INTRODUCTION

The acetylation of lysine residues is an important secondary modification that regulates protein function and is controlled by the action of acetylase and deacetylase enzymes. Class I histone deacetylases (HDACs) are recruited to, and activated by, cognate corepressor proteins that target HDAC activity to particular chromatin loci, resulting in the spatial and temporal control of gene expression. These large multiprotein complexes silence target genes through the removal of the acetyl groups from lysine residues in histone tails. The deacetylation of chromatin results in the formation of a higher-order, more condensed structure, leading to the repression of gene transcription (Grunstein, 1997; Struhl, 1998; Shogren-Knaak et al., 2006).

Class I HDACs have been reported to be associated with at least four major corepressor complexes. HDAC3 is recruited uniquely to the SMRT/NCoR repression complex (Guenther et al., 2000; Wen et al., 2000), whereas HDAC1 and HDAC2 are activated through recruitment into several corepressor complexes including the Sin3A (Laherty et al., 1997), CoREST (Humphrey et al., 2001), and NuRD (Xue et al., 1998; Zhang et al., 1999) complexes. Understanding the assembly of HDAC complexes is important since inhibitors of HDACs have an increasing number of therapeutic applications. Currently, inhibitors of class I HDACs are used in the clinic to treat cutaneous T cell lymphoma and may be useful in the treatment of other cancers and Alzheimer’s disease (Marks and Xu, 2009; Xu et al., 2011). The development of selective inhibitors that are specific for particular complexes may be important for effective clinical application.

Class I HDACs Share a Common Mechanism of Regulation by Inositol Phosphates

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SUMMARY

Class I histone deacetylases (HDAC1, HDAC2, and HDAC3) are recruited by cognate corepressor proteins into specific transcriptional repression complexes that target HDAC activity to chromatin resulting in chromatin condensation and transcriptional silencing. We previously reported the structure of HDAC3 in complex with the SMRT corepressor. This structure revealed the presence of inositol-tetraraphosphate [Ins(1,4,5,6)P4] at the interface of the two proteins. It was previously unclear whether the role of Ins(1,4,5,6)P4 is to act as a structural cofactor or a regulator of HDAC3 activity. Here we report the structure of HDAC1 in complex with MTA1 from the NuRD complex. The ELM2-SANT domains from MTA1 wrap completely around HDAC1 occupying both sides of the active site such that the adjacent BAH domain is ideally positioned to recruit nucleosomes to the active site of the enzyme. Functional assays of both the HDAC1 and HDAC3 complexes reveal that Ins(1,4,5,6)P4 is a bona fide conserved regulator of class I HDAC complexes.

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We recently reported the structure of HDAC3 in complex with the extended SANT domain from the SMRT corepressor (Watson et al., 2012). This structure revealed that the interaction between HDAC3 and the SMRT-SANT domain requires the presence of a D-myo-inositol-1,4,5,6-tetrakisphosphate [Ins(1,4,5,6)P4] molecule sandwiched at the interface of the two proteins. We showed that Ins(1,4,5,6)P4 is essential for the interaction of HDAC3 with the SMRT-SANT domain, but it remained unclear whether Ins(1,4,5,6)P4 is simply a structural cofactor or a bona fide regulator of complex assembly (and hence HDAC3 activity). However, it is important to note that there is no evidence for a pool of free HDAC3 awaiting Ins(1,4,5,6)P4 to mediate assembly with corepressors.

Sequence conservation suggests that other class I HDAC complexes may also bind inositol phosphates. To explore this possibility, we investigated the reported interaction between HDAC1 and metastasis-associated protein 1 (MTA1) from the NuRD complex (Toh et al., 2000; Manavathi and Kumar, 2007) and determined the structure of HDAC1 in complex with the adjacent ELM2 and SANT domains from MTA1. The structure reveals that this complex also has what appears to be an inositol phosphate binding pocket at the interface between the MTA1-SANT domain and HDAC1, suggesting that this is a common feature in class I HDAC:corepressor complexes.

Importantly, we found that the ELM2 domain from MTA1 mediates assembly of the HDAC1 complex independently of inositol phosphate. By analogy, we found that a similarly extended region of SMRT is able to mediate interaction with HDAC3 in the absence of Ins(1,4,5,6)P4. Addition of Ins(1,4,5,6)P4 to both complexes results in a dramatic increase in HDAC activity. Together,
these findings suggest that class I HDACs are constitutively assembled into corepressor complexes and that their activity is regulated by freely dissociating inositol phosphates. The role of the inositol phosphate is likely to involve “engaging” the SANT domain with the HDAC catalytic domain.

Comparison of the HDAC1 complex with that of HDAC3 reveals the stereochemical basis for the specificity of complex assembly. The ELM2 domain mediates dimerization of the complex and also has a conserved arm that wraps completely around the HDAC that is ideally positioned to allow the adjacent amino-terminal BAH domain to present nucleosomal substrates to the catalytic site of HDAC1, hence determining substrate specificity.

### RESULTS

#### Structure of the HDAC1:MTA1 Complex

To determine the structure of the HDAC1:MTA1 complex, HDAC1 was coexpressed with the ELM2-SANT domain from MTA1 (residues 162–335) in suspension grown HEK293 cells. Small crystals (15 μm) of the purified complex were obtained in 2 M ammonium sulfate. Diffraction data from three crystals were merged giving a complete data set to 3 Å (Table 1). The structure was solved by molecular replacement using the structure of HDAC3:SMRT (PDB code 4A69). The resulting maps were resolved by difference refinement using the starting model of the MTA1-SANT domain and HDAC1 when the two proteins were coexpressed in mammalian cells (Figures 3A and 3B), raising the possibility that inositol phosphate may be required to mediate interaction of the HDAC1:MTA1-SANT complex. Indeed the mutation of residues in MTA1 that we expect to coordinate Ins(1,4,5,6)P4 leads to a reduced interaction with HDAC1 (Figure S2).

While the isolated MTA1-SANT domain only forms a complex with HDAC1 when coexpressed in mammalian cells, the extended interaction between the ELM2 domain of MTA1 and HDAC1 would suggest that this region of the interface should mediate interaction independently of bound Ins(1,4,5,6)P4. Indeed, pull-down assays show that the ELM2 domain is sufficient to mediate interaction with HDAC1 when the complex is reconstituted in the absence of Ins(1,4,5,6)P4 (Figures 3C and 3D).

#### The Role of Inositol Phosphate in HDAC1 and HDAC3 Corepressor Complexes

We previously demonstrated that Ins(1,4,5,6)P4, which copurified with the complex from mammalian cells, was essential for the interaction between HDAC3 and the extended SANT domain from the SMRT corepressor (Watson et al., 2012). In a similar fashion, we were only able to obtain a complex between the isolated MTA1-SANT domain and HDAC1 when the two proteins were coexpressed in mammalian cells (Figures 3A and 3B), raising the possibility that inositol phosphate may be required to mediate interaction of the HDAC1:MTA1-SANT complex. Indeed the mutation of residues in MTA1 that we expect to coordinate Ins(1,4,5,6)P4 leads to a reduced interaction with HDAC1 (Figure S2).

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### Table 1. Data Collection and Refinement Statistics

| Data Collection | Space group | P 32 2 1 |
|-----------------|-------------|-----------|
| Cell dimensions: a, b, c (Å) | 108.2, 108.2, 133.2 |
| Cell dimensions: α, β, γ (°) | 90, 90, 120 |
| Resolution (Å) | 76.8–3.0 (3.18–3.0) |
| Rmerge or Rmerge | 16.3 (65.7) |
| Completeness (%) | 96.1 (97.6) |
| Redundancy | 3.0 (3.1) |

See also Figure S1 for information about protein expression and crystallization.

*The highest resolution shell is shown in parentheses.*

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| Redundancy                       | 3.0 (3.1) |

See also Figure S1 for information about protein expression and crystallization.

*The highest resolution shell is shown in parentheses.*
This means that MTA1 will be bound to HDAC1 whether or not inositol phosphate is present at the interface with the SANT domain. Thus, the SANT domain is “tethered” to but not “engaged” with HDAC1 in the absence of Ins(1,4,5,6)P₄.

As mentioned above, the conserved ELM2 specific motif of MTA1 interacts in an extended nonpolar groove on the surface of HDAC1. Significantly, this groove is essentially identical to a corresponding groove in the surface of HDAC3. This prompted us to ask whether there might be a similar motif in the SMRT corepressor that could interact in an analogous fashion. Figure 3E shows an alignment of the ELM2 specific motif with a motif in the amino-terminal region of SMRT (residues 351–363) and NCoR that we proposed might contribute to the interaction with HDAC3. To test this possibility, we made a number of extended constructs of the SMRT corepressor and explored their ability to interact with, and activate, wild-type HDAC3 (Figures 4A–4C). We also tested whether the longest of these SMRT constructs (residues 350–480) could interact with various mutant...
HDAC3 proteins that we have previously shown no longer interact with the extended SANT domain (residues 389–480). These HDAC3 mutants also lack HDAC activity, presumably as a consequence of impaired Ins(1,4,5,6)P4 binding. Significantly, we found that this longer SMRT construct is able to interact with all the HDAC3 mutants tested (Figures 4D and 4E). This supports the hypothesis that in vivo, SMRT will be tethered to HDAC3 even in the absence of Ins(1,4,5,6)P4.

When characterizing this HDAC3 complex with the extended SMRT domain, we observed a gradual loss in HDAC activity with time (Figure 5A). HDAC activity could be partially recovered by addition of Ins(1,4,5,6)P4 to the "aged" complex (Figure 5A), suggesting that the aging is in part through loss of Ins(1,4,5,6)P4. Similarly, dialysis of fresh complex against high salt led to an immediate loss in HDAC activity (Figure 5B). Strikingly, activity can be fully restored (and indeed increased further) by the addition of exogenous Ins(1,4,5,6)P4 (Figure 5C). Mutations in HDAC3 and SMRT that would be expected to impair Ins(1,4,5,6)P4 binding to the complex abolished the ability of added Ins(1,4,5,6)P4 to enhance HDAC activity (Figure 5D). The activation characteristics suggest an apparent Kd for activation of HDAC1:MTA1 by Ins(1,4,5,6)P4 is 5 μM—similar to that of the HDAC3:SMRT complex.

To further characterize Ins(1,4,5,6)P4 binding to the two HDAC complexes, we used native and tandem mass spectrometry to explore the constitution of the complexes (Figures 5H and 5I). The experimental mass of the HDAC1:MTA1 complex matched the calculated mass for the proteins (with bound ions). In contrast, the HDAC3:SMRT complex was found to contain an additional mass of 496 Da, corresponding to bound Ins(1,4,5,6)P4. The difference in the Ins(1,4,5,6)P4 retention by the HDAC1 and HDAC3 complexes may result from MTA1 having one fewer Ins(1,4,5,6)P4-interacting lysine residues than SMRT. Taken together, our findings strongly support the hypothesis that Ins(1,4,5,6)P4 has a regulatory role in class I HDAC complexes in vivo (Figure 5J).

A Folding Transition Mediates Dimerization of HDAC1 in the NuRD Complex

The ELM2 dimerization domain in MTA1 is located immediately amino-terminal to the SANT domain and is largely helical in character. In the structure of the complex with HDAC1, this domain is sandwiched between its homodimeric partner and the deacetylase, making extensive interactions with both proteins. Interestingly, circular dichroism studies show that in isolation this domain undergoes a dramatic folding transition on binding to HDAC1 so as to adopt a helical structure that mediates homodimerization of the whole complex.
The ELM2 dimerization domain is composed of four α-helices. The interface with HDAC1 is mediated by helices H1 and H3 and excludes 1,278 Å² from solvent exposure. In addition to important nonpolar interactions made by Trp199, Phe252, and Met255, there are a number of significant electrostatic interactions. Interestingly, mutation of the residues corresponding to either Trp199 or Phe252 in the related ELM2-SANT-containing corepressor protein MIER1 resulted in attenuated recruitment of HDAC1 (Ding et al., 2003).

Helices H1 and H4 of the ELM2 dimerization domain form the primary dimer interface with a smaller contribution from helix H2 (Figures 1D and 1E). In total, 28 nonpolar side chains are buried at the interface (14 from each monomer). The largely nonpolar and complementary nature of this dimer interface, as well as the extensive solvent excluded surface (2,332 Å²), indicates that this is a physiologically relevant interface and that the complete NuRD complex probably contains two HDAC enzymes. Given the similarity between HDAC1 and HDAC2, we envisage that the NuRD complex could contain either a homodimer of HDAC1 or HDAC2 or a heterodimer of the two enzymes. Consistent with this, HDAC1 and HDAC2 have often been reported to be both associated with the NuRD complex (e.g., Xue et al., 1998, Bantscheff et al., 2011). Importantly, the arrangement of the dimeric complex means that the active sites of the two HDACs are located on approximately the same face of the dimer, oriented approximately 50° away from the two-fold axis (Figure 1D). This may be functionally important, as such an orientation could potentially allow the complex to simultaneously target more than one nucleosome.

The overall sequence conservation for the ELM2 dimerization domain is low (Figure S4). However, the pattern of conservation suggests that the domain will adopt a similar fold in the related ELM2-containing corepressors—although helix H2 is lacking in the CoREST proteins (Figure S4). It remains to be seen whether all the related ELM2-containing corepressors form dimers and how many of the HDAC contacts are conserved.

Specificity of HDAC Complex Assembly
HDAC1 and HDAC2 are sister enzymes (83% identity) that are recruited to the same corepressor complexes (Laherty et al., 1997; Zhang et al., 1999; Humphrey et al., 2001). In contrast, HDAC3, which is 57% identical to HDAC1, is recruited uniquely to the SMRT and NCoR complexes (Guenther et al., 2000; Li et al., 2000; Yoon et al., 2003; Oberoi et al., 2011). Comparison of the structure of the HDAC3:SMRT complex with that of HDAC1:MTA1 reveals key differences between the two HDACs and their corepressors that dictate the specificity of complex assembly (Figure 6).

Given the similarity of the SANT domains of MTA1 and SMRT, we sought to determine whether HDAC1 and HDAC3 can discriminate between the SANT domains. Coimmunoprecipitation assays show that HDAC1 appears to be able to bind the SANT domains from both MTA1 and SMRT. In contrast, HDAC3 appears to bind exclusively to the SMRT-SANT domain (Figure 6A). Examination of the structures suggests that in the HDAC1:MTA1 structure there is a key electrostatic interaction between Glu325 in MTA1 and Arg36 in HDAC1 (Figure 6B). In the HDAC3:SMRT complex, the equivalent residues are Leu468 and Ala30. Calculation of the surface charge of HDAC3...
reveals a negatively charged surface that could not accommodate the negative charge of Glu325 in the MTA1-SANT domain and explains the specificity of HDAC3 for the SMRT-SANT. Interestingly, the bulkier side chains of Glu325 (MTA1) and Arg36 (HDAC1) cause helix H3 in MTA1-SANT to be slightly tipped away from the HDAC compared with the SMRT-SANT. This change is propagated to the supporting helices H1 and H2. This difference in SANT orientation matches with other amino acid differences (i.e., Gln26/Ala20, Tyr23/His17, and Asp104/Pro98 in HDAC1 and HDAC3, respectively).

DISCUSSION

The HDAC1:MTA1 structure provides insights into how the combined ELM2-SANT domains are able to recruit histone deacetylase enzymes. Together, the two domains make very extensive interactions with HDAC1, wrapping completely around the
The ELM2 and SANT domains are juxtaposed in at least 13 human corepressor proteins, most of which have been shown to recruit HDAC1 and/or HDAC2 (Toh et al., 2000; Ding et al., 2003; Lee et al., 2006; Wang et al., 2006; Bantscheff et al., 2011; Hao et al., 2011) (Figure S5). Sequence conservation strongly suggests that the mode of HDAC binding, as well as the involvement of an inositol phosphate cofactor, will be common to all these corepressor complexes. The conserved ELM2-specific motif binds in a conserved nonpolar groove on the surface of the HDAC. In MTA1, the region between the ELM2-specific motif and the SANT domain mediates dimerization of the complex, but sequence conservation suggests that this may not be the case for all ELM2-SANT-containing proteins.

Intriguingly, within the different classes of corepressors, the protein context of the ELM2-SANT domain differs significantly (Figure S5). The MTA and RERE corepressors have an amino-terminal BAH (bromo-adjacent homology) domain immediately adjacent to the ELM2 domain. The BAH domain has been shown to mediate interaction with nucleosomes and histone tails, consistent with a role in targeting repression complexes to chromatin (Callebaut et al., 1999). A crystal structure of the SIR3 BAH domain in complex with a nucleosome revealed a mode of binding that involves recognition of the tail of histone H4 (Armache et al., 2011). The finding that the BAH domain

Figure 5. Regulation of HDAC Complexes by Ins(1,4,5,6)P4

(A) Time course showing that HDAC activity of the HDAC3:SMRT complex declines over 12 days. The “aged” complex can be partially reactivated upon addition of Ins(1,4,5,6)P4, suggesting that the “aging” is in part due to dissociation of Ins(1,4,5,6)P4.

(B) HDAC activity of HDAC3:SMRT is decreased after salt displacement of Ins(1,4,5,6)P4 (1 M NaCl).

(C) Activation of 1 M NaCl-treated HDAC3:SMRT upon addition of Ins(1,4,5,6)P4.

(D) Wild-type HDAC3:SMRT is activated upon the addition of Ins(1,4,5,6)P4, whereas complexes with surface mutations within the Ins(1,4,5,6)P4 binding pocket do not respond to Ins(1,4,5,6)P4. Mutations in HDAC3, H17C, G21A, K25I, R265P, L288M, and R301A; mutations in SMRT, Y470A and Y471A.

(E) Ins(1,4,5,6)P4 is readily lost from the HDAC1:MTA1 complex during purification since the activity is unchanged after washing with 1 M NaCl. NaCl-treated HDAC1:MTA1 (1 M) can be activated by exogenous Ins(1,4,5,6)P4, but this activity is lost on further NaCl treatment.

(F) Ins(1,4,5,6)P4 stimulates the HDAC activity of HDAC1:MTA1.

(G) Wild-type HDAC1:MTA1 is activated upon the addition of Ins(1,4,5,6)P4, whereas complexes with surface mutations within the Ins(1,4,5,6)P4 binding pocket do not respond to Ins(1,4,5,6)P4. In (A)–(G), error bars indicate the SEM. Mutations in HDAC1, R270A and R306P; mutations in MTA1, Y327A, Y328A, and K331A.

(H) Native MS spectra of HDAC3 (bottom) in complex with SMRT and MS/MS of the 15+ charge state (top) showing additional mass corresponding to one Ins(1,4,5,6)P4 molecule bound.

(I) Native MS spectra of HDAC1 (bottom) in complex with MTA1 and MS/MS of the 17+ charge state (top) confirming no additional mass in the complex.

(J) Model to show engagement of the SANT domain on the addition of Ins(1,4,5,6)P4.
from ORC1 also recognizes histone H4 tails supports the concept that BAH domains are chromatin recognition modules (Kuo et al., 2012). If we assume that the MTA1-BAH domain interacts with nucleosomes in a similar fashion to that of SIR3, then it is immediately clear that the BAH domain would serve as a substrate presentation module (Figure 7A) for HDAC1. Of course the BAH domains of MTA1 may well act in combination with other chromatin-targeting domains to contribute histone binding specificity to the NuRD complex. This model explains why HDAC enzymes themselves appear to lack substrate specificity since chromatin recognition modules within the specific corepressor complexes would control substrate selection.

The RCOR1, RCOR2, and RCOR3 corepressors also contain an ELM2-SANT domain that recruits HDAC1 and HDAC2 to the CoREST complex. RCOR1 does not contain an amino-terminal BAH domain; instead, there is a second SANT domain that has been shown to mediate nucleosome binding (Yang et al., 2006). Between the ELM2-SANT domain and this second SANT domain is a region that forms a coiled-coil with the lysine demethylase LSD1. This coiled-coil is separated from the ELM2-SANT domain by a 70-amino-acid linker. Combination of the ELM2-SANT domain with the HDAC1:ELM2-SANT structure suggests that the demethylase and deacetylase enzymes are closely associated such that they can target histone tails on the same nucleosome (Figure 7B).

While it was previously thought that HDAC complexes are constitutively active, we recently showed that a potentially regulatory inositol tetraphosphate molecule [Ins(1,4,5,6)P$_4$] is sandwiched between HDAC3 and its cognate corepressor, SMRT. HDAC activity of both the HDAC3-SMRT and HDAC1:MTA1 complexes. On the basis of sequence conservation, we presume that inositol phosphates will activate all class I HDAC complexes that contain ELM2-SANT or equivalent domains. Interestingly, a major HDAC complex that does not contain an identifiable SANT domain is the ubiquitous Sin3A:HDAC1 complex. It remains to be established how HDAC1 is activated in this complex.

The apparent $K_d$ of both HDAC3 and HDAC1 complexes for Ins(1,4,5,6)P$_4$ is around 5 μM. If Ins(1,4,5,6)P$_4$ were to act as a physiologically relevant regulator of these HDAC complexes, then Ins(1,4,5,6)P$_4$ levels in the relevant cellular compartment would need to fluctuate around this concentration. We could not identify any reports of Ins(1,4,5,6)P$_4$ levels in the nucleus; however, the average concentration of InsP$_4$ (both enantiomers) in the cell has been reported to vary between 3.6 and 10.5 μM, depending upon the stage of the cell cycle (Barker et al., 2004). On the basis of our in vitro assays, this would equate to at least a 2-fold change in HDAC activity. The match between the apparent $K_d$ of binding to HDAC complexes with the cellular concentration of Ins(1,4,5,6)P$_4$ would seem to strongly suggest that the Ins(1,4,5,6)P$_4$ is regulatory in vivo.

It is interesting to compare the Ins(1,4,5,6)P$_4$ regulation of HDACs with the well-established regulatory activity of Ins(1,4,5,3)P$_3$ in the opening of calcium channels. The concentration of Ins(1,4,5)P$_3$ needed to give maximal calcium release is about 2 μM (Marchant and Taylor, 1997). The reported average cellular concentration of Ins(1,4,5)P$_3$ is 0.8–2.7 μM. The similarity in the ratio of these concentrations with those needed to activate the HDAC3 and HDAC1 complexes provides further support that Ins(1,4,5,6)P$_4$ is a bona fide regulator of HDAC activity.
**Molecular Cell**

**HDAC Activity Is Regulated by Inositol Phosphates**

![Diagram showing the interaction of the HDAC1:MTA1 dimeric complex](image)

Since Ins(1,4,5,6)P₄ and Ins(1,4,5)P₃ are both biologically relevant signaling molecules and differ only in a single phosphate group, it is important to ask how these signaling pathways are kept distinct, i.e., what is the specificity of Ins(1,4,5)P₃ receptors versus HDAC complexes. It is very clear from the structure of the HDAC1:MTA1-SANT domain to the coiled-coil (LSD1 interaction) domain. Sequence alignments suggest that an IP molecule (red) will mediate the interface between HDAC1 and MTA1-SANT.

**EXPERIMENTAL PROCEDURES**

**Protein Expression and Purification**

The process of substrate presentation and specificity is determined by the adjacent BAH domain serving as a chromatin recognition module.

**Crystallography**

Diffracting crystals of 15 μm were obtained by sitting-drop vapor diffusion at 20 °C against wells containing 0.1 M NaHEPES (pH 7.5), 2 M ammonium sulfate, and 5% PEG400. Single crystals were frozen in mother liquors with the addition of 15% glycerol (cryoprotectant), and data were collected at the Diamond synchrotron microfocus beamline I24, with use of a grid-scan tool to center the crystals (Aishima et al., 2010). Diffraction data from three crystals were processed with MOSFILM (Leslie, 2006) and then with AIMLESS. The structure was solved by molecular replacement with HDAC3:SMRT (PDB code 4A69) as a search model using Phaser (McCoy et al., 2007). The HDAC1:MTA1 structure was built through multiple rounds of rebuilding and refinement with REFMAC (Collaborative Computational Project, Number 4, 1994) and COOT (Emsley et al., 2010).
The final model contains amino acids 165–333 of MTA1 (chain A), amino acids 8–376 of HDAC1 (chain B), one zinc ion, two potassium ions, four sulfate molecules, and one acetate molecule.

**Immunoprecipitation**

Myc-MTA1 constructs and HDAC1-Flag, or Myc-SMRT constructs and HDAC3-Flag, were transfected individually or cotransfected into HEK293F cells as described above. Cells were lysed by sonication in buffer containing 50 mM Tris-Cl (pH 7.5), 100 mM potassium acetate, 5% v/v glycerol, 0.3% v/v Triton X-100, and Roche Complete Protease Inhibitor, and the insoluble material was removed by centrifugation. The supernatant was applied to BSA-blocked FLAG resin for 2 hr before three washes with 50 mM Tris-Cl (pH 7.5), 100 mM potassium acetate, and 5% v/v glycerol. For the reconstitution experiments, Myc-MTA1 supernatant was incubated with the resin bound HDAC1-Flag for 2 hr before three washes with 50 mM Tris-Cl (pH 7.5), 100 mM potassium acetate, and 5% v/v glycerol.

**Circular Dichroism**

MTA1 (residues 205–310) was expressed in E. coli Rosetta (Novogen). Initial purification was carried out with NIN TA and followed by ion-exchange chromatography. Protein was dialyzed into 50 mM Tris-Cl (pH 8), 100 mM NaCl, and 1 mM DTT and concentrated to 1 mg/ml. A circular dichroism spectrum was measured from 200–250 nm at 20°C with a spectropolarimeter (Jasco J-715). A melting curve was obtained by monitoring of ellipticity CD at 210 nm as the temperature was increased from 5°C to 95°C.

**HDAC Activity Assays**

HDAC3 and SMRT (residues 350–411) were coexpressed in HEK293F cells and purified on FLAG resin. HDAC3 activity was measured with the HDAC Activity Kit (Active Motif) and read with a Victor X5 plate reader (Perkin Elmer) as described (Watson et al., 2012).

HDAC1 and MTA1 (residues 162–335) were coexpressed in HEK293 cells, and HDAC1 activity was measured with the HDAC Activity Assay Kit (Millipore). In brief, purified HDAC1:MTA1 was incubated with 25,000 cpm [3H]-acetate biotinylated-histone H4 peptide captured on streptavidin agarose in buffer containing 50 mM Tris-Cl (pH 7.5), 50 mM NaCl, and 5% v/v glycerol for 2 hr at 37°C. The reaction was quenched with acid before the beads were pelleted by centrifugation for 2 min at 14,000 g. Released [3H]-acetate was measured with a scintillation counter (Beckman LS 6500).

**HDAC Activity Assay with Ins(1,4,5,6)P4 Titration**

HDAC3:SMRT (residues 350–480, 428–480, 350–427, 350–411) were coexpressed in HEK293 cells and purified on FLAG resin. HDAC3 activity was measured with the HDAC Activity Assay Kit (Active Motif) and read with a Victor X5 plate reader (Perkin Elmer) as described (Watson et al., 2012).

**Supplemental Information**

Supplemental Information includes five figures and can be found with this article online at http://dx.doi.org/10.1016/j.molcel.2013.05.020.

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