Histone deacetylases: silencers for hire

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Over the past few years, the long-standing idea that covalent modification of chromatin can play a role in determining states of gene activity has been confirmed. Eukaryotic genes can be silenced by deacetylation of acetyllysine moieties in the N-terminal tails of histones. Recent work links histone deacetylases with an increasing number of repressors, suggesting that deacetylation might be a rather pervasive feature of transcriptional repression systems.

EUKARYOTIC DNA IS packed with core histones H2A, H2B, H3 and H4 into nucleosomes, which form the basic repeating units of chromatin. More than thirty years ago it was noted that transcriptionally active cells were enriched in acetylated histone forms, and this led to speculation that gene expression might be regulated by covalent modification of histone proteins. Over the past few years, these speculations have been dramatically vindicated, as studies of transcription have made clear that the acetylation status of histones is a key determinant of transcriptional activity. Transcriptional activators are often associated with histone acetyltransferases (HATs), and repressors can interact with histone deacetylases (HDACs) (reviewed in Ref. 5). The link between histone deacetylation and repression first became apparent when a mammalian histone deacetylase, HDAC1, purified based on its affinity for the histone deacetylase inhibitor trapoxin, turned out to be related to a known yeast transcriptional repressor, called RPD3 (Ref. 4). Subsequent work has greatly strengthened the link, as deacetylases are of particular interest as histone deacetylases with an increasing number of repressors, suggesting that deacetylation might be a rather pervasive feature of transcriptional repression systems.

The Sin3 histone deacetylase complex

Several nuclear proteins, including the unliganded heterodimeric nuclear hormone receptors Mad/Max and Mxi/Max and the yeast Ume6 transcriptional repressor, have been shown to repress transcription in vitro via an association with a histone deacetylase complex that contains the protein Sin3. That deacetylation results from these proteins binding to the Sin3 complex in vivo has been clearly demonstrated in the yeast system. Ume6 interacts with the Sin3–RPD3 complex leading to localized deacetylation of nucleosomal histones in vitro and transcriptional repression. Importantly, histone deacetylation depends on the presence of both Sin3 and RPD3. These studies provide evidence for the notion that DNA-binding factors recruit the Sin3–HDAC complex and bring about histone deacetylation, leading to transcriptional silencing. The mammalian Sin3 complex comprises at least seven subunits, including the two deacetylases HDAC1 and HDAC2, plus Sin3, RbAp48, RbAp46, SAP10 and SAP18. Several nuclear proteins, including the nuclear hormone receptors, are of particular interest as they can bind directly to helix 1 of histone H4 in vitro. These proteins might therefore be involved in silencing phenomena that involve deacetylation.

Further the range of gene silencing phenomena that involve deacetylation.

The NuRD complex

The Sin3 complex can deacetylate nucleosomal histones in vitro, although free core histones are effective substrates. The Sin3 complex is now extensive (Fig. 1a). Among the best understood are the nuclear hormone receptors, which, in the absence of hormone, bind to specific promoters and repress transcription. Hormone binding causes a conformational change in the bound receptor, converting it, with the help of other recruited proteins, to a powerful transcriptional activator. The unliganded retinoic acid receptor (RAR), thyroid hormone receptor (TR), antagomist-bound estrogen and progesterone receptors and the homeodomain repressor Rpx, all interact with the corepressor proteins SMRT (silencing mediator of retinoid and thyroid hormone receptor) and N-CoR (nuclear receptor corepressor). NuRD and the NuRD-like complex can deacetylate nucleosomal histone H4 in vivo.

The core-histone-binding subunits of the histone deacetylase complex. Accordingly, Xenopus RbAp48 has recently been shown to interact only with histone H4 in vitro. However, this region of H4 is normally buried within the nucleosome, making it unlikely that RbAp48 and RbAp46 subunits can bind to assembled nucleosomes alone. Indeed, the Sin3 complex can not deacetylate nucleosomal histones in vitro, although free core histones are effective substrates. Perhaps, additional factor(s) are required to open up the nucleosome, so that the complex can deacetylate nucleosomal core histones. DNA-binding factors that are responsible for recruiting the Sin3 complex to chromatin (see Table 1) could be involved in this, as Ume6 interacts with both DNA and the Sin3–HDAC complex to bring about targeted deacetylation of nucleosomal histones in vitro.

The list of mammalian proteins that repress via the Sin3 complex is now extensive (Fig. 1a). Among the best understood are the nuclear hormone receptors, which, in the absence of hormone, bind to specific promoters and repress transcription. Hormone binding causes a conformational change in the bound receptor, converting it, with the help of other recruited proteins, to a powerful transcriptional activator. The unliganded retinoic acid receptor (RAR), thyroid hormone receptor (TR), antagomist-bound estrogen and progesterone receptors and the homeodomain repressor Rpx, all interact with the corepressor proteins SMRT (silencing mediator of retinoid and thyroid hormone receptor) and N-CoR (nuclear receptor corepressor). NuRD and the NuRD-like complex can deacetylate nucleosomal histone H4 in vivo.
and NCOR (nuclear hormone receptor corepressor) (reviewed in Ref. 11). Bound SMRT and NCOR bring about transcriptional repression by recruiting the Sin3–HDAC complex: a parallel mechanism has been observed in non-vertebrate systems. The hormone ecysyne is not found in mammals, yet the transcriptional corepressor (SMRT), which associates with the Drosophila ecysyne receptor (Ecr), ultra-sensitive (USP) heterodimer, turns out to be related to the mammalian SMRT and NCOR (Ref. 12). SMRT interacts with Ecr and contains several repression domains, one of which can interact in vivo with both the Drosophila and mammalian Sm3. Thus, recruitment of Sm3–HDAC complex by various hormone receptors to repress transcription appears to be evolutionarily conserved.

### The c-Ski, Smad, NCOR and HDAC connections

Recently, the Sin3–HDAC complex has been implicated as a mediator of the effects of the hormone transforming growth factor β (TGF-β) on gene expression. The story began when a protein called c-Ski, which is the cellular homologue of the avian viral oncogene v-ski, was shown to associate with NCOR, Sin3A and HDAC1 (Ref. 13). Microinjection of anti-c-Ski antibodies and overexpression of c-Ski and its truncation products can relieve Mad–Mbp-induced repression, indicating that c-Ski plays a role in transcriptional repression by these proteins. A strong indication that the Mad-dependent repression pathway for this gene is defective in the absence of c-Ski comes from the in vivo finding that a c-ski knockout mouse embryo shows ectopic expression of the ornithine decarboxylase gene, which is a target for myc. These findings predict that c-ski should function as a transcriptional repressor when tethered to a reporter gene and this is indeed found to be the case14. c-Ski itself is not a DNA-binding protein, but the purified c-Ski complex binds preferentially to an 8-bp palindromic DNA sequence element, GTCCTAGAC (Ref. 14). This sequence is identical to the Smad-binding element (SBE) to which Smad3 and Smad4, two intracellular components of the TGF-β signalling pathway, bind. Upon stimulation by TGF-β, the type-I TGF-β receptor phosphorlyates Smad2 and Smad3, triggering a conformational change that causes both proteins to associate with Smad4. The heteromeric complex then translocates into the nucleus and activates downstream target genes (Fig. 2). Could it be that Smad proteins are the DNA-binding components of the c-Ski complex and mediate transcriptional repression via histone deacetylases, that are also part of the complex, in response to TGF-β signalling? The answer appears to be yes. Affinity purification of Smad3/4-associated proteins identifies c-Ski as an interacting partner and co-immunoprecipitation experiments reveal that c-Ski can bind to Smad2 and Smad3 (Ref. 15,16). In addition, overexpression of c-Ski leads to repression of a promoter containing a TGF-β-responsive element (Fig. 2), and cells whose growth is normally inhibited by TGF-β become refractory to growth-arrest in the presence of c-Ski. Thus, overexpressed c-Ski can antagonize the normal downstream consequences of TGF-β activity, perhaps by biasing the transcriptional response to the hormone towards the repression rather than the activation of genes. Smad2 also associates with HDAC1 in a complex with the homeodomain protein TGIF ('TGF-β-interacting factor')21. TGF-β expression, like c-Ski expression, attenuates the activation of a TGF-β-responsive reporter and represses Smad-activated transcription. The Smad-TGF-β-HDAC1 complex appears to be recruited to a TGF-β-responsive promoter through an interaction with DNA-binding transcription factors Fast1 or Fast2 (for ‘forkhead activin signal transducer’). Activin is a member of the TGF-β superfamily of secreted polypeptides. Fast1 and Fast2 both bind to related activin-response elements (ARE) and are essential for the activating signalling cascade. In addition, the Fast proteins interact with Smad2, and the interaction is required for activation/TGF-β-induced transcriptional activity of certain promoters containing SBE and ARE sequences. The interaction between TGF and Fast appears to be bridged by Smad2 (Ref. 17) (Fig. 2), and the formation of the Fast–Smad2–TGF–HDAC complex depends upon interaction with TGF-β and coexpression of TGIF. Importantly, treatment with the histone deacetylase inhibitor trichostatin A (TSA) is able to nullify the TGF-dependent repression of a TGF-β-responsive promoter.
in the presence of TGF-β, indicating that histone deacetylation is a critical component of repression by TGF-β.

These findings beg the question: what determines whether the nuclear Smad complex associates with coactivators such as p300 or corepressors such as c-Ski or TGIF? One possibility is that both coactivators and corepressors in the cells compete for the activated Smad complex, as coexpression of TGF-β leads to a reduction in the p300-Smad complex. The emerging complexity of the TGF-β signalling cascade illustrates the intricate regulation of gene expression that can be achieved by partitioning a DNA-binding protein between binding partners that are either coactivators or corepressors.

**p53, Ikaros and the REST**

The tumour suppressor protein p53 has a dual function as a transcriptional activator and repressor (reviewed in Ref. 18). The repression of previously activated genes by p53 (Ref. 19). A recent report sheds light on the mechanism of repression by p53, suggesting that histone deacetylation might be involved in p53-dependent repression. Co-immunoprecipitation experiments revealed that p53 exists in a complex containing Sin3 and HDAC1, but not SMRT. More directly, chromatin immunoprecipitation assays showed that Sin3 and p53 are associated with the Map4 promoter in vivo. Inactivation of p53 by a disrupted allele of the gene results in an increase in histone H3 acetylation at the Mad4 promoter. This result further strengthens the functional link between p53 and histone deacetylase. However, at the moment it is not clear whether p53 or another DNA-binding factor is responsible for targeting the Sin3-HDAC complex to p53-repressed genes. Interestingly, it has previously been shown that the sequence-specific DNA-binding activity of p53 can be enhanced through acetylation by its coactivator, p300 (Ref. 20). It is possible therefore that the biological activities of p53 itself can be modulated by histone deacetylase. In other words, HDACs might not only target histones, but might also deacetylate other proteins such as p53 in vivo.

The transcription factors Ikaros and Aiolos are implicated in the determination of the lymphoid lineage, and they too have been shown to associate with Sin3 by yeast two-hybrid and co-immunoprecipitation assays21. To test for the in vivo relevance of the interaction, Ikaros has been fused to a LexA DNA-binding domain and its ability to repress a reporter with LexA-binding sites tested in yeast. The feasibility of this experiment was suggested earlier as a LexA-HDAC fusion protein can repress transcription in the yeast system22. Similarly, LexA-Ikaros represses a LexA-lacZ reporter, albeit weakly. However, in an isogenic yeast strain with a disrupted sin3 gene, the repressive activity of LexA-Ikaros is lost, suggesting that repression by Ikaros is dependent on functional Sin3. In addition, repression is sensitive to deacetylase inhibitors, and chromatin immunoprecipitation experiments show that the targeted reporter gene is deficient in acetylated histone H3.

These data indicate that transcriptional repression by Ikaros involves histone deacetylation. The list of transcriptional regulators found to interact with histone deacetylases continues to grow. Many neuron-specific genes contain a RE-1 domain (repressor element-1) that restricts their expression in non-neuronal tissues. Accordingly, RE-1 is bound by a transcriptional repressor, called RE-1...
silencing transcription factor (REST). REST has recently been shown to interact with the Sin3-HDAC1 complex. Repression of RE-1-containing genes by REST plays a role in repression by REST through RE-1. Other nuclear proteins known to interact with histone deacytlaters (HDAC1 or HDAC2, or both) include the Drosophila co-repressor Groucho, the transcription factor Sp1 (Ref. 25), CBF1/RBP-J (Refs 26,27), Rb-associated protein 1 (RBP1) (Ref. 28) and breast cancer susceptibility protein 1 (BRCA1) (see Table 1). Repression by the members of the TF1 (transcriptional intermediary factor 1) and HP1 (heterochromatin-binding protein 1) families has been shown to be relieved by TSA, suggesting that these repressor proteins can also recruit histone deacytlaters to repress transcription. It begins to look as though deacytlation is a regular feature of transcriptional repression. The 6-2-containing histone deacytlaters complex (NuRD) There are other histone deacytlaters complexes besides the Sin3-HDAC complex. Most prominent among these is the NuRD (nucleosome remodelling histone deacytlaters) complex, which contains the distinctive Mi-2 protein. Mi-2 contains several recognizable sequence motifs, including PHD (plant homeodomain) zinc fingers, chromodomains and, most strikingly, a SWI2/SNF2 helicase/ATPase domain (Fig. 1b). The mammalian NuRD complex has been affinity-purified and has seven subunits: Mi-2, MT2, HDAC1, HDAC2, RhaAp48, RhaAp46 and MBD3 (Ref. 31). The Xenopus NuRD complex comprises the same subunits, plus a novel polypeptide, p66 (Ref. 32). MT2a is related to MTA1 (metastasis-associated protein 1), which was originally identified as the gene that is highly expressed in metastatic cells. MBD2 (methyl-CpG-binding domain protein 2) belongs to the family of proteins with a region homologous to the methyl-CpG-binding domain of MeCP2 (methyl-CpG-binding protein 2). This raises the tantalizing possibility that the nucleosome remodelling and histone deacytalysates activities of the NuRD complex might be targeted to methylated sites in the genome. However, the data on this issue are inconclusive at present. Although Xenopus MBD2 shows a strong preference for binding to methylated DNA in vitro, mammalian MBD2 discriminates weakly between methylated and non-methylated DNA (Ref. 33). Indeed, a fusion between mammalian MBD3 and green fluorescent protein (GFP) does not localize to densely methylated heterochromatic foci in living cells, and the mammalian NuRD complex, of which MBD3 is a component, does not bind selectively to methylated DNA in vitro. An MBD2-related protein has recently been observed in Drosophila melanogaster, whose genome is thought not to contain methylated cytosine. The methyl-CpG-binding domain of this protein is highly diverged in sequence (Ref. 33). The protein does not bind to methylated DNA in vitro, but can associate with Drosophila HEC1 and Mi-2, suggesting that it is part of the Drosophila NuRD complex. Thus, the function of the NuRD complex might not depend on an intrinsic DNA-binding subunit, but upon recruitment by other DNA-binding proteins. A mechanistic link between DNA methylation and histone deacytlaters was established initially for the methyl-CpG-binding protein MeCP2, which was shown to recruit the Sin3-HDAC complex (reviewed in Ref. 35). MBD2 is also a methyl-CpG-binding domain protein and is closely related to NuRD-complex component MBD3. However, unlike MBD3, mammalian MBD2 shows a strong preference for binding to methylated DNA in vitro and in vivo. It has recently been shown that MBD2 is a component of the long-sought methyl-CpG-binding complex MeCP1 and can repress transcription in a TSA-resistant manner. In addition to Mi-2, the MeCP1 complex contains histone deacytlaters. MBD2 also interacts with the mammalian NuRD complex in vivo, raising the possibility that MBD2 might recruit the NuRD complex to methylated DNA. Like the Sin3-HDAC complex, the NuRD complex appears to be recruited to DNA via DNA-binding factors. For example,
Ikaros and Aiolos, which are involved in the determination of the lymphoid lineage, have been shown to associate with the NuRD complex in T cells27. Ikaros and Aiolos were also found to interact with Mi2 in a yeast two-hybrid screen. Upon the activation of T cells, Ikaros, Aiolos, Mi2 and HDAC1 undergo dramatic relocalization to discrete structures in the vicinity of the heterochromatin, suggesting that Ikaros and Aiolos are involved in the recruitment of the NuRD complex to transcriptionally silenced regions of the genome40. Aiolos has also been shown to interact with the Sin3–HDAC complex, which is biochemically distinct from the NuRD complex41. These transcription factors might organize repressive domains in the nucleus by attracting different HDAC complex.

Other deacetylases

In addition to RPD3, yeast has several other histone deacetylases: HDAI, HOS1, HOS2 and HOS3 (Ref. 38). Database searching has led to the identification of mammalian homologues of HDAI, HDAC1–HDAC4, HDAC5–HDAC8 (Ref. 39) and HDAC6/mHDA2 (Refs 39–41). Two recent reports show that HDAC4 is associated with the MEF2 (myocyte-enhancer factor 2) family of transcription factors42,43, which are involved in the regulation of several muscle-specific genes. A MEF2-activated reporter is repressed by coexpression of HDAC4, and this repression is sensitive to TSA. Interestingly, ectopically expressed GFP–HDAC4 is predominantly localized in the cytoplasm42. Treatment of the cells with an inhibitor of nuclear export leads to an influx of cytoplasmic GFP–HDAC4 to the nucleus, indicating that HDAC4 is actively exported to the cytoplasm. Coexpression of nuclear HDAC2 results in colocalization of the two proteins in the nucleus. The data suggest that HDAC4 is recruited to appropriate promoters by the binding of MEF2 to DNA.

Mechanisms for repression by histone deacetylation

We know that recruitment of HDAC to a promoter can result in localized histone deacetylation44 and leads to transcriptional silencing. It is not known, however, whether histone deacetylation represses by specifically preventing the activation of transcription factors, or by inducing a higher-order chromatin structure that is incompatible with transcription (Fig. 3). The mechanism of transcriptional activation by acetylated histones is marginally better understood. The bromodomain of coactivator P/CAP (p300/CBP-associated factor) can interact specifically with acetyl-lysine, suggesting that acetyl-lysine residues in histone tails could serve as a signal for bromodomain-containing transcriptional regulators and thereby facilitate transcription45. Conversely, removal of this signal by deacetylation might impede the assembly or recruitment of transcriptional activators (Fig. 3). Recently, a functional link has been made between histone acetylation and the polyamines, which are abundant cellular polyamines46. Polyamines promote the oligomerization of nucleosomal arrays in vitro, but histone hyperacetylation can inhibit the polyamine-dependent condensation process. It is possible, therefore, that domains of deacetylated chromatin interact favourably with polyamines to form a higher-order chromatin structure that is incompatible with transcription (Fig. 3). Whether hypoaacetylated nucleosomal arrays form in vivo is not yet known.

Do histone deacetylases only deacetylate histones?

Although core-histone tails are physiologically substrates for histone modifying enzymes, such as the HATs and HDACs, mounting evidence points to the importance of acetylation and deacetylation of non-histone proteins in gene regulation. For example, acetylation of p53 and GATA-1 modulates their DNA-binding properties47,48. The nuclear hormone receptor coactivator ATRX can be acetylated by p300/CBP (Ref. 47). Acetylation of ATRX leads to dissociation of the coactivator complex with consequent transcriptional downregulation, suggesting that protein acetylation is involved in auto-regulation of hormone induction. As our awareness of nuclear proteins that are modified by acetylation increases, we can expect many more examples of transcriptional regulation by the addition or removal of acetyl groups.

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