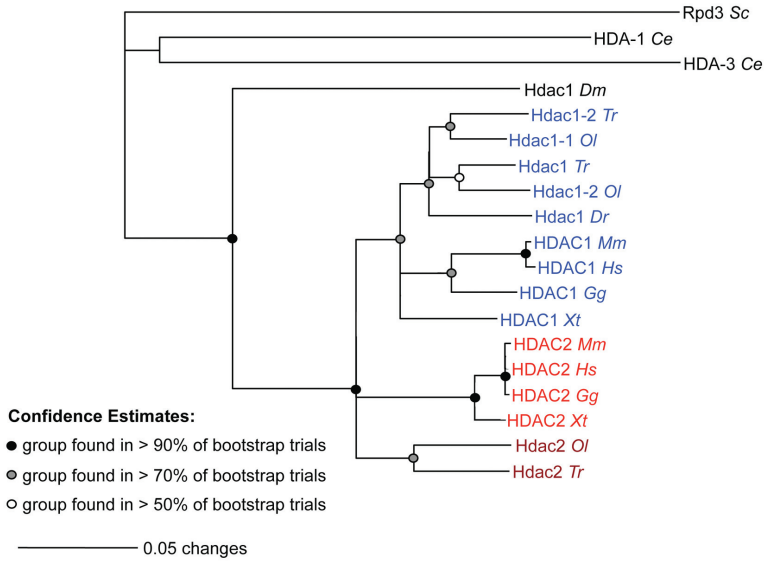


**Fig. 1. Phylogenetic relationship between animal HDAC1/HDAC2 proteins.** *Bootstrapped neighbour-joining phylogenetic tree of selected eukaryotic HDAC1/HDAC2 protein sequences.* Caenorhabditis elegans *harbours two genes encoding HDAC1/HDAC2-like proteins originating from an ancient gene duplication event (HDA-1; HDA-3),* Drosophila melanogaster *a single one (Hdac1). Vertebrates included in the analysis possess paralogs falling into an HDAC1-like (blue) or an HDAC2-like (red) subgroup. Note that HDAC2-like sequences from fish (*Oryzies lapides*; Takifugu rubripes; dark red) do not unambiguously group with HDAC2 proteins from higher vertebrates with the methods employed. Alignment of complete protein sequences was performed using ClustalW with default settings, tree building by PAUP 4.0. Bootstrap analyses (1000 trials) provide a measure of confidence for the detected relationship. The sequence of* Saccharomyces cerevisiae *Rpd3 was used as outgroup. Sc,* Saccharomyces cerevisiae*; Ce,* Caenorhabditis elegans*; Dm,* Drosophila melanogaster*; Dr,* Danio rerio*; Ol,* Oryzias lapides*; Tr,* Takifugu rubripes*; Xt,* Xenopus tropicalis*; Gg,* Gallus gallus*; Mm,* Mus musculus*; Hs,* Homo sapiens*. Protein sequences were derived from www.ensembl.org.*

duplication event in the common ancestor of all vertebrates. Therefore most vertebrates harbour one copy of each gene (e.g. *Xenopus tropicalis*, *Gallus gallus*, *Mus musculus*, *Homo sapiens*). In fish, the situation is more complex. In *Danio rerio* only one *HDAC1/HDAC2*-like gene has been identified, whereas the genome of other fish species comprehend two (*Salmo salar, Gasterosteus aculeatus*) or even three (*Oryzias latipes, Takifugu rupripes*) putative *HDAC1/HDAC2*-like genes. This might be the result of a genome duplication followed by secondary loss (Fig. 1).

Gene duplication usually leads to functional diversification in the form of paralogs, so that one paralog can acquire new functions or gain tissue-specific distribution. Given the fact that the duplication of the *HDAC1/HDAC2* gene was a relatively recent event in evolution and taking into account that HDAC1 and HDAC2 proteins exhibit high sequence similarity (e.g. 82% amino acid identity between human HDAC1 and HDAC2), there should still be a high degree of functional overlap. This seems to be valid for the majority of biological processes, however it becomes evident from knockout studies (described in detail in the following chapters) that HDAC1 and HDAC2 also have distinct and non-redundant biological functions.

### *Functional domains and posttranslational modifications of HDAC1 and HDAC2*

Both HDAC1 and HDAC2 contain several domains with defined function (Fig. 2): (1) The catalytic domain common to all class I HDACs is formed by a stretch of more than 300 amino acids constituting a large portion of the protein. The active site comprises a pocket containing two adjacent histidine residues, two aspartic acid residues and one tyrosine residue forming a charge-relay system with a $Zn^{2+}$ ion as an essential component (de Ruijter *et al.*, 2003). (2) This catalytic domain partly overlaps with an N-terminal HDAC association domain (HAD; residues 1 to ~50) which is essential for homo- and heterodimerisation (Taplick *et al.*, 2001). (3) The C-terminal part contains an IAC(E/D)E motif involved in the interaction with the pocket proteins pRb, p107 and p130 (Brehm *et al.*, 1998; Magnaghi-Jaulin *et al.*, 1998). (4) Two amino-acid residues within the catalytic domain of HDAC1 are essential for the interaction with Chfr, an ubiquitin ligase regulating protein degradation (Oh *et al.*, 2009). Finally, there are domains specific for either HDAC1 or HDAC2: A nuclear localisation signal (NLS) at the C-terminus can only be found in HDAC1 (Taplick *et al.*, 2001), whereas a C-terminal coiled-coil domain (possibly enabling additional protein-protein associations) seems to be specific for HDAC2 (Gregoretti *et al.*, 2004). Distinct additional domains are found only in certain species, for example a C-terminal extension of unknown function in *Drosophila*.

The HDAC1/HDAC2 protein is a target for various posttranslational modifications: *In vitro* studies indicate that phosphorylation of serine residues in the C-terminal portion of HDAC1/HDAC2 enhances its activity, promotes corepressor complex formation and regulates nuclear import (Cai *et al.*, 2001; Pflum *et al.*, 2001; Smillie *et al.*, 2004). Another study demonstrated that phosphorylated HDAC2 is preferentially incorporated into corepressor complexes found at promoters (Sun *et al.*, 2007). However, the exact role of HDAC1/HDAC2 phosphorylation *in vivo* is still not fully clarified (Karwowska-Desaulniers *et al.*, 2007). Another modification, acetylation of lysines within the HDAC domain and the C-terminus leads to reduced enzymatic activity (Qiu *et al.*, 2006). The biological consequence of HDAC1 sumoylation has been a matter of debate (Colombo *et al.*, 2002; David *et al.*, 2002), but there is recent evidence that sumoylation modulates the interaction with other proteins (Gocke and Yu, 2008). Finally, Nott *et al.* found that S-nitrosylation of HDAC2 regulates its release from chromatin (Nott *et al.*, 2008).

Many of the sites modified in either HDAC1 or HDAC2 are conserved between both proteins. Therefore it will be of interest to dissect, which modifications are indeed shared between the two homologs or are occurring in an enzyme-specific manner. That way, distinct roles for each protein will be more precisely defined.

### *HDAC1 and HDAC2 – redundancy versus specificity*

Several facts confound the definition of the distinct roles of *HDAC1* and *HDAC2* in organisms harbouring both genes. Upon depletion of *HDAC1* or *HDAC2*, protein levels of the paralog are

consistently increased in a number of murine tissues and cell lines (Lagger *et al.*, 2002; Senese *et al.*, 2007). Interestingly, this cross-regulation seems to occur at the translational or post-translational level rather than at the level of transcription, as the abundance of *HDAC2* mRNA is not influenced upon *HDAC1* inactivation (Montgomery *et al.*, 2007; Senese *et al.*, 2007; Zupkovitz *et al.*, 2006). One possible mechanism responsible for the regulation of HDAC1/HDAC2 protein levels could be the modulation of protein stability either by protein-protein interaction or by chemical modification of the proteins.

As a consequence, several observed phenotypic effects accompanying loss of either *HDAC1* or *HDAC2* could originate from upregulation of its paralog, rather than inactivation of the gene itself. First insights regarding this issue have been gained in gene expression studies performed in mouse ES cells by Zupkovitz and colleagues (Zupkovitz *et al.*, 2006): Loss of HDAC1 can have completely opposing effects on transcription of individual target genes. The expression of many genes is induced because HDAC2 cannot compensate for HDAC1 depletion. In other cases, increased expression and ectopic recruitment of HDAC2 to promoters leads to transcriptional repression in the absence of HDAC1.

### *HDAC1 and HDAC2 as members of multiprotein complexes*

HDAC1 and HDAC2 proteins have the ability to homo- and heterodimerise. The fact that class I and II enzymes either dimerise or contain two catalytic domains (e.g. HDAC6), strongly suggests that the presence of two active centres within a deacetylase complex is required for protein deacetylation of some substrates (Zhang *et al.*, 2006). HDAC1/HDAC2 is unable to bind to DNA by itself, but becomes recruited by transcription factors such as SP1/SP3 (Doetzlhofer *et al.*, 1999) and YY1 (Yang *et al.*, 1996), E2F proteins in complex with the pocket proteins pRb, p107, p130 (Brehm *et al.*, 1998; Ferreira *et al.*, 1998; Magnaghi-Jaulin *et al.*, 1998), and the tumour suppressors p53 (Juan *et al.*, 2000) and BRCA1 (Yarden and Brody, 1999). Alternatively, HDAC1/HDAC2 can be tethered to DNA as part of large multiprotein complexes. Several HDAC1/HDAC2-containing complexes have been characterised in mammals: (1) the SIN3 corepressor complex, (2) the nucleosome remodelling and deacetylase complex (NuRD), (3) the CoREST complex, (4) the Nanog and Oct4 associated deacetylase complex (NODE) and (5) the SHIP1 containing complex (see Fig. 3 for protein composition of complexes).

(1) The SIN3 core complex contains eight proteins including HDAC1/2 (Silverstein and Ekwall, 2005). Additionally it serves as a platform for numerous other proteins responsible for targeting the complex to specific promoters (transcription factors, corepressors) or for extending the enzymatic function of the core. Some complexes contain nucleosome remodelling proteins such as BRG1 and BRM (Sif *et al.*, 2001), while others contain O-linked N-acetylglucosamine transferases or histone methyltransferases such as ESET, which can both deliver HDAC-independent silencing activity (Yang *et al.*, 2003; Yang *et al.*, 2002).

(2) The NuRD complex couples histone deacetylase activity with other chromatin modifying activities (Denslow and Wade, 2007). In addition to HDAC1/HDAC2, the NuRD complex contains Mi-2, a helicase-like ATPase of the SWI/SNF family possessing chromatin remodelling activity, as well as a methyl CpG-binding domain (MBD) protein, which links the deacetylase complex to DNA methylation. Similar to SIN3, numerous subtypes of the complex containing specific isoforms of the core

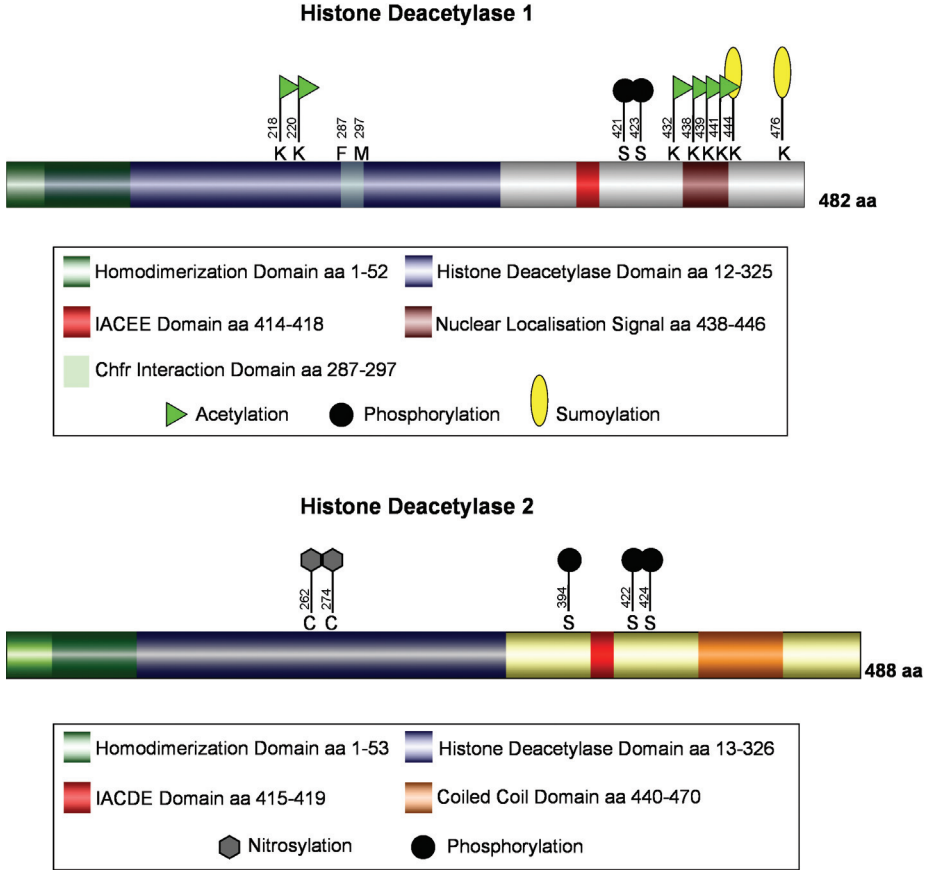


**Fig. 2. Functional domains of HDAC1/HDAC2 proteins.** *Schematic view of the human HDAC1 and HDAC2 proteins as representatives for HDAC1-like and HDAC2-like proteins. Both share a highly conserved N-terminal part harbouring the HDAC association domain (HAD) important for protein-protein interaction as well as the catalytic domain (HDAC domain) essential for enzymatic function. Common to HDAC1/HDAC2 is an IAC(E/D)E motif required for the interaction with pocket proteins. Interaction between HDAC1 and the ubiquitin ligase Chfr depends on two amino acids within the HDAC domain (F287; M297). The C-terminus exhibits more diversity: while HDAC1 harbours a nuclear localisation signal (NLS), HDAC2 contains a coiled-coil domain for protein-protein interaction. Both enzymes are targets for posttranslational modifications such as phosphorylation, acetylation and sumoylation, which are thought to have influence on enzyme activity, protein stability, complex formation and nuclear import.*

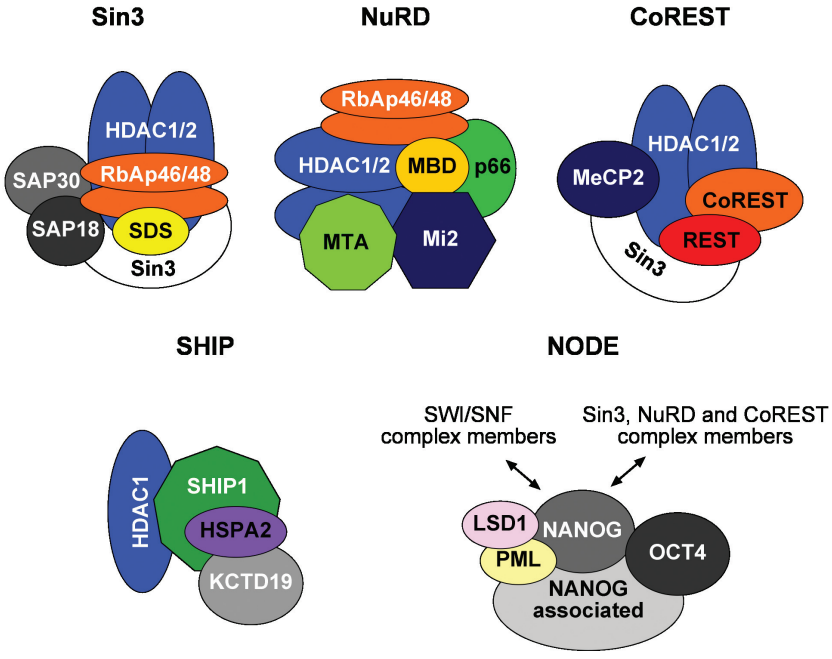


**Fig. 3. Composition of HDAC1/HDAC2 containing multiprotein complexes.** *The SIN3 complex is comprised of HDAC1 and HDAC2 delivering enzymatic activity and the structural components SIN3, RbAp46, RbAp48, SAP18, SAP30 and SDS3. RbAp46, RbAp48, HDAC1 and HDAC2 are also found in the NuRD complex, together with the NuRD specific factors MTA, Mi-2, p66 and MBD. Both complexes are tethered to DNA* via *numerous transcription factors (not illustrated). The CoREST complex consists of CoREST, MeCP2, SIN3, HDAC1, HDAC2 and is targeted to DNA* via *the REST protein. Besides those three classical HDAC complexes, a testis specific complex containing SHIP1, HDAC1, HSPA2 and KCTD19 has been identified recently. In ES cells the transcription factors Nanog and Oct4, together with associated proteins interact with HDAC1 and HDAC2 and several other members of the SIN3, NuRD, CoREST and SWI/SNF complexes. Note that the schematic view does not mirror physical interaction of the subunits.*

components have been described. Furthermore, additional chromatin modifying enzymes such as the arginine histone methyltransferase PRMT5 (Le Guezennec *et al.*, 2006) can be recruited. Tethering of the complex to target promoters is accomplished by several transcription factors.

(3) In contrast to SIN3 and NuRD complexes, which can associate with different transcription factors, the CoREST complex is recruited to chromatin by a specific DNA binding factor. The REST/NRSF (RE1 silencing transcription factor/neuronal restricted silencing factor) protein binds to a conserved 23bp DNA motif known as RE1 (repressor element 1, also known as NRSE) (Chong *et al.*, 1995; Schoenherr and Anderson, 1995), which is found in a large number of genes encoding fundamental neuronal traits. Again, as a common theme, the core complex can be extended by other chromatin modifiers such as the histone demethylase LSD1 (Lee *et al.*, 2005) or the histone methyltransferases SUVAR39H1 and G9a (Lunyak *et al.*, 2002; Roopra *et al.*, 2004; Shi *et al.*, 2004). Related to the CoREST complex is the BRAF-HDAC complex (BHC), containing HDAC1/HDAC2, BRAF35, CoREST, LSD1 (=BHC110), and the Mi-2-like protein BHC80 as core components.

(4) The NODE complex is specifically found in embryonic stem (ES) cells and controls ES cell fate by repressing Nanog/Oct4 target genes. It contains proteins found in nucleosome remodelling complexes, the NuRD and SIN3 complex, as well as the demethylase LSD1 and the transcription factor Pml (Liang *et al.*, 2008).

(5) Recently, a complex comprising HDAC1, the heat shock protein HSPA2, SHIP1, a testis specific protein possessing putative DNA binding and chromatin remodelling properties and KCTD19, another testis specific protein, has been identified in spermatocytes (Choi *et al.*, 2008).

Besides those HDAC1/HDAC2 containing protein complexes, a number of other multiprotein complexes with different intrinsic enzymatic activities have been shown to recruit HDAC1/HDAC2: the Polycomb repressive complex 2 (PRC2) confers histone H3 lysine K27 trimethylation (H3K27me3) to Polycomb group (PcG) target genes and can also recruit HDAC1/HDAC2 (Grimaud *et al.*, 2006). A variant of the Polycomb repressive complex 1 (PRC1) called CHRASCH, responsible for maintaining a repressive chromatin structure and inhibiting transcription, also contains HDAC1 (Huang and Chang, 2004). Moreover, interaction between *Drosophila* Hdac1 and a complex including the histone demethylase Lid, a trithorax group (trxG) protein has recently been described (Lee *et al.*, 2009). DNMT1, together with pRb, E2F1 and HDAC1 forms a complex that represses transcription from E2F-responsive promoters (Robertson *et al.*, 2000). Finally, HDAC1/HDAC2 interacts with a variety of repressive chromatin modifiers such as histone methyltransferase SUV39H1 (Czermin *et al.*, 2001; Vaute *et al.*, 2002) and DNA methyltransferases DNMT1 (Fuks *et al.*, 2000), DNMT3A (Fuks *et al.*, 2001), DNMT3B (Geiman *et al.*, 2004) and DNMT3L (Deplus *et al.*, 2002).

In summary, several key features of HDAC1/HDAC2 recruitment become apparent: (1) HDAC1/HDAC2-mediated deacetylase activity is tethered to its targets by various chromatin-binding proteins. These include transcription factors with DNA sequence specificity but also proteins recognising particular chromatin modifications. The latter scenario appears to support feedback loops between histone modifications and histone modifiers. (2) If not directly recruited by specific transcription factors, HDAC1/HDAC2 is delivered as part of a multiprotein complex. (3) These complexes often contain proteins with additional chromatin remodelling or histone modifying activities. Therefore the exact composition of the complex fine-tunes the specificity and biological outcome of HDAC1/HDAC2 recruitment and is likely to differ between target genes. (4) One function of HDAC1/HDAC2 as member of a multiprotein complex may be to “prepare” the chromatin for subsequent modifications performed by other enzymes associated with the same complex (e.g. prior to histone methylation, acetyl moieties have to be removed). (5) Finally, it is notable that silencing by multiprotein complexes with varying enzymatic activities ensures a certain robustness and results in multiple layers of repression.

Despite a high degree of variability and crosstalk within HDAC1/HDAC2-containing complexes, significant insight into HDAC1/HDAC2-dependent processes has been gained in recent years. Our current understanding how HDAC1/HDAC2 contributes to the regulation of developmental processes will be summarised in the following chapters.

## Early embryonic expression of HDAC1 and HDAC2

Expression of HDAC1/HDAC2 during embryogenesis has been studied in a variety of different organisms. HDAC1/HDAC2 is generally believed to be ubiquitously expressed, however mRNA and protein levels vary between species and early embryonic stages. In model systems analysed so far, HDAC1/HDAC2 is maternally provided and can be detected as soon as embryonic transcription is initiated (Dufourcq *et al.*, 2002). Throughout later embryogenesis, HDAC1/HDAC2 expression becomes spatially defined to distinct regions of ongoing organogenesis. In several species, HDAC1 expression is pronounced in the developing head region, including the central nervous system and brain (Damjanovski *et al.*, 2000; Mannervik and Levine, 1999; Mottus *et al.*, 2000; Pillai *et al.*, 2004). Most notably, in the chicken *G. gallus,* HDAC1 reveals a prominent expression "hotspot" at the tail end of the open neural tube, which diminishes after neural tube closure (C. Murko and O. Pusch, Medical University of Vienna, personal communication). Interestingly, HDAC2 is more broadly distributed, pointing towards functional diversification of the two paralog genes.

## Early embryonic patterning

### *HDA-1 in the development of* Caenorhabditis elegans

As mentioned above, *C. elegans* harbours two genes (*hda-1* and *hda-3*), which display similarity to HDAC1/HDAC2, but so far only the function of HDA-1 has been analysed in detail: inhibition of maternal and zygotic HDA-1 by RNAi leads to arrest in embryonic development at the one-fold stage and to embryonic lethality (Shi and Mello, 1998), although most cells exhibit tissue-specific differentiation and proper organisation of tissue layers. When the HDA-1 protein is depleted in first stage larvae by soaking in dsRNA, worms are sterile and develop ectopic vulvas (the multivulval or Muv phenotype) (Solari and Ahringer, 2000). The authors showed *via* genetic interaction studies that *hda-1* acts in both of the two functionally redundant synMuv pathways (synMuvA and synMuvB), together with other members of the NuRD complex. Remarkably, neither *hda-3* nor the HDAC3 homolog *hda-2* seem to be involved in this process. Dufourcq and co-workers analysed the consequence of zygotic inactivation of HDA-1 in *C. elegans*. They identified an *hda-1* genetic mutant and demonstrated that the protein is essential for proper gonadogenesis and vulval induction; cells of the somatic gonads were present and differentiated, however, the corresponding tissue was disorganised and the animals were sterile. Vulval induction was disturbed in two ways: the lineage decision of certain cells was affected and the multivulval phenotype already described was recapitulated. Another report from the same laboratory suggests that HDA-1 may play a role in the regulation of genes associated with tissue-specific functions (Whetstine *et al.*, 2005). Microarray analysis of mutant worms reveals HDA-1 as key regulator of genes involved in extracellular matrix (ECM) biology. Finally, by depletion of HDA-1 in L3 larvae *via* RNAi, Choy and colleagues defined a role for hda-1 in development of the male sensory ray (Choy *et al.*, 2007).

### *Hdac1 in the development of* Drosophila melanogaster *and polycomb group-mediated silencing*

Early embryonic patterning along the anterior-posterior axis in *Drosophila* is governed by expression of transcription factors in a highly defined spatial and temporal order: first, maternal effect genes establish a gradient along the anterior-posterior axis, which results in the expression of gap genes in broad bands. The different concentrations of gap gene products cause the transcription of pair-rule genes that divide the embryo into periodic units (i.e. stripes), which are further refined by the periodic expression of segment polarity genes. At the same time, those transcription factors interact to regulate homeotic genes, which determine the fate of each segment. Finally, maintenance of a homeotic gene expression state is taken over by Polycomb and trithorax group proteins, after the initial transcriptional regulators disappear.

From several recent studies we have learned that *Hdac1* (the only *HDAC1/HDAC2*-like gene in *Drosophila*; also known as *Rpd3*) plays an important role in multiple levels of developmen-

TABLE 1

***DROSOPHILA HDAC1* SHOWS GENETIC INTERACTION WITH VARIOUS GENES IMPORTANT IN DEVELOPMENT**

| Gene | Reference | Interaction | | Function | Targets |
|---|---|---|---|---|---|
| | | genetic | biochemical | | |
| *Groucho* | (Chen *et al.*, 1999) | + | + | Corepressor | *ND* |
| *SAP18/Bicoid* | (Singh *et al.*, 2005) | + | + | Corepressor/TF | *Hunchback* |
| *Knirps* | (Struffi and Arnosti, 2005) | + | + | TF | *Even skipped* |
| *Su(var)3-9* | (Czermin *et al.*, 2001) | + | + | HMT | *White* reporter gene |
| *Polycomb* | (Chang *et al.*, 2001) | + | + | PcG/PRC1 | *Sex Comb Reduced, Ultrabithorax* |
| *Posterior Sex Combs* | (Chang *et al.*, 2001) | + | + | PcG/PRC1 | *Abdominal-b* |
| *Polycomblike* | (Tie *et al.*, 2003) | + | + | PcG cofactor | *ND* |
| *Trithorax like* | (Canudas *et al.*, 2005) | + | *ND* | trxG | Fab-7 element |
| *Atrophin* | *(Wang et al., 2008)* | + | + (m) | Corepressor | *ND*, EGFR pathway |

Included are corepressors, transcription factors (TFs), histone methyltransferases (HMTs), and members of the Polycomb (PcG) and trithorax (trxG) gene groups. In most cases, (direct or indirect) biochemical interaction was demonstrated. *ND*, not determined; PRC1, Polycomb repressive complex 1; (m), interaction of mammalian homologs.

tal processes. Homozygous null mutations are lethal in the larval stage. The lethality is accompanied by a variable pair rule phenotype where usually odd numbered abdominal segments are lost (Mannervik and Levine, 1999). The pair rule genes fushi tarazu (*ftz*) and odd skipped (Balasubramaniyan *et al.*, 2006) are misregulated, pointing towards a role of Hdac1 as cofactor for the even skipped (*eve*) repressor, another pair rule gene regulating *ftz* and *odd* expression. However, others showed that Hdac1 is not necessary for *eve*-mediated repression of *odd* and suggest that the main role of Hdac1 is the maintenance of *eve*- and *runt*-induced repression rather than the establishment of repression patterns itself (Wheeler *et al.*, 2002). Recent screens identified Hdac1 to be involved in imaginal disc regeneration (McClure and Schubiger, 2008) and silencing of the proapoptotic genes *reaper* and *hid* during embryogenesis (Zhang *et al.*, 2008).

During later stages of *Drosophila* development, distinct functions of Hdac1 have mainly been investigated *via* genetic interaction screens. Using this approach, numerous genetic interaction partners of *Hdac1* have been identified, including corepressors, transcription factors, Polycomb group genes and others (see Table 1). Most of these genetic interactions have been confirmed by biochemical studies, verifying the presence of Hdac1 and its interaction partner in the same complexes.

Strikingly, *Drosophila Hdac1* cooperates with PcG genes in the repression of some target genes. It is not only found in complex with several PcG proteins but there is also a significant overlap in chromosomal localisation on polytene chromosomes (Chang *et al.*, 2001; Tie *et al.*, 2001; Tie *et al.*, 2003). However, it is noteworthy that this overlap is not complete and numerous PcG target genes are obviously not dependent on HDAC activity. Also, Hdac1 localisation is not restricted to PcG target genes, underlining its role as a more general regulator of transcription. Detailed analyses of high-resolution chromosome-wide localisation patterns (e.g. *via* ChIP-sequencing) will help to clarify the exact role of Hdac1 in PcG-mediated gene silencing in *Drosophila* and other organisms.

### *HDAC1 and HDAC2 in the development of* Mus musculus

Murine *Hdac1* and *Hdac2* are closely related genes that encode two enzymes with high sequence similarity (Khier *et al.*, 1999). Both enzymes are often found in the same multiprotein complexes, suggesting that HDAC1 and HDAC2 might have largely redundant functions. Interestingly, disruption of *Hdac1* (Lagger *et al.*, 2002; Montgomery *et al.*, 2007) or expression of a C-terminal mutated HDAC1 version (Ashe *et al.*, 2008) is sufficient to cause severe effects on embryonic development. Embryos are growth retarded, show several developmental defects such as disturbed head and allantois formation and die before day 10.5 of gestation. Embryonic lethality is accompanied by reduced proliferation rates and increased levels of the cyclin dependent kinase (CDK) inhibitors $p21^{WAF1/CIP1}$ and $p27^{KIP1}$ in ES cells (Lagger *et al.*, 2002). Interestingly, additional loss of $p21^{WAF1/CIP1}$ rescues the proliferation phenotype of HDAC1-deficient ES cells (Zupkovitz *et al.*, manuscript submitted). In contrast, the developmental phenotype of HDAC1-deficient embryos is not affected by additional ablation of $p21^{WAF1/CIP1}$, suggesting that other genes contribute to the developmental abnormalities. Along this line many genes involved in differentiation and development have been identified as HDAC1 targets (Zupkovitz *et al.*, 2006). Therefore, the origin of the developmental failure of HDAC1-deficient mice demands further investigation. In stark contrast to the essential role of HDAC1 during embryogenesis, depletion of HDAC2 does not display a phenotype during early development (Montgomery *et al.*, 2007; Zimmermann *et al.*, 2007). These findings point towards a set of crucial target genes exclusively regulated by HDAC1 during embryogenesis. Alternatively, HDAC1 and HDAC2 have similar functions, but the amount of HDAC2 protein is not sufficient to compensate for the loss of HDAC1. Accordingly, HDAC1 protein is expressed at significantly higher levels than HDAC2 in ES cells (Jennifer Jurkin and Christian Seiser, unpublished data). Deletion of either HDAC1 or HDAC2 in a wide range of tissues does not affect viability (Montgomery *et al.*, 2007) (Christian Seiser, unpublished results), suggesting redundant functions of HDAC1 and HDAC2 in many cell types.

## Neurogenesis

### In vitro *systems*

The first reports implicating HDACs in neuronal differentiation were based on experiments performed with the use of the general HDAC inhibitor (HDI) trichostatin A in the mid 90s in the PC12 cell line (Futamura *et al.*, 1995; Sano and Kitajima, 1996). To date a variety of HDIs has been tested in several cell culture systems displaying either inhibiting or promoting effects on neuronal outgrowth.

Recent studies directly implicate HDAC1/HDAC2 in nerve growth factor (NGF)-mediated differentiation, repression of neuronal gene expression and apoptosis or survival of neuronal cells. In 2005, Bai and co-workers linked HDAC2 to a specific function in NGF-induced differentiation of rat PC12 cells (Bai *et al.*, 2005). A different component of the epigenetic silencing machinery, DNA methyltransferase 3b (DNMT3b), was shown to be critical for neurite outgrowth. DNMT3b-dependent differentiation is mediated by recruitment of HDAC2. Most notably, the repressive action of the DNMT3b/HDAC2 complex was found to be independent of DNMT3b enzymatic activity, thereby suggesting a central role for HDAC2. The underlying mechanism most likely involves recruitment of HDAC2 and DNMT3b to target gene promoters in order to maintain silencing of genes that prevent neuronal differentiation. In a follow-up report, the same authors verified their hypothesis that the DNMT3b/HDAC2 repressor complex favours neuronal differentiation by identifying *T-Cadherin* (*T-Cad*) as a DNMT3b/HDAC2-regulated target gene (Bai *et al.*, 2006). Also working on NGF-mediated differentiation in PC12 cells, Zhang and colleagues showed that HDAC1/HDAC2 is recruited by a truncated version of p73, a member of the p53 tumour suppressor protein family, thereby repressing the promoter of the NGF-high affinity receptor TrkA (Zhang and Chen, 2007). The authors demonstrated that suppression of TrkA *via* the ΔNp73/HDAC1/HDAC2 complex attenuates the NGF-mediated MAP kinase pathway and leads to a block in neurite outgrowth. This finding may significantly contribute to understanding the mechanism of neuroblastoma pathogenesis, since high levels of TrkA and ΔNp73 have been linked to bad prognosis in neuroblastoma patients.

HDAC1 also seems to participate in regulating neuronal survival *via* E2F-mediated gene repression in cortical neurons and rat PC12 cells (Liu and Greene, 2001). Several studies have

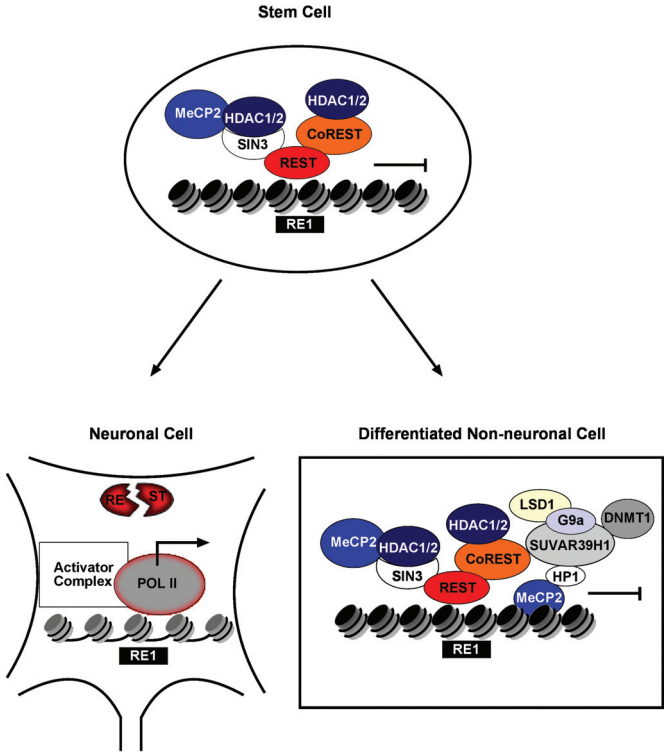


**Fig. 4. Chromatin state of neuronal genes in distinct stages of differentiation.** *In embryonic stem cells, neuronal genes with an internal RE-1 DNA element are associated with REST, its corepressor CoREST and additional factors such as SIN3A, HDAC1/HDAC2 and MeCP2. Although neuronal gene expression is almost entirely abolished, the chromatin is poised for consecutive activation. In addition to the core CoREST complex, inducers of heterochromatin are recruited to neuronal genes in differentiated non-neuronal cells. Due to recruitment of heterochromatic protein-1 (HP1), histone demethylase LSD1, histone methyltransferases SUVAR39H1/G9a as well as DNA methyltransferase-1 (DNMT1), neuronal specific gene expression is completely inhibited and heterochromatin formation is induced. In terminally differentiated neuronal cells, REST is proteasomally degraded, thereby relieving the repressive chromatin state. Polymerase recruitment by activator complexes leads to expression of neural specific genes. This schematic view does not mirror physical interaction of the subunits.*

shown that apoptotic stimuli like DNA damage promote CDK4/6 activity and consequent hyperphosphorylation of pRb leads to derepression of apoptotic E2F target genes in neuronal cells (Liu and Greene, 2001; Park *et al.*, 1997; Park *et al.*, 1996). Liu and colleagues revealed that the pocket protein family member p130 in complex with E2F4 recruits HDAC1 and the histone methyltransferase SUV39H1 thereby repressing pro-apoptotic genes such as *B-myb*. Following apoptotic stimuli in post-mitotic neurons, the p130/E2F4/HDAC1/Suv39H1 complex dissociates from the pro-apoptotic *B-myc* promoter triggering apoptosis and neuronal death. Recently, the histone-deacetylase related protein (HDRP) has been shown to exhibit neuroprotective functions in cultured cerebellar granule neurons *via* its interaction with HDAC1 (Morrison *et al.*, 2006). HDRP acquires HDAC activity by the recruitment of HDAC1, and in cooperation the apoptosis-associated expression of *c-jun* is prevented.

### *HDAC1 and HDAC2 in neuronal multiprotein complexes*

HDAC1/HDAC2-containing multiprotein complexes exhibit important functions in the regulation of neuronal gene expression. Among those, the CoREST complex acts as a crucial regulator of neuronal specific genes (Chong *et al.*, 1995; Schoenherr and Anderson, 1995) encoding for instance ion channels, synaptic vesicle proteins and neurotransmitter receptors. CoREST regulates transitions from pluripotent stem cells to neuronal progenitors and from neuronal progenitors to mature neurons (Ballas *et al.*, 2005). The DNA binding component of CoREST, REST/NRSF, mediates transcriptional repression of neuronal target genes *via* recruitment of HDAC1/HDAC2 through the corepressors mSIN3 and CoREST in non-neuronal cells (Ballas *et al.*, 2001; Grimes *et al.*, 2000; Humphrey *et al.*, 2001; Naruse *et al.*, 1999; Roopra *et al.*, 2000; You *et al.*, 2001). Furthermore, other epigenetic silencing factors like MeCP2, HP1, G9a, SUVAR39H1, LSD1 (Lunyak *et al.*, 2002; Roopra *et al.*, 2004; Shi *et al.*, 2004) and DNMT1 (Ballas and Mandel, 2005) are recruited to REST/NRSF-responsive genes in order to irreversibly silence the expression of neuronal genes in non-neuronal cells (Fig. 4). Interestingly, the chromatin of REST/NRSF-responsive genes in embryonic stem cells and neuronal progenitors lacks DNA methylation and recruitment of heterochromatin inducers such as HP1 or histone methyltransferases (Ballas and Mandel, 2005). Since HDAC1/HDAC2, mSIN3 and REST/NRSF are still associated with RE1 elements in stem cells, these epigenetic modifiers mediate a reversibly silenced, but permissive chromatin state, poised for subsequent activation. This model is supported by the fact that inhibition of HDAC activity leads to expression of REST/NRSF-responsive genes (e.g. neuroD) in adult rat neural progenitor cells (Hsieh *et al.*, 2004) but not in differentiated non-neuronal cells (Ballas *et al.*, 2005). Therefore HDAC1 and HDAC2 appear to be essential players in maintaining plasticity of REST/NRSF responsive neuronal genes.

The BRAF-HDAC complex (BHC) (Hakimi *et al.*, 2002) is a distinct HDAC1/HDAC2-containing multiprotein complex, related to the canonical CoREST complex and involved in neuronal gene silencing. The BHC complex is also recruited to RE1 sites of REST-responsive genes *via* the corepressor CoREST, suggesting a related function for both complexes.

### *HDAC1 and HDAC2 in the embryonic nervous system*

Most of our knowledge about the function of HDAC1/HDAC2 in the embryonic nervous system has been gained from studies using zebrafish. In 2002, results from an insertional mutagenesis study suggested *hdac1* as a regulator of multiple developmental processes in the embryo (Golling *et al.*, 2002). Two studies in 2004 verified Hdac1-dependent developmental defects by detailed characterization of the pleiotropic phenotype (Cunliffe, 2004; Pillai *et al.*, 2004). The analysis revealed severe neuronal abnormalities such as failure of neuron and glia cell formation in the hindbrain, loss of segmental organisation of post-mitotic neurons and associated glia cells, accumulation of neuronal precursors as well as a dramatic deficit in branchiomotor neurons (Cunliffe, 2004). The authors identified Hdac1 as a crucial repressor of the Notch-responsive transcriptional repressor her6, resulting in inhibition of proneural gene expression.

Loss of HDAC1 leads to impaired proliferation in several contexts of vertebrate development (Lagger *et al.*, 2002) and HDIs have been shown to induce growth arrest in various tumours and cell lines (Johnstone and Licht, 2003; Yang and Seto, 2007). However, this effect is proposed to be region- and tissue-specific, since Hdac1 promotes cell cycle exit and consequent neuronal differentiation in the zebrafish retina (Stadler *et al.*, 2005; Yamaguchi *et al.*, 2005). Yamaguchi and coworkers described an Hdac1 zebrafish mutant, displaying severe neuronal retinal differentiation defects and continuously proliferating neuronal progenitor cells. The authors suggest a model where Hdac1 represses the canonical Wnt pathway as well as Notch signalling in the developing zebrafish retina, thereby promoting cell cycle exit and initiation of retinal neurogenesis in zebrafish. In accordance with these data, another zebrafish mutant could be completely rescued by overexpression of canonical Wnt pathway antagonists (Nambiar and Henion, 2004). Recently, strong evidence was provided that Hdac1 not only regulates canonical Wnt signalling but also acts as a positive regulator of the non-canonical Wnt/PCP pathway controlling axial extension (Nambiar *et al.*, 2007). Furthermore Hdac1 is necessary for the differentiation of neural crest derived melanophores *via* repression of the *foxd3* gene (Ignatius *et al.*, 2008).

Finally, Hdac1 has also been implicated in the regulation of developmental signalling cascades such as Hedgehog and Fgf8 during embryonic neurogenesis (Cunliffe, 2004; Cunliffe and Casaccia-Bonnefil, 2006; Plaster *et al.*, 2007). Plaster and colleagues provided evidence for an *in vivo* function of REREa/Hdac-mediated transcriptional repression in promoting Fgf signalling, thereby patterning the telencephalon and maintaining the midbrain-hindbrain boundary (Plaster *et al.*, 2007). Cunliffe and Casaccia identified Hdac1 as an essential factor for differentiation of neuronal precursor cells to oligodendrocytes by facilitating hedgehog-mediated expression of the oligodendrocyte marker *olig2*.

In the mouse system MacDonald and Roskams reported that both HDAC1 and HDAC2 are expressed in neural progenitor and stem cells (MacDonald and Roskams, 2008). Strikingly, upon lineage specification into either glial cells or neuroblasts and post-mitotic neurons, HDAC1 and HDAC2 are found to be mutually exclusive expressed. Therefore, this report provides first evidence that following combined expression in a common progenitor, HDAC1 and HDAC2 gain specific functions throughout lineage specification.

Zinovyeva and colleagues identified an important function for *C. elegans hda-1* during neurogenesis in the developing worm embryo. The *hda-1* mutation produced a highly penetrant, uncoordinated locomotion phenotype with severe defects in axon pathfinding, fasciculation of the nerve cord and neuronal cell migration (Zinovyeva *et al.*, 2006).

## Myogenesis and cardiogenesis

### *HDAC1 and HDAC2 in skeletal muscle differentiation*

The transition from undifferentiated, proliferating skeletal muscle cells into mature multinuclear myotubes is governed by a set of transcription factors, which have the potential to turn on transcription of differentiation-specific genes. Among them, MyoD plays an important role in withdrawing differentiating cells from the cell cycle by upregulation of cyclin dependent kinase (CDK) inhibitors and in the activation of muscle specific genes. MyoD is constitutively expressed in undifferentiated myoblast cells as well as in differentiated myotubes; nevertheless it is unable to function as a transcriptional activator in undifferentiated myoblasts. The exact mechanism how MyoD activity is regulated remains unclear, though recent work has demonstrated that the control of MyoD activity is at least in part accomplished by an HDAC1/HDAC2-dependent mechanism. Exploiting a murine cellular model system for myogenesis, Mal and colleagues found that the MyoD protein is acetylated only upon differentiation. HDAC1 and HDAC2 were shown to bind to MyoD in myoblasts (Mal *et al.*, 2001; Puri *et al.*, 2001) and to inhibit acetylation of the MyoD protein and the chromatin of MyoD target genes (Mal and Harter, 2003), thereby maintaining a silenced state. Upon differentiation, HDAC1/HDAC2 is dissociated from MyoD by the hypophosphorylated pRb protein (Puri *et al.*, 2001), and this dissociation allows binding of the histone acetyltransferase PCAF to MyoD. The resulting acetylation of MyoD as well as the chromatin of MyoD target genes induces transcriptional activation of muscle-specific genes. In turn, pRb binds E2F, leading to repression of E2F-dependent genes implicated in cell cycle progression.

Another transcription factor essential for myogenesis, MEF2, has been demonstrated to recruit class II HDACs to repress muscle specific genes in myoblasts. However, there are reports also implicating HDAC1/HDAC2 in this process through CABIN1/MITR-dependent recruitment to MEF2 (Backs and Olson, 2006).

### *HDAC1 and HDAC2 in heart development*

Two reports define a crucial role of HDAC1/HDAC2 in heart morphogenesis, growth and function (Montgomery *et al.*, 2007; Trivedi *et al.*, 2007). Interestingly, while both laboratories used knock out techniques to inactivate HDAC2 in the mouse, one group reported postnatal death of all pups, while the other observed that only half of the pups died during the first 25 days. The survivors were reported to be smaller, lethargic and recovered after 2 months to develop normally. In both cases, the postnatal lethality was attributed to abnormalities of heart morphology such as thickening of the ventricular wall (due to increased proliferation), decreased size of the ventricular cavity and altered foetal cardiac gene expression. The remaining discrepancy concerning the penetrance of the postnatal lethal phenotype has not been resolved. However, possible reasons might be different genetic backgrounds of the mice used in the two studies. Alternatively, the mutation of HDAC2 created by partial deletion of the gene could lead to the formation of a hypomorphic allele or the expression of a truncated protein possessing dominant negative properties in one of the reported cases. To ask, whether the lethal phenotype of HDAC2 knockout mice reflects a cell-autonomous function of HDAC2 in cardiomyocytes Eric Olson's group conditionally deleted the HDAC2 gene in cardiomyocytes. The targeted mice were viable and showed no gross cardiac abnormalities, suggesting a vital function of HDAC2 in multiple cell lineages within the heart. In contrast, combined deletion of HDAC1 and HDAC2 in cardiomyocytes resulted in a strong phenotype: mice died 14 days after birth and displayed cardiac arrhythmias and severe

right and left ventricular dilation. The cause appears to be the increased expression of defined $Ca^{2+}$ ion channels resulting in pathological influx of $Ca^{2+}$ and increased expression of skeletal muscle-specific contractile proteins.

Together, these data demonstrate an important and partially redundant role for HDAC1/HDAC2 in heart development.

## Haematopoiesis

During haematopoiesis all blood and immune cells of an organism are generated from common lymphoid or myeloid progenitor cells. Haematopoietic transcription factors, such as GATA-1, GATA-2, NF-E2, EKLF, Tal-1/SCL, C-myb or PU.1 are recruited to lineage-specific genes and thereby determine the fate of progenitor cells. The majority of these transcription factors operate as transcriptional activators or repressors by equally recruiting histone acetyltransferases (HATs) and HDACs and controlling gene expression switches crucial for cell commitment.

The use of HDIs clearly showed that reversible acetylation is an essential mechanism for terminal differentiation e.g. for B cells (Lee, 2003). In addition, HATs and HDACs are not restricted to modifying histones, but are also capable of acetylating/deacetylating haematopoietic transcription factors. As a consequence, the function of these transcription factors is modulated due to acetylation-dependent changes in protein-protein interactions, DNA-binding affinity or transcriptional activation/repression (Huo and Zhang, 2005). The action of histone deacetylases is also essential to regulate fast on/off switching gene expression strategies of genes responsible for proper immune responses. A recent report implicated HDAC1 as a member of a ZEB1/CtBP2 repressor complex in the regulation of cytokine IL-2 in resting T cells (Wang *et al.*, 2009). Interestingly, the administration of bacterial superantigens leads to HDAC1 recruitment, silenced IL-2 expression and thereby promotion of T cell unresponsiveness and anergy (Kametani *et al.*, 2008).

### *Haematopoietic regulators influenced by HDAC1 and HDAC2*

*The IKAROS zinc finger protein family: IKAROS and AIOLOS*

A master regulator of early hematopoiesis is the haematopoietic cell-specific zinc finger DNA-binding protein family IKAROS, consisting of IKAROS, AIOLOS, HELIOS, EOS and PEGASUS. IKAROS regulates hematopoietic stem cell (HSC) activity and enables differentiation along the lymphoid lineage but restricts determination of myeloid fates (Georgopoulos, 2002). Therefore, IKAROS may act as a transcriptional repressor in addition to its function as an activator of transcription. In differentiating thymocytes and mature T cells, the majority of IKAROS protein is incorporated into the NuRD complex and to a lesser degree into the SWI/SNF complex comprising also HDAC1/HDAC2 (Kim *et al.*, 1999). Since the NuRD complex contains chromatin remodelling as well as HDAC1/HDAC2 deacetylase activities, "bivalent" chromatin regulation has been proposed. This bivalent Ikaros/NuRD complex may provide the potential for positive as well as negative transcriptional regulation of target genes, allowing lineage plasticity and the ability for differentiation of haematopoietic progenitor cells (Ng *et al.*, 2007).

Recently, HDAC2 was identified as a regulator of immunoglobulin *IgM H-chain and L-chain* genes in the chicken B cell line DT40. The authors reported that HDAC2 has a positive impact on the expression of IKAROS, AIOLOS, PAX5 and EBF1, which consequently repress transcription of *IgM H/L chain* mRNA (Nakayama *et al.*, 2007). Further studies also revealed a role for HDAC2 in the modulation of gene conversion frequencies of Ig genes (Lin *et al.*, 2008), but it remains unclear if HDAC2 is directly involved or acts through indirect regulation of other factors.

Disruption of AIOLOS in mice leads to an increase in pro-B and immature B cells and to a severe reduction in cycling B cells (Wang *et al.*, 1998). AIOLOS has also been suggested to control apoptosis (Romero *et al.*, 1999), exhibit tumour suppressor functions and affect autoimmune disease (Wang *et al.*, 1998). Interestingly, several splice variants of AIOLOS are differentially expressed and incorporated into SIN3 and Mi-2/NuRD complexes, leading to increased specificity and fine-tuning of AIOLOS action (Caballero *et al.*, 2007).

*Erythroid Krüppel-like factor (EKLF)*

The EKLF transcription factor is expressed in erythroid, megakaryotic and mast cells (Turner and Crossley, 1999). It has been shown that EKLF is crucial for providing a switch to high levels of adult $\beta$ *globin* expression during human and murine erythroid cell maturation. Direct acetylation of EKLF by CBP/P300 stimulates SWI/SNF chromatin remodelling complex recruitment and results in formation of the ERC-1 (EKLF co-activator remodelling complex 1) complex (Zhang and Bieker, 1998). ERC-1 recruitment leads to transcriptional activation of the $\beta$ *globin* locus. Conversely, Chen and co-workers demonstrated that EKLF alternatively interacts with mSIN3A/HDAC1 thereby acting as a transcriptional repressor. Since EKLF-mediated transcriptional repression can be relieved by the HDI TSA, HDAC activity seems to be essential for inhibition of target gene expression (Chen and Bieker, 2001).

*Growth factor independence-1 (GFI-1)*

*GFI-1* and *GFI-1b* encode zinc finger transcriptional repressors, proposed to function as regulators of cell death and cell cycle in hematopoietic cells. Recently, Saleque and colleagues showed that CoREST, LSD1, and HDAC1/HDAC2 are recruited by GFI-1B and consequently repress GFI target genes *in vivo* (Saleque *et al.*, 2007). This mechanism seems to ensure lineage-specific differentiation of cells arising from common myeloid progenitors.

### *Diseases associated with haematopoietic transcription factors*

*PU.1*

The Ets family transcription factor PU.1 is predominantly expressed in B cell and macrophage lineages, whereas its expression in erythroid precursors can lead to erythroleukemia in mice (Ben-David and Bernstein, 1991). It is generally believed that PU.1 balances proliferation and differentiation of haematopoietic precursor cells, most probably by regulating the acetylation state of haematopoietic transcription factors and histones (Huo and Zhang, 2005). A biochemical study in human 293T cells revealed that PU.1 associates with SIN3A/

HDAC1 and efficiently represses target genes such as *c-myc* (Kihara-Negishi *et al.*, 2001). It was later reported that PU.1 directly interacts with the methyl CpG-binding protein MeCP2, which in turn recruits SIN3A/HDAC1 (Suzuki *et al.*, 2003). This complex was proposed to prevent *β globin* gene expression in undifferentiated murine erythroleukemia cells.

*Stem cell leukemia factor TAL1 (SCL)*

The basic helix-loop-helix transcription factor TAL1 is highly expressed in erythroid cells and plays a crucial role during erythroid differentiation. Aberrant expression of TAL1 in T-lymphocytes has been linked to acute T cell leukaemia (T-ALL). Huang and colleagues have shown that TAL1 interacts with SIN3A/HDAC1 in murine erythroleukemia and human T-ALL cells preventing cellular differentiation (Huang and Brandt, 2000). Therefore, transcriptional repression mediated by TAL1/SIN3A/HDAC1 has implications for erythroid differentiation as well as leukemogenesis. In summary, HDAC1/HDAC2 is widely implicated in the regulation of haematopoietic gene expression, mainly *via* its presence in corepressor complexes. Epigenetic modifications seem to provide an essential means to confer lineage specification and cell commitment during haematopoietic development. In 2005, Cowley and colleagues identified a component of chromatin modifying complexes, mSIN3A, to be essential for T cell development (Cowley *et al.*, 2005). It will be of particular interest to deplete other members of chromatin modifying complexes, such as HDAC1/HDAC2 in the haematopoietic system.

## Epithelial differentiation

Epithelial structures are found throughout the organism and generally line cavities and surfaces in order to maintain essential functions such as secretion, selective permeability or protection of tissues. In 2004, Tou and colleagues specified a function for HDAC1/HDAC2 during mammalian epithelial and intestine development (Tou *et al.*, 2004). Since HDAC1/HDAC2 protein levels were significantly decreased around embryonic day E15, the authors hypothesized that at this time point tissue maturation required the expression of lineage specific genes, formerly repressed by HDAC1/HDAC2. Indeed, upon overexpression of HDAC1/HDAC2 in an *ex vivo* gut explant model, epithelial intestine-specific marker genes were significantly decreased whereas HDAC inhibition accelerated gut differentiation. Therefore, Tou and co-workers suggested that downregulation of HDAC1/HDAC2 during epithelial gut differentiation switches the chromatin state towards an active conformation allowing the expression of epithelial lineage specific marker genes.

### *Epithelial-mesenchymal transition*

An interesting feature of epithelial cells is their ability to perform epithelial-mesenchymal transitions (EMTs) (Moustakas and Heldin, 2007). During EMT, epithelial cells disassemble their junctional structures, express mesenchymal specific proteins, remodel their extracellular matrix and become migratory in order to generate novel tissue types in ongoing embryogenesis. One necessary feature during EMT is functional loss of *E-Cadherin*, which not only occurs in the gastrulating embryo, but also in malignant tissue transformations (Peinado *et al.*, 2007). Potent regulators of *E-Cadherin* are members of the Snail superfamily transcriptional repressors. In order to achieve repression of the *E-Cadherin* promoter and consequent induction of EMT, SNAIL1 recruits a SIN3A/HDAC1/HDAC2 complex *via* its SNAG domain in mouse cells (Hemavathy *et al.*, 2000; Peinado *et al.*, 2004). In *Drosophila* embryos, Snail associates with the corepressor CtBP (C-terminal binding protein) to exert its repressor functions (Nibu *et al.*, 1998) whereas the CtBP interaction domain is absent in mouse and human SNAIL proteins. The CtBP repressor complex also contains HDAC1/HDAC2, CoREST, G9a and LSD1 which are able to induce changes in histone modifications and consecutive repression of the *E-Cadherin* gene *in vivo* (Shi *et al.*, 2003).

### *Wnt signalling* via *β-Catenin*

Wnt signalling *via* β-Catenin and its nuclear partner T cell factor/lymphoid enhancer factor (TCF-LEF) is also implicated in EMT during gastrulation, morphogenesis, as well as in a variety of developmental processes and malignant transformations (Hoppler and Kavanagh, 2007; Kikuchi *et al.*, 2006; Logan and Nusse, 2004; Luo *et al.*, 2007; Willert and Jones, 2006). An important feature of canonical Wnt signalling is the stabilisation of nuclear β-Catenin and its association with TCF-LEF high mobility group transcription factors that mediate transcriptional regulation of Wnt/β-Catenin target genes. In the absence of Wnt signalling these genes are kept silent by CtBP or Groucho/TLE corepressor complexes (Willert and Jones, 2006). The Groucho/TLE repressor complex contains TCF, Groucho, histone H1 and HDAC1 and was originally identified in *Drosophila* (Chen *et al.*, 1999). The activation of TCF/LEF target genes by β-Catenin requires a two-step mechanism. The first step involves HDAC1 dissociation from LEF-1 and, as a consequence, the target gene promoter is inactive but poised for activation. Once HDAC1-dependent repression has been relieved, β-Catenin binds LEF-1 and activates downstream target genes of Wnt signalling (Billin *et al.*, 2000). In 2006, Olson and colleagues identified another unexpected strategy for β-Catenin to regulate cell-lineage determination in the pituitary gland (Olson *et al.*, 2006). In this tissue β-Catenin interacts with the tissue specific homeodomain factor Prop1, rather than TCF/LEFs, thereby activating the *Pit1* gene. Simultaneously, β-Catenin represses the paired like homeodomain factor *Hesx1* gene by recruitment of TLE/Groucho/HDAC1/Reptin corepressor complexes. This mechanism is suggested to generate diverse cell types from pluripotent precursor cells in response to Wnt signalling during organogenesis.

### *Deregulation of epithelial differentiation*

Due to high turnover rates of epithelial cells in an organism, a constant pool of epithelial stem and progenitor cells has to be present. One crucial hallmark of stem cells is self-renewal, promoted for example by Wnt signalling in gut epithelial cells. An increasing body of work suggests that these self-renewing embryonic stem cells might be the source of mutant cells, leading to hyperproliferation and cancer formation (Reguart *et al.*, 2005).

In a variety of human tumours such as gastric or prostate cancer, HDAC1 and HDAC2 have been found overexpressed,

indicating a possible function of HDAC1/HDAC2 in the onset and progression of cancer. Furthermore, HDIs have been used in tumour treatment, leading to cell cycle arrest, apoptosis and cellular differentiation of cancer cells. Consistently, a study revealed that HDAC2 deficient mice exhibited a decrease in intestinal tumour formation (Zimmermann *et al.*, 2007).

## Outlook

Thanks to the work of numerous laboratories we have gained considerable knowledge on the role of HDAC1/HDAC2 in development and differentiation in recent years. It is becoming clear that an extensive number of developmental decisions and differentiation programs depend on HDAC1/HDAC2 as co-factors. Often they act as part of bigger multiprotein complexes and HDAC activity is only one of several enzymatic activities. It will be a major challenge in the future to dissect the exact mode how individual target genes are regulated by multilayered silencing mechanisms and how different enzymatic activities interact and depend on each other. Combining systematic interaction screens, biochemical analyses and high-throughput technologies (as e.g. ChIP-sequencing) will be of great value to precisely define the contribution of HDAC1/HDAC2. The use of novel, more specific inhibitors will also be instrumental for a comprehensive and straightforward identification of target genes in different organisms, lineages and developmental stages. As a complementary strategy, analysing the increasing number of genetic mutants for HDAC1/HDAC2 in different organisms and tissues – from null alleles to point mutations – will allow to specifically address open questions of HDAC1/HDAC2 biology as e.g. substrate specificity, cell type specific functions, cross regulatory mechanisms, regulation of protein stability, activity and the influence of posttranslational modifications.

Finally, a better understanding of developmental decisions depending on HDAC1/HDAC2 will enable a rational use of HDAC inhibitors to modulate differentiation processes perturbed in numerous pathological situations.

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