Structure of a Rpd3/HDAC holoenzyme complex bound to the nucleosome

Xuejuan Wang (✉ xuejuan@ustc.edu.cn)
University of Science and Technology of China

Yueyue Zhang
The First Affiliated Hospital of USTC, MOE Key Laboratory for Membraneless Organelles and Cellular Dynamics, Division of Life Sciences and Medicine, University of Science and Technology of China
https://orcid.org/0000-0003-2522-111X

Gang Cai
The First Affiliated Hospital of USTC, MOE Key Laboratory for Membraneless Organelles and Cellular Dynamics, Division of Life Sciences and Medicine, University of Science and Technology of China
https://orcid.org/0000-0001-8622-3907

Biological Sciences - Article

Keywords:

Posted Date: March 16th, 2023

DOI: https://doi.org/10.21203/rs.3.rs-2667786/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License. Read Full License

Additional Declarations: There is NO Competing Interest.

Version of Record: A version of this preprint was published at Cell Research on October 16th, 2023. See the published version at https://doi.org/10.1038/s41422-023-00884-2.
Abstract

The Sin3/HDAC complexes are highly conserved from yeast to humans and function by deacetylating histones, condensing chromatin, and modulating gene expression. Despite the continued analysis of HDAC inhibitors in clinical trials, our mechanistic understanding of the HDAC activity is hindered by the lack of a high-resolution structure of complexes from this family. Here we report the cryo-electron microscopy structure of Saccharomyces cerevisiae Rpd3S/Sin3B bound to a nucleosome, at atomic resolution. In the structure, two copies of Rco1 and two copies of Eaf3 are integrated through the scaffold subunit Sin3, which wraps the catalytic Rpd3/HDAC. Rco1 and Eaf3 recognize the H3 tails with a specific methylation pattern. N-terminal disordered region of Rco1 contacts the histones at the acidic patch. Furthermore, Rco1, Eaf3 and the Sin3 subunits also bind to extranucleosomal and nucleosomal DNA. These multivalent interactions not only facilitate detaching histone tails from nucleosomes but also optimally orient the complex to H3 tail, catalyzing deacetylation of H3K18 in our structure. The complex architecture and dimeric Rco1-Eaf3 heterodimer offer the ergonomics allowing one Rpd3S simultaneously engages two neighboring nucleosomes. Our study elucidates the central roles played by the non-catalytic subunits in a class I HDAC complex provides a structural framework for the understanding of HDAC regulation by histone modifications.

Full Text

Histone deacetylases (HDACs) are evolutionally conserved enzymes that remove acetyl modifications from histones and play a central role in epigenetic gene silencing\(^1,2\). Class I HDACs are promising targets for epigenetic therapies for a range of diseases such as cancers, inflammations, infections and neurological diseases\(^3\). Yeast Rpd3 is the founding member of class I HDACs, which forms two distinct complexes: the \(\sim 1.2\) MDa Rpd3L deacetylates histones at promoter regions, and the \(\sim 0.6\) MDa Rpd3S targets transcribed regions to suppress intragenic transcription initiation\(^4,5\). The presence of additional components and the acquisition of paralogs and isoforms in higher eukaryotes further aggravates the heterogeneous nature of HDAC complexes, limiting our understanding of the functional attributes of these complexes.

The yeast Rpd3S complex is a prototype for studying HDAC complexes in eukaryote. Rpd3S consists of three core proteins: Rpd3, Sin3, and Ume1, which is a chromatin stabilization module\(^6\). Sin3 is the scaffold subunit of the Rps3S complex\(^7\). Eaf3 and Rco1 are two dedicated chromatin binding proteins, which operate in a combinatorial manner in targeting the Rpd3S complex to transcribed chromatin\(^8\). Eaf3 chromodomain (CHD) recognizes methylated H3-K36 tails\(^9\) and the two adjacent plant homeodomains (PHD) of Rco1 subunit independently engage the unmodified N terminus of H3\(^10\). It was recently reported that Rco1 exists as a dimer within the Rpd3S complex and both subunits are required for full functionality of Rpd3S\(^7\). Until now, the subunit stoichiometry, complex architecture and the mechanism by which Rpd3S is targeted to chromatin and mediates its function is still poorly understood.
Here, we report the cryo–electron microscopy (cryo-EM) structure of the Rpd3S holoenzyme binding a nucleosome at 3.5 Å resolution (Extended Data Table).

Endogeneous Rpd3S holoenzyme purified to homogeneity (Extended Data Fig. 1) was used because a large quantity of high-quality complex is needed for the assembly. For our structural studies of Rpd3S interactions with chromatin, we decided to explore a specific functional configuration, with a structurally tractable substrate: a mononucleosome containing methyl-lysine analogs (H3K36me3) with a long link at one end. Binding of the nucleosome to Rpd3S was tested using electrophoretic mobility shift assay (EMSA) (Extended Data Fig. 2). The Rpd3S preparation displays HDAC activity, which could be stimulated by incubation with the H3K36me3 nucleosome (Extended Data Fig. 1c). After careful 3D classification, we obtained a cryo-EM reconstruction of Rpd3S to an average resolution of 3.7 Å (Fig. 1a, and Extended Data Fig. 3-4). This resolution enabled de novo model building of the Rpd3S-Nuc complex (Fig. 1b).

Notably, the structure of the Rpd3S complex shows that it contains two copies of Rco1 and two copies of Eaf3 (Fig. 2a, b). Therefore, one Rpd3S has at least six conserved chromatin-binding domains: two Eaf3 CHD and four PHDs, two per copy of Rco1. Two copies of Eaf3-Rco1 dimerize through Rco1 C-terminal coiled-coil and the dimer interface packs against Sin3 PAH3 (paired amphipathic α-helix) (Fig. 2b, c and Extended Data Fig. 5). The first Rco1-Eaf3 heterodimer binds to Sin3 extensively through PAH3 (paired amphipathic α-helix) and CTD (SIN3B C-terminal domain) (Fig. 2c, d). Whereas, the second heterodimer only loosely interact with Sin3 through PAH3, which enable a more flexible binding surface for nucleosomes (Fig. 2b, c). Strikingly, Sin3 wraps around the catalytic Rpd3/HDAC with its HID (the HDAC interacting domain), MD (Middle-domain) and CTD (SIN3B C-terminal domain) (Fig. 2b, d and f). Ume1 only peripherally contacts Sin3, which is visible only at low threshold (Extended Data Fig. 10). Moreover, Eaf3 MRG (MORF4 related gene) (Fig. 2f) and Rco1 PHD2 (Fig. 2d) also contribute to Sin3-Rpd3 interaction, implicating the possible allosteric regulation on HDAC activity. Rco1 interact with Eaf3 through its EBR (Eaf3 binding region) (Fig. 2e). Rco1 binds to Sin3 through two interfaces: an extended loop (SIR, Sin3 interacting region) of Rco1 with Sin3 MD (Fig. 2b) and SID1 in Rco1 with Sin3 gating loop (Extended Data Fig. 7). Our structure shows Rco1 interacts extensively with all other subunits and Rco1 homodimerization brings two Eaf3 molecules into the complex, which is the key in defining the Rpd3S architecture.

Multivalent interactions are a prevalent mode of chromatin recognition. Eaf3 and Rco1 synergistically recognize nucleosomes methylated at H3K36 (H3K36me) with high affinity and strong specificity. However, the Eaf3 CHD itself only binds very weakly and with little specificity to histone peptides. Intriguingly, Eaf3 DBR (DNA binding region) and CHD are self-contained in the full-length protein. In the Rpd3S-Nuc complex, Eaf3 CHD extend from the Eaf3 MRG and binds to H3K36me (Fig. 3a, b). In addition, CHD also harbors intrinsic DNA binding ability and make extensive interactions with the backbone of wrapped DNA at SHL (+0.5~+1.5). The extended conformation of the Eaf3 upon nucleosome binding, could also facilitate the its DBR to interact with linker DNA. These multivalent interactions with DNA and histone tails promote H3K36me accessibility on the nucleosome and Eaf3 engagement.
Sensing extranucleosomal DNA is critical for the ability of Rpd3S to bind DNA and grab histone tails for modification. Eaf3 and Rco1 PHD-SID (260-370) constitute a minimal chromatin recognition module of Rpd3S, which only interacts with nucleosomes containing linker DNA. Rco1 SID (322-370) stimulates preferential binding of Eaf3 to H3K36-methylated peptide, however, the 12 amino acids (310-321) between PHD and SID suppress SID-mediated Eaf3 activation. Notably, Rco1 residues K321 and K328 (within EBR, Eaf3 binding region) directly bind the backbone of extranucleosomal DNA (Fig. 3a, b). Mutation of K320-K321 significantly increased H3K36me-dependent histone peptide binding, which is probably recapitulated in the DNA binding of Rco1 EBR in the intact Rpd3S complex resulting in Eaf3 activation.

The function of the N-terminal of Rco1 is still uncharacterized. Surprisingly, the 18 residues (50-67) extend from the Rpd3S core onto the surface of the nucleosome and directly bind to histone acidic patch (AP) and we refer this region as AP binding region (ABR) (Figs. 2b, and 3a, c). This acidic patch could be employed by Rpd3S to stably anchor the complex to target the H3 tail substrate. Rco1 SIR (85-124) intertwines with the surface of Sin3 MD (Figs. 1 and 2b). Rco1 indeed plays a critical role in Rpd3 assembly and nucleosome binding. Consistently, deletion of almost any part of Rco1 imposes detrimental effects on Rpd3 functionality.

The Sin3 scaffold not only mediates the whole Rpd3S complex assembly but also directly interacts with DNA. Sin3 residues K936, K940, K941 and K1244 within CTD bind the backbone of the DNA at SHL -2 (Fig. 3a, d). Both the Eaf3 CHD-DNA interacting region (SHL +0.5/+1.5) and the Sin3 CTD-DNA contact point (SHL -2) are largely overlapping with the regions known to be covered by the histone H3 tails lying along the nucleosomal DNA helix. These features highlight the ergonomics and functional significance of the Rpd3S architecture. The length and slight curvature of the Rpd3S complex is such that the clamping grip of the enzyme on the nucleosome disk has the proper design to inherently induce the detachment of the histone tail from the nucleosomal DNA. Furthermore, the Sin3 CTD contact wrapped DNA at SHL -2 locks the Rpd3/HDAC into a fixed position pointing to the SHL -1, where the H3 tail extend through the DNA gyre. The Sin3 CTD-DNA and Eaf3 CHD-DNA interactions not only facilitate detach H3 tails from nucleosomal DNA but also orient the complex to a favorable position to optimally access the H3 tails for catalysis.

The side chains of the H3 tail substrate (1-24) penetrating into the substrate binding groove could be clearly identified from the cryo-EM density map (Fig. 3a, e). Only the H3 tail extended from SHL -1 in its fully extended conformation could satisfy the distance from H3K36 to active site (Extended Data Fig. 9). The ε-amino group of H3K4 side chain directly interacts with the carboxyl group of the D261 residue within Rco1 PHD finger (Fig. 3f), which recognizes unmodified N terminus of H3. Methylation of the H3K4 residue shall destabilizes the electrostatic interaction. Therefore, only the H3K4me0 tail could be grabbed by Rco1 which explain why Rpd3S is restricted from binding to promoter nucleosomes normally marked with H3K4me3. In addition, H3Q5 also interact with Rpd3 K98 residue. Through recognition of H3K4me0 by Rco1 and H3Q5 by Rpd3, an unmodified H3 tail was delivered into Rpd3/HDAC active site.
for catalysis. Although the Sin3 gating loop is in proximity of the active site, it doesn't interact with the histone tail substrate (Fig. 3g). Unexpectedly, the H3K18 residue is poised for catalysis which catalytic site was previously unknown by Rpd3/HDAC (Fig. 3g). The H3K18Ac is a marker of cancer progression and potential target of anti-cancer therapy. As H3K18Ac is deacetylated by Rpd3, class I HDACs are indeed promising targets for cancers therapy.

To track the conformational changes in Rpd3S upon nucleosome binding, we also determined the cryo-EM structures in its apo state (Fig. 4c and Extended Data Fig. 3). The resolution of the cryo-EM reconstructions was limited to 8-9 Angstrom due to the substantial flexibility and didn't allow model building. Without contacting nucleosome, the extended structures of the Eaf3 CHD and Rco1 ABR/SIR are invisible. The whole surface supposed to bind nucleosomes are concealed in the apo state Rpd3S, thereby the complex is immobilized in autoinhibition state (Extended Data Fig. 10). Since a large portion of Sin3 (1-655) is disordered, the allosteric modulation of the nucleosomal binding surface could probably result from the highly dynamic Sin3 N-terminal. H3K36me modified nucleosome are needed to stimulates synergistic conformational changes in Rpd3S that increases its affinity for chromatin, stimulating its HDAC enzymatic activity.

Rpd3S prefers dinucleosome substrates in vivo and in vitro and its deacetylase activity is further promoted when these nucleosomes are properly spaced from one another. In our cryo-EM reconstruction, only one copy of Eaf3 and Rco1 interacts with the substrate nucleosome. It is interesting to observe the dimerization interface of Eaf3-Rco1 is rigid in both apo state and nucleosomal binding state (Fig. 4a, c, and Extended Data Fig. 10). The dimeric Rco1-Eaf3 can function as a protein ruler and the distance between the two Eaf3-Rco1 heterodimers could allow Rpd3S simultaneously engages two neighboring nucleosomes. After superimposing our Rpd3S-Nuc structure in the dinucleosome model, the idle Eaf3-Rco1 copy is optimally positioned to interact with the neighboring nucleosome (Fig. 4b, d). The Rpd3S complex architecture and dimeric Rco1-Eaf3 heterodimer offer the ergonomics preferring dinucleosome substrates.

Understanding the assembly and function of specific HDAC complexes is important since HDAC inhibitors have steadily increasing therapeutic applications. However, currently available HDAC inhibitors are all pan-inhibitors with little or no HDAC isoform selectivity, exhibiting undesirable side effects. Sin3 HDAC complexes are conserved from yeast to humans. Human SinA/SinB HDAC are closely homologous to yeast Rpd3L/Rpd3S, respectively. Our strategy to utilize a structurally tractable nucleosomal substrate (H3K36me3, H3K4me0 and long linker DNA at one end) may facilitate further research to elucidate the unique functions of the distinct classes of Sin3 complexes. Our findings highlight the central roles played by the non-catalytic subunits in a specific HDAC complex and a more thorough mechanistic understanding will reveal novel approaches to target these complex-specific subunits with therapeutic intent.

Declarations
Acknowledgements: This study was supported by the Strategic Priority Research Program of the Chinese Academy of Sciences (XDB37010000), the National Natural Science Foundation of China (31922035, 32030057, 31890783 and 32271255) and the Fundamental Research Funds for the Central Universities (WK9100000032 and YD2070002012). Single particle cryo-EM data of Rpd3S were collected at the Center for Bio-imaging, Institute of Biophysics, Chinese Academy of Sciences. We thank X. Huang for technical help and support with electron microscopy and thank Z. Zhang for the cryo-EM data collection. Single particle cryo-EM data of Rpd3S-nucleosome complex was collected at the Cryo-EM Center at University of Science and Technology of China. We thank Y. Gao and P. Tang for technical help and support with electron microscopy. We thank L. Wang and L. Sheng for the initiation of this projection. We also thank T. Tian, J. Zang and Y. Shi for the help of nucleosome reconstitution and purification. Y.Z. prepared the Rpd3S-Nuc assembly, determined the structure, Y.Z. built the models with X.W.; X.W. designed experiments, analyzed data and wrote the manuscript with G.C. The authors declare no conflicts of interest. Coordinates and EM maps are deposited in the Protein Data Bank under accession codes EMD-35456 (Rpd3S apo state 1), EMD-35457 (Rpd3S apo state 2), EMD-35458 (Rpd3S apo state 3), EMD-35449, PDB ID 8IHM (Eaf3 CHD domain bound to the nucleosome), EMD-35450, PDB ID 8IHN (Rpd3S core complex), EMD-35455, PDB ID 8IHT (Rpd3S bound to the nucleosome).

References


https://doi.org/10.1074/jbc.M116.720193

https://doi.org/10.1016/j.str.2008.08.008

https://doi.org/10.1016/j.celrep.2014.12.027


https://doi.org/10.1016/j.molcel.2013.08.024

https://doi.org/10.1074/mcp.RA120.002078

https://doi.org/10.1038/nature03686

**Figures**
Figure 1

Cryo-EM structure of the Rpd3S-nucleosome complex. a, Front (left) and back (right) views of the cryo-EM composite map (see Methods) of the Rpd3S-nucleosome complex. Mononucleosomes containing methyl-lysine analogs (H3K36me3) with a long link at one end are used in the assembly. b, Corresponding views of the structural model of the Rpd3S-nucleosome complex. Rpd3S subunits and histones are colored as in Fig. 2a.
Figure 2

**Structural organization of Rpd3S.** a, Schematics showing domains and functional regions of Rpd3S subunits. Proteins are color coded, with those not structurally resolved in white. WD, tryptophan-aspartic acid; CHD, chromatin binding domain; DBR, DNA binding region; MRG, MORF4 related gene; ABR, acidic patch binding region; SIR, Sin3 interaction region; PHD, plant homeodomain; EBR, Eaf3 binding region; SID, Sin3 interacting domain; CC, coiled coil; PAH, paired amphipathic α-helix; MD, middle domain; HID,
HDAC interacting domain; CTD, C-terminal domain. The Eaf3-Rco1 copy II is in less saturated color than the Eaf3-Rco1 copy I. Color scheme for Rpd3S subunits and histones is used throughout all figures. b, Overall architecture of Rpd3S highlighted in transparent surface representation within the Rpd3S-nucleosome complex. c-f, Detailed interactions in the Sin3PAH3 – Rco1CC (c), the Sin3CTD-Rco1 I PHD2 (d), the Rco1 I EBR - Eaf3 I MRG (e) and the Sin3MD - Rpd3 - Eaf3 I MRG (f) interfaces.

Figure 3

Rpd3S-nucleosome interactions. a, An overview of the Rpd3S-nucleosome complex highlighting the interface of the Eaf3, Rco1 and Sin3 with the nucleosome. Dotted lines indicate regions enlarged in b, c and d. b, Close-up view of interactions between Eaf3 I CHD and Rco1 I with H3K36 tail, nucleosomal and extranucleosomal DNA. The electrostatic surface potential (-/+3.000) is shown on Eaf3 I CHD. The positively charged surface of the Eaf3 I CHD pack against the nucleosome DNA between SHL +0.5 and SHL +1.5. Several lysine residues of Rco1 I bind the extranucleosomal DNA. c, Close view showing the Rco1 I ABR contacts the acidic patch of the nucleosome (dotted red circle). d, Close view showing several lysine residues of Sin3 bind the nucleosome DNA at SHL -2. e, Structure of H3 tail (1-24 aa) penetrating into the active center of Rpd3S are shown as transparent EM density map fitted with the corresponding model. f, Close-up view of H3 tail depicting how the H3K4 and H3Q5 are gripped by Rco1 I and Rpd3, respectively. g, Close view showing the ε-amino group of H3K18 residue in the active site is poised for
catalysis. Rpd3 residues involved in interactions with H3 tail are depicted. Zn ion is highlighted in green. Sin3 gating loop doesn’t interact with the H3 tail substrate.

**Figure 4**

**Model of histone deacetylating by Rpd3S complex and two copies of Rco1-Eaf3 heterodimer is a unique feature.** **a**, A cartoon depicting the molecular architecture of the Rpd3S-nucleosome complex. Eaf3, Rco1 and Sin3 are involved in contacting DNA, binding to the H2A/H2B acidic patch and H3 tails. **b**, Superimposing our Rpd3S-nucleosome structure in a dinucleosomes model (PDB 1ZBB). The second nucleosome was slightly twisted to make the linker DNA aligned with the extranucleosomal DNA in our structure. **c**, Before engagement with the nucleosome, the Eaf3, Rco1 and Sin3 are flexible. The nucleosome binding surface is concealed in autoinhibition state. **d**, A cartoon depicting the Rpd3S complex architecture and dimeric Rco1-Eaf3 heterodimer offer the ergonomics allowing one Rpd3S simultaneously engages two neighboring nucleosomes.

**Supplementary Files**
This is a list of supplementary files associated with this preprint. Click to download.

- Extendeddata23Mar08.pdf