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Differential Properties of Transcriptional Complexes Formed by the CoREST Family

Álvaro P. Barrios,[a] Andrea V. Gómez,[b] Julián E. Sáez,[a] Giuseppe Ciossani,[b] Emanuela Toffolo,[c] Elena Battaglioli,[c,d] Andrea Mattevi,[b] María E. Andrés[a]

Nucleus Millennium in Stress and Addiction, Department of Cellular and Molecular Biology, Faculty of Biological Sciences, Pontificia Universidad Católica de Chile, Santiago, Chile[a]; Department of Biology and Biotechnology, University of Pavia, Pavia, Italy[b]; Department of Medical Biotechnology and Translational Medicine, University of Milan, Milan, Italy[c]; Consiglio Nazionale delle Ricerche, Institute of Neuroscience, Milan, Italy[d]

Mammalian genomes harbor three CoREST genes. *rcor1* encodes CoREST (CoREST1), and the paralogues *rcor2* and *rcor3* encode CoREST2 and CoREST3, respectively. Here, we describe specific properties of transcriptional complexes formed by CoREST proteins with the histone demethylase LSD1/KDM1A and histone deacetylases 1 and 2 (HDAC1/2) and the finding that all three CoRESTs are expressed in the adult rat brain. CoRESTs interact equally strongly with LSD1/KDM1A. Structural analysis shows that the overall conformation of CoREST3 is similar to that of CoREST1 complexed with LSD1/KDM1A. Nonetheless, transcriptional repressive capacity of CoREST3 is lower than that of CoREST1, which correlates with the observation that CoREST3 leads to a reduced LSD1/KDM1A catalytic efficiency. Also, CoREST2 shows a lower transcriptional repression than CoREST1, which is resistant to HDAC inhibitors. CoREST2 displays lower interaction with HDAC1/2, which is barely present in LSD1/KDM1A-CoREST2 complexes. A nonconserved leucine in the first SANT domain of CoREST2 severely weakens its association with HDAC1/2. Furthermore, CoREST2 mutants with increased HDAC1/2 interaction and those without HDAC1/2 interaction exhibit equivalent transcriptional repression capacities, indicating that CoREST2 represses in an HDAC-independent manner. In conclusion, differences among CoREST proteins are instrumental in the modulation of protein-protein interactions and catalytic activities of LSD1/KDM1A-CoREST-HDAC complexes, fine-tuning gene expression regulation.

Gene expression regulation is exerted by activator or repressor transcriptional complexes that are targeted to specific loci in the chromatin. CoREST was initially described as a transcriptional corepressor for REST (repressor element 1-silencing transcription factor [1]) and later purified as part of a transcriptional corepressor complex, comprising also the histone demethylase LSD1/KDM1A and the histone deacetylases 1 and 2 (HDAC1/2) (2, 3), here referred to as the LCH complex. REST recruits CoREST to specifically repress the expression of neuronal genes in nonneuronal cells and neural stem cells (4, 5). However, in neural stem cells, CoREST targets a group of genes which are not REST targets, suggesting other relevant mechanisms in neuronal specification (6, 7). A variety of transcription factors have indeed been shown to interact with CoREST and/or LSD1/KDM1A, thus bringing the LCH complex to transiently repress or silence target genes in both neuronal and nonneuronal systems (8, 9). For instance, in hematopoietic cells, the zinc finger transcription factor Gfi (growth factor independent) recruits CoREST to regulate the expression of target genes during erythroid differentiation (9, 10). CoREST also controls the expression of certain genes during physiopathological processes, as exemplified by its participation in repressing the heat shock (11) and proinflammatory (12) responses.

The components of the LCH complex operate sequentially and interdependently to epigenetically modify histones in the vicinity of regulated genes. First, HDAC1/2 deacetylates the H3 histone tail, allowing LSD1/KDM1A to demethylate mono- and dimethylated lysine 4 of histone H3 (13–15). The presence of CoREST in the complex is essential for the recognition of the nucleosomal substrate and its deacetylation and demethylation by HDAC1/2 and LSD1/KDM1A, respectively (14–17). CoREST is highly preserved throughout evolution, and homologs in *Drosophila* (18), *Xenopus* (19), and *Caenorhabditis elegans* (20), where it regulates specific cell phenotypes acquisition (18, 20–22), have been described. In humans, CoREST is encoded by the gene *rcor1* and has 482 amino acids with a molecular mass of 66 kDa (1). CoREST harbors three highly conserved domains: one ELM2 (Egl-27 and MTA homology 2) and two SANT (SWI/SNF, ADA, NCoR, and TFIIIB) domains (14). The ELM2 and the first SANT domain (SANT1) participate in the interaction with HDAC1/2 (3, 16, 23–25), whereas the second SANT domain (SANT2) binds to LSD1/KDMA1, forming a very tight protein complex (15, 17, 26).

Two other genes, *rcor2* and *rcor3*, with strong homology to the CoREST (CoREST1 hereafter) gene have been found in humans and in other species (27, 28). In humans, *rcor2* encodes CoREST2, a protein of 523 amino acids, whereas *rcor3* encodes CoREST3, a protein with four isoforms of 436, 449, 495, and 553 amino acids generated by alternative splicing. It has been shown that CoREST2 is capable of interacting with LSD1/KDM1A (29), and CoREST3 has been copurified in LSD1/KDM1A complexes (15). Although the three proteins exhibit high sequence identity, little is known about CoREST2 and CoREST3 transcriptional activities, their

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Address correspondence to Andrea Mattevi, andrea.mattevi@unipv.it, or María E. Andrés, mandres@bio.puc.cl.
Á.P.B. and A.V.G. contributed equally to this work.
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functional partnerships, and their expression profile in mammalian brain. Here we show the specific properties and functional partnerships of each CoREST protein and their expression in the adult rat brain.

MATERIALS AND METHODS

Animals. Adult male Sprague-Dawley rats weighing 250 to 300 g were used. Rats were maintained on a 12-h-light/12-h-dark cycle with food and water available *ad libitum*. The procedures were conducted in accordance with national and institutional policies (Comisión Nacional de Investigación Científica y Tecnológica [CONICYT] and Pontificia Universidad Católica de Chile).

Cell culture conditions. HEK293 and HEK293T cell lines were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum and 1% penicillin-streptomycin and maintained at 37°C and 5% CO_2.

Antibodies. Polyclonal anti-Gal4 DNA binding domain (DBD) (sc-577) and monoclonal anti-Myc (9E10) antibodies were obtained from Santa Cruz Biotechnology. Two monoclonal anti-CoREST1 antibodies were used: K72/8 from Neuromab and 612146 from BD Transduction Laboratories. Anti-HDAC1 (10E2), anti-HDAC2 (3F3), polyclonal anti-Myc (ab9106), polyclonal anti-CoREST3 (ab76921), and polyclonal anti-LSD1/KDM1A (ab17721) antibodies were obtained from Abcam. Polyclonal antibodies against LSD1/KDM1A (2139) and CoREST1 (07-455) were obtained from Millipore. Polyclonal anti-CoREST2 (HPA021638) and anti-α-tubulin (T5168) antibodies were obtained from Sigma.

Plasmids. To obtain recombinant Gal4-CoREST expression plasmids, full-length rat CoREST2 cDNA (NM_001013994.1) and rat CoREST3 cDNA (NM_001134985.1) were generated by PCR from pEXPRESS and cloned into the vector pSG424 in the KpnI and XbaI sites. The human Gal4-CoREST1 was described previously (1). Human Myc-CoREST1 was subcloned from pcDNA-CoREST (1) into the vector pCS2+MT, which encodes a 6× Myc epitope. Myc-CoREST2- and Myc-CoREST3-expressing plasmids were generated by PCR from pEXPRESS and cloned into pCS2+MT. Myc-CoREST2 lacking the ELM2 domain, Myc-CoREST2 with the ELM2 of CoREST1, and CoREST2 containing point mutations were generated using previously described protocols (30). Mutants of CoREST2 were subcloned from pCS2+MT into pSG424 using standard cloning methods. All plasmids encoding fusion protein constructs were sequenced across the junction to confirm that inserts were in frame. All constructs generated by PCR were completely sequenced.

Coimmunoprecipitation and Western blotting. HEK293T cells (3.5×10^6 cells) were transfected with 2.5 μg of Myc-CoREST1, Myc-CoREST2, or Myc-CoREST3 and harvested 48 h after transfection. Coimmunoprecipitation assays and Western blot assays were carried out as described previously (11). To quantify the amount of coprecipitated proteins (HDACs and LSD1/KDM1A), specific bands of Western blots were quantified with ImageJ software, and values were corrected by the relative amount of each Myc-CoREST protein precipitated.

Transient-transfection and reporter gene assays. Transient-transfection and reporter gene assays were performed as we described previously (30). We used the reporter plasmid G5S4tkLuc, which encodes firefly luciferase and is driven by a system containing 5 upstream activation sequences (UAS) and four elements for the SP1 transcriptional activator, upstream of the thymidine kinase promoter (0.102 μg), at molar ratios of 1:1, 1:0.5, and 1:0.25 relative to Gal4-CoREST expression vectors. Control experiments were carried out using equivalent molar amounts of the empty vector (pSG424) that encodes Gal4-DBD (amino acids 1 to 147). Suberoylanilide hydroxamic acid (SAHA; 0.5, 2.0, and 5.0 μM) and trichostatin A (TSA; 0.03, 0.1, 0.5, 1.0, and 2.0 μM) solutions or vehicle (dimethyl sulfoxide [DMSO]) was added to cultured cells 24 h before cell harvesting.

***In vitro* histone deacetylation assay (HDAC assay).** Immunoprecipitates with anti-Myc antibodies obtained from 2 mg of whole HEK293 cell extracts were tested for their associated histone deacetylase activity as we described previously (31). Briefly, immunoprecipitates were incubated for 60 min with a short peptide substrate containing an acetylated lysine residue that can be deacetylated by class I, II, and IV HDAC enzymes. We used fluorescent acetylated histone peptides as the substrate (Active Motif, Inc., Carlsbad, CA) and measured HDAC activity as relative fluorescence units (RFU)/h. HeLa nuclear extract (5 μg) was used as a positive control. Myc immunoprecipitates from mock-transfected cells were used as a negative control (see Fig. S2B in the supplemental material). The reaction was read using a Wallac 1420 Victor3 V microplate reader (PerkinElmer, Waltham, MA).

TABLE 1 Kinetic parameters of LSD1/KDM1A-CoREST1 and LSD1/KDM1A-CoREST3 complexes[a]

Complex	k_{cat} (min^{-1})	K_M (μM)
LSD1/KDM1A-CoREST1	7.35 ± 0.28[b]	5.12 ± 1.04[b]
LSD1/KDM1A-CoREST3	3.76 ± 0.15	16.39 ± 1.88

[a] Apparent steady-state kinetic parameters were determined by using a 21-amino-acid H3 peptide monomethylated at Lys4, as described previously (47).
[b] Data taken from reference 26.

Immunofluorescence assays. Immunofluorescence assays were performed essentially as described previously (32). Brain slices were incubated with anti-CoREST1 (612146 from BD Transduction Laboratories) for 48 h at 4°C, and then anti-CoREST2 (HPA021638 from Sigma) was added, followed by incubation for 12 h. Subsequently, slices were mounted on glass slides and incubated with secondary anti-rabbit IgG coupled to Alexa Fluor 594 and anti-mouse IgG coupled to Alexa Fluor 488. Finally, analysis and photomicrography were carried out with a confocal microscope (Olympus FV-1000).

Enzymatic studies. *Escherichia coli* overexpression, purification, and crystallization of the human LSD1/KDM1A-CoREST3 complex were carried out following the procedures previously described for LSD1/KDM1A-CoREST1 (26). Briefly, a His-SUMO-tagged recombinant form of LSD1/KDM1A (residues 123 to 852) was copurified with a glutathione *S*-transferase (GST)-tagged CoREST3 protein (residues 258 to 418 with reference to NP_001129695.1) by tandem-affinity chromatography followed by gel filtration on a Superdex200 column (GE Healthcare). The enzymatic activity of purified LSD1/KDM1A-CoREST3 was measured on a 21-residue H3-monomethylated peptide on Lys4 (Thermo Electron Corporation) by the peroxidase-coupled assay at 25°C using a Cary 100 UV/visible spectrophotometer (Varian Inc.) (33). The demethylation reaction was performed by adding 1 μM protein solution to the reaction mixtures (150 μl) consisting of 50 mM HEPES-NaOH buffer (pH 7.5), 0.1 mM 4-aminoantipyrine, 1 mM 3.5-dichloro-2-hydroxybenzenesulfonic acid, 0.35 μM horseradish peroxidase, and variable concentrations (2 to 100 μM) of the monomethylated H3-K4 peptide. The best fit was obtained with the equation describing a competitive inhibition by using GraphPad Prism 5 software (Table 1).

Structural biology. The LSD1/KDM1A-CoREST3 complex was crystallized by the hanging-drop vapor diffusion method at 20°C following previously described protocols (26). The resulting crystals were screened at beamlines of the SLS and ESRF synchrotrons, and data processing and scaling were carried out using MOSFLM (34) and programs of the CCP4 package (1994). The structure of the LSD1/KDM1A-CoREST1 complex (26) (PDB entry 2V1D) was used as the initial model for refinement after removal of all water atoms. Crystallographic refinement (Table 2) was performed with Refmac5 (35), and manual rebuilding was done with Coot (36). Pictures were produced with CCP4MG software (http://www.ccp4.ac.uk/MG/). Vertebrate CoREST protein sequences available at National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/protein) were compared. Protein sequence alignment (CoREST1, NP_055971.1; CoREST2, NP_775858.2; and the longest spliced variant of CoREST3 [CoREST3a], NP_001129695.1) was performed using the tools at the website of the European Bioinformatics Institute (http://www.ebi.ac.uk/Tools/msa/clustalw2/), and identity was calculated with the follow-

TABLE 2 Data collection and refinement statistics for LSD1/KDM1A-CoREST3 complex

Parameter	Result[a]
Space group	I222
Unit cell (Å)	$a = 118.0$, $b = 177.4$, $c = 235.8$
Resolution (Å)	3.0
R_{sym}[b] (%)	18.7 (82.3)
CC 1/2[b] (%)	99.2 (53.3)
Completeness (%)	99.8 (100)
Redundancy	5.1 (5.3)
I/σ	6.5 (1.3)
R_{cryst}[cd] (%)	19.7
R_{free}[d] (%)	23.6
RMS[e] bond length (Å)	0.012
RMS bond angles (°)	1.6

[a] Values in parentheses are for reflections in the highest-resolution shell.
[b] $R_{sym} = \Sigma_h\Sigma_i|I(h)i - \langle I(h)\rangle|/\Sigma_h\Sigma_i I_{(h)}$, where $I(h)i$ is the scaled observed intensity of the ith symmetry-related observation for reflection h and $\langle I(h)\rangle$ is the average intensity. CC 1/2 is the half-data-set correlation.
[c] The final model consists of residues 171 to 836 of LSD1/KDM1A, a FAD molecule, and residues 273 to 405 of CoREST3.
[d] $R_{cryst} = \Sigma|F_{obs} - F_{calc}|/\Sigma F_{obs}$, where F_{obs} and F_{calc} are the observed and calculated structure factor amplitudes, respectively. The set of reflections used for R_{free} calculations and excluded from refinement was extracted from the structure factor file relative to PDB entry 2V1D (26).
[e] RMS, root mean square.

ing formula: % amino acid identity = (number of identical amino acids/number of aligned amino acids) × 100. To search for functional structural domains, we used the UniProt database (http://www.uniprot.org/) and tools from EBI InterProScan (http://www.ebi.ac.uk/Tools/pfa/iprscan).

Statistical analysis. The nonparametric Mann-Whitney U test for comparison of 2 groups and two-way analysis of variance (ANOVA) followed by a Bonferroni *post hoc* test for comparison of multiple groups were used to determine the statistical significance of differences.

Protein structure accession number. Coordinates and structure factors for LSD1/KDM1A-CoREST3 have been deposited with the Protein Data Bank as entry 4CZZ.

RESULTS

CoREST2 and CoREST3 behave as transcriptional repressors. To evaluate whether CoREST2 and CoREST3 are transcriptional repressors, like CoREST1, we performed luciferase reporter assays using a promoter harboring 5 upstream activation sequence (UAS) elements for the yeast transcription factor Gal4 and 4 elements for the mammalian transcription factor SP1, which confers a high basal transcriptional rate. All CoRESTs fused to the Gal4 DNA binding domain (DBD) significantly repressed the expression of the reporter (Fig. 1A). CoREST1 showed the highest transcriptional repression among the three proteins. CoREST2 and CoREST3 exhibited transcriptional repression activities, although they were significantly lower than that of CoREST1 at all reporter/

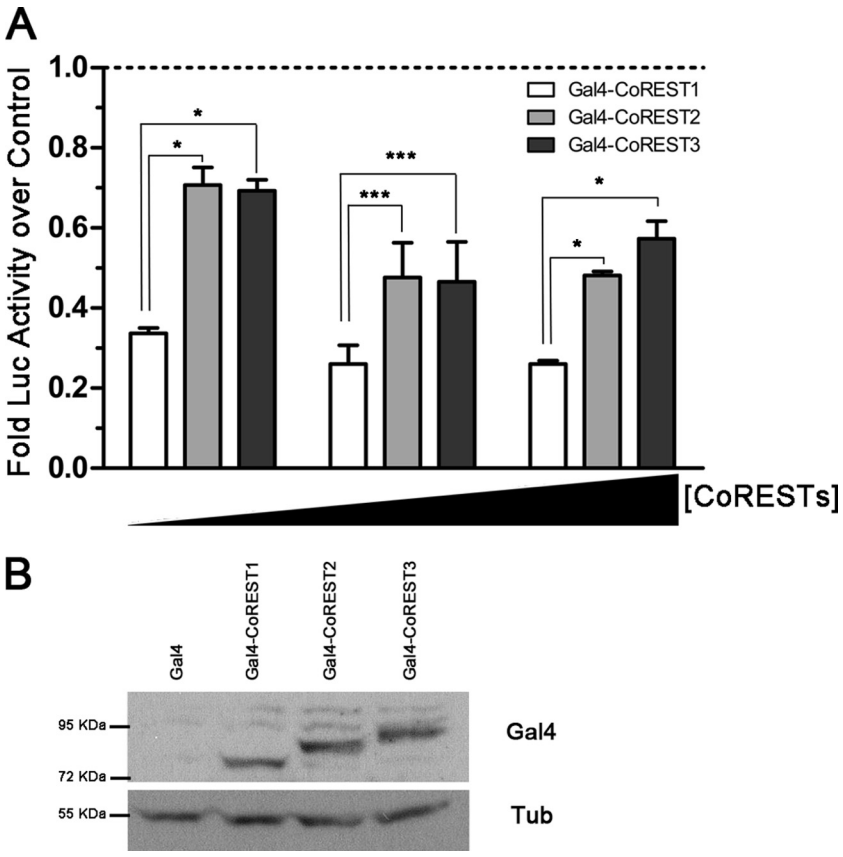

FIG 1 CoREST2 and CoREST3 are transcriptional repressors with lower repressive capacity than CoREST1. (A) Recombinant Gal4-DBDs fused to full-length CoREST1, CoREST2, and CoREST3 were assayed for their ability to repress the luciferase reporter gene (G5S4tkLuc) in HEK293T cells at increasing reporter/repressor molar ratios (1:0.25; 1:0.5, and 1:1). Values are means ± standard errors of the mean (SEM), expressed as the increase in luciferase (Luc) activity over the control (Gal4-DBD empty vector). *, $P < 0.05$, and ***, $P < 0.001$, according to the nonparametric Mann-Whitney U test. (B) Plasmids encoding Gal4-CoREST fusion proteins were transfected into HEK293T cells, and their expression was detected by Western blotting using an anti-Gal4-DBD antibody. Tubulin (Tub) was used as a loading control.

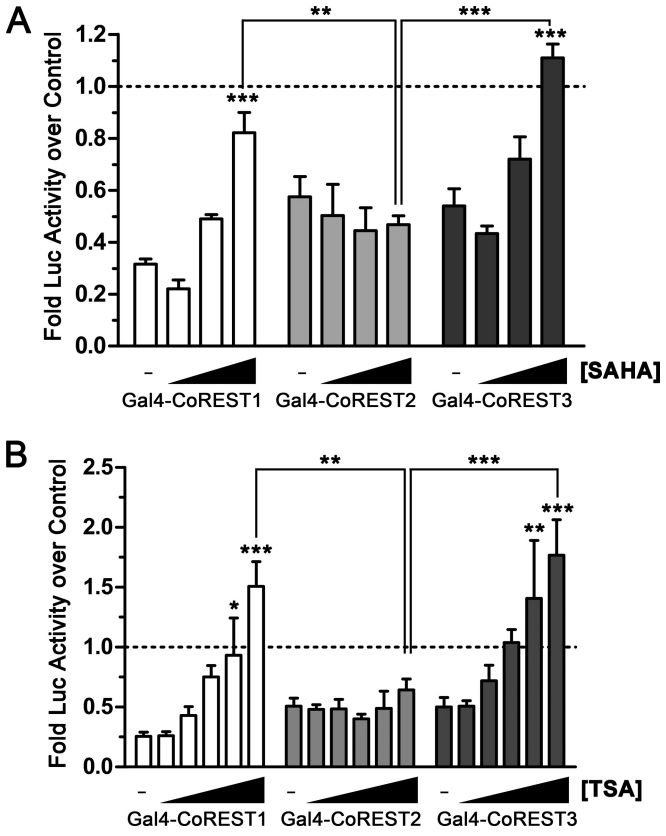

FIG 2 CoREST2-dependent transcriptional repression is insensitive to HDAC inhibition. The reporter plasmid G5S4tkLuc was cotransfected with each Gal4-CoREST-expressing plasmid in HEK293T cells. Twenty-four hours after transfection, cells were treated with different SAHA (0.5, 2.0, and 5.0 μM) (A) or TSA (0.03, 0.1, 0.5, 1.0, and 2.0 μM) (B) concentrations, and they were harvested 24 h later. Values are means ± SEM, expressed as the increase over control luciferase (Luc) activity values (Gal4-DBD empty vector) at every indicated inhibitor concentration. *, $P < 0.05$, **, $P < 0.01$, and ***, $P < 0.001$, according to two-way ANOVA.

repressor molar ratios (Fig. 1A). Gal4-CoREST protein levels in HEK293T cells were equivalent, and the proteins were located in the nuclei of the cells, demonstrating that the differences observed in transcriptional repression were not due to differences in the expression levels and/or subcellular distribution of the proteins (Fig. 1B; also, see Fig. S1 in the supplemental material).

Epigenetic modifications mediated by CoREST1 depend on its interaction with HDAC1/2 and LSD1/KDM1A. In order to determine whether histone deacetylation activity is involved in CoREST2- and CoREST3-dependent transcriptional repression, we studied the effect of HDAC inhibitors (37) on the transcriptional repression of Gal4-CoREST recombinant proteins. As shown in Fig. 2A (also, see Table S1 in the supplemental material), SAHA reversed the transcriptional repressor effect of Gal4-CoREST1 and Gal4-CoREST3 in a concentration-dependent manner. Conversely, Gal4-CoREST2 maintained its transcriptional repression in the presence of SAHA (Fig. 2A). Similar results were observed using TSA (Fig. 2B). These data suggest that the repressor capacities of CoREST1 and CoREST3 but not of CoREST2 depend on HDACs sensitive to TSA and SAHA.

Differential interaction of CoREST proteins with HDAC1/2. These first observations prompted us to test the ability of CoREST2 and CoREST3 to interact with HDAC1/2 by coimmunoprecipitation assays. In line with data presented above, Myc-CoREST2 precipitated a significantly smaller amount of HDAC1 and HDAC2 than Myc-CoREST1 and Myc-CoREST3, which coprecipitated the deacetylases with similar efficiencies (Fig. 3A and B; also, see Fig. S2A in the supplemental material). To further verify the different capacity of each CoREST protein to interact with HDAC1/2, we carried out *in vitro* histone deacetylase activity assays with each Myc-CoREST-isolated immunocomplex. We found that Myc-CoREST2 immunoprecipitates showed a significantly lower HDAC-associated enzymatic activity than Myc-CoREST1 and Myc-CoREST3 immunoprecipitates (Fig. 3C; Myc-CoREST2, 2.77- ± 0.26-fold greater than the control [$P = 0.0011$ versus Myc-CoREST1 and $P = 0.023$ versus Myc-CoREST3]), whereas Myc-CoREST1 and Myc-CoREST3 immunocomplexes exhibited HDAC-associated enzymatic activities 7.52- ± 0.62-fold and 10.37- ± 2.05-fold greater than those of the controls, respectively (Fig. 3C). Taken together, the data show that CoREST2 has a significantly lower capacity to form complexes with HDAC1/2.

A nonconserved leucine on SANT1 domain weakens the interaction of CoREST2 with HDACs. CoREST proteins are highly conserved through evolution, showing sequence identity ranging from 90% for CoREST1 to 95% for CoREST3, among reported mammal sequences, suggesting specific functions for each of them. To uncover particular features of CoREST2 explaining its lower interaction with HDACs, we compared human CoREST family members (Fig. 4A; also, see Fig. S3 in the supplemental material for complete CoREST sequence alignment).

First, we validated the notion that the ELM2 domain is essential for HDAC1/2 recruitment (23). As was reported for CoREST1 (24), we found that CoREST2 protein lacking the ELM2 domain (CoREST2ΔELM2) completely lost its weak ability to precipitate HDAC2 (Fig. 4B), and as expected, Myc-CoREST2ΔELM2 immunoprecipitates displayed barely detectable HDAC enzymatic activity (Fig. 4C). To reveal whether the differences in the ELM2 domain of CoREST2, compared with the ELM domains of CoREST1 and CoREST3, are responsible for its lower interaction with HDAC2, we switched the ELM2 domains between CoREST1 and CoREST2. The chimera CoREST2/ELM2(CoREST1), in which we replaced the ELM2 domain of CoREST2 with the ELM2 of CoREST1, had an increased capacity to precipitate HDAC2, which, however, was not accompanied by an equal increase in HDAC enzymatic activity (Fig. 4B and C). To uncover the residues of CoREST2 responsible for its lower capacity to interact with HDACs, we replaced four amino acids in the ELM2 domain of CoREST1 (Fig. 4A) with those found in CoREST2 (F169L, N174D, I175V, and P183A). These residues stand out in the sequence alignment because they are located in the highly conserved C-terminal part of the ELM2 and are identical in CoREST1 and CoREST3 but not in CoREST2 (Fig. 4A). However, these amino acid replacements on CoREST1 did not produce any effect on its interaction with HDAC1/2 (data not shown). Next we looked into the highly conserved SANT1 domain of CoRESTs, considering that many proteins that interact with class I HDACs also display the ELM2-SANT domain organization (23, 25). From the sequence alignment of the ELM2-SANT1 domain of CoREST proteins (Fig. 4A), we noticed that amino acid 165 in CoREST2 is a leucine, whereas in CoREST1,

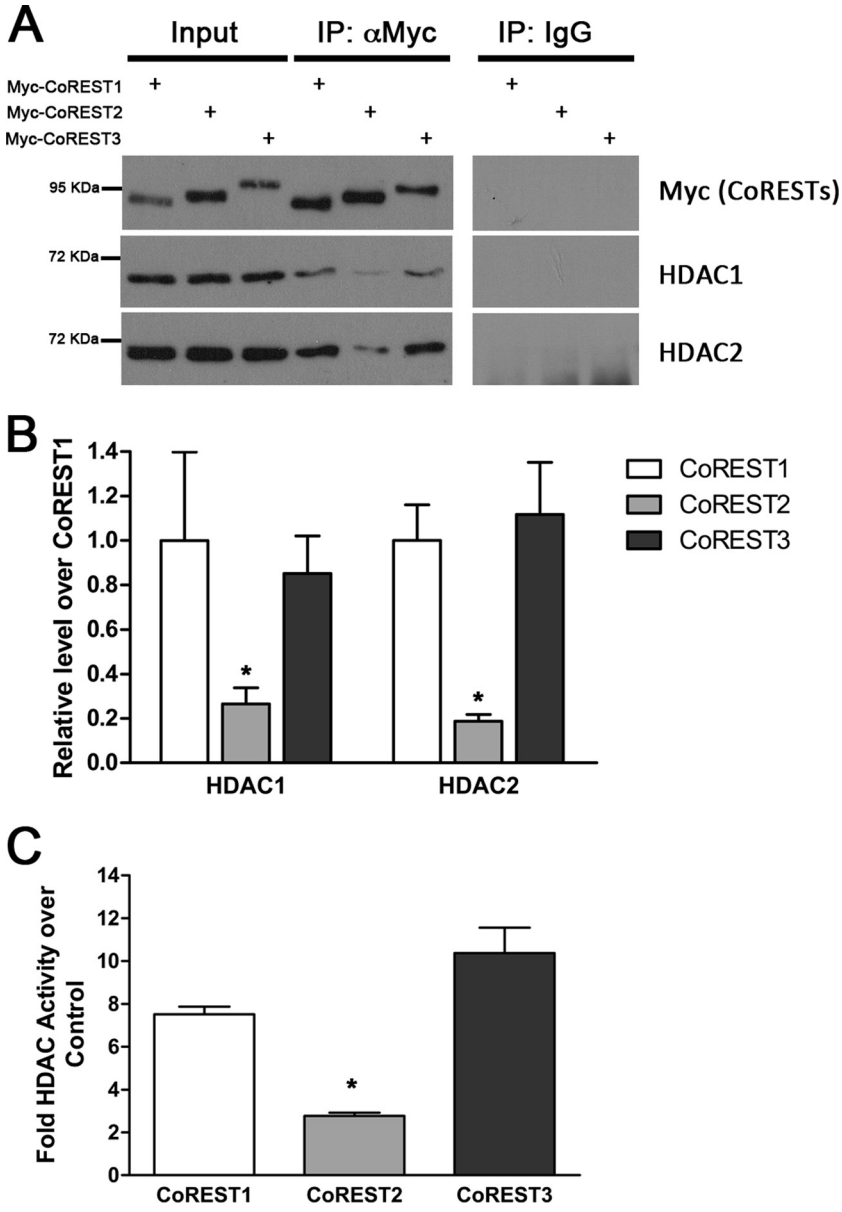

FIG 3 CoREST2 recruits less HDAC1/2 than CoREST1 and CoREST3. (A) Whole-cell extracts of HEK293T cells transfected with Myc-CoREST1, Myc-CoREST2, or Myc-CoREST3 were immunoprecipitated with anti-Myc antibody, and complexes were fractionated on SDS-PAGE. Specific anti-HDAC1 or anti-HDAC2 antibodies were used to detect endogenous HDAC1 and HDAC2 in Western blots. (B) Densitometry quantification of 3 independent experiments performed as described for panel A. The amount of HDAC1/2 precipitated is expressed relative to the amount precipitated with Myc-CoREST1, and values were corrected for the amount of each Myc-CoREST (CoREST1, -2, and -3) found in the precipitation. (C) HDAC activity associated with precipitated Myc immunocomplexes from whole extracts of HEK293 cells transfected as described for panel A. Values are means ± SEM from 3 independent experiments, expressed as the increase over the value obtained with mock transfection (transfection with Myc empty vector) and corrected by the amount of Myc-CoREST (CoREST1, -2, and -3) found in each precipitation. *, $P < 0.05$, according to the nonparametric Mann-Whitney U test.

CoREST3, and other HDAC-interacting proteins, the corresponding amino acid is a serine or a threonine (38). Remarkably, the mutation of leucine 165 to a serine in CoREST2 exhibited a drastic effect, inducing a significant increase in the capacity of CoREST2 to interact with HDAC2 (Fig. 4B), which was corroborated by a notable HDAC activity associated with this mutant (Fig. 4C). Altogether, the data indicate that leucine 165 in SANT1 is the main factor limiting the interaction of the ELM2-SANT1 domains of CoREST2 with HDAC1/2.

We studied whether the increased interaction and HDAC activity associated with the CoREST2L165S mutant resulted in a greater transcriptional repression capacity and/or sensitivity to the HDAC inhibitor SAHA. As shown in Fig. 4D, Gal4-CoREST2L165S displayed a transcriptional repression similar to that of wild-type Gal4-CoREST2, which is also insensitive to the presence of SAHA (Fig. 4D). Furthermore, CoREST2 without the ELM2 domain displayed the same transcriptional repression as the wild type and was similarly insensitive to the presence of SAHA (Fig. 4D). Taken together, the data indicate that CoREST2 transcriptional repression is independent of

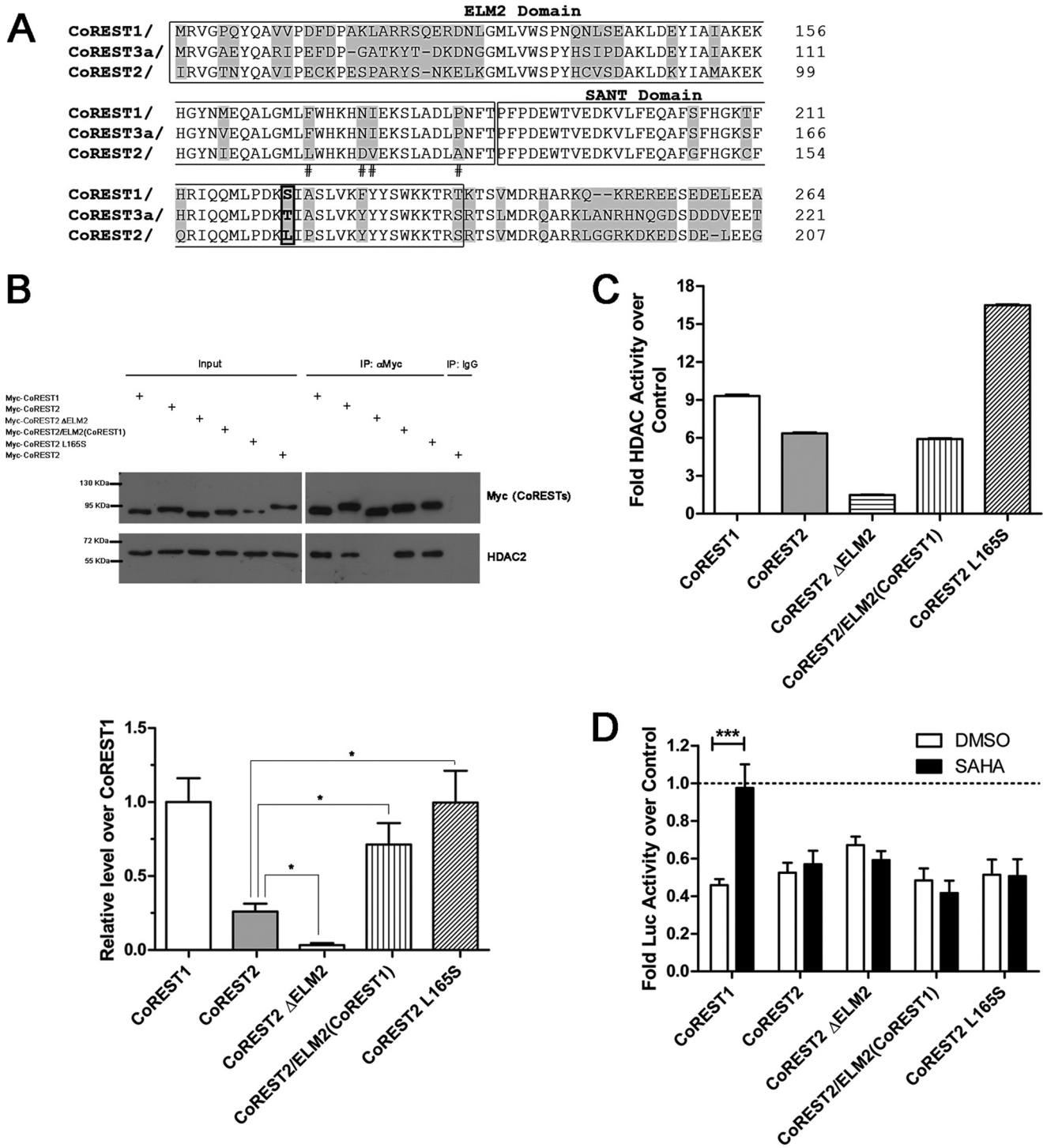

FIG 4 Leucine 165 of SANT1 domain controls CoREST2 interaction with HDAC2. (A) Sequence alignment of the ELM2-SANT1 region of *Homo sapiens* CoREST (CoREST1, -2, and -3) protein (NP_055971.1, NP_775858.2, and NP_001129695.1). Nonconserved amino acids in the three CoRESTs are shown in gray. #, amino acid corresponding to the site of a point mutation in CoREST1. The L165S mutation studied in CoREST2 and the amino acids in the equivalent position on CoREST1 and CoREST3 are boxed and in bold. (B) Whole-cell extracts of HEK293T cells transfected with recombinant wild-type Myc-CoREST1, wild-type Myc-CoREST2, chimeric Myc-CoREST2, and CoREST2 mutants were immunoprecipitated with anti-Myc antibody and fractionated on SDS-PAGE. (Top) Western blot assays of immunoprecipitates were carried out with anti-HDAC2 and anti-Myc antibodies. (Bottom) Densitometry quantification from 3 independent experiments. *, $P < 0.05$, according to the nonparametric Mann-Whitney U test. (C) HDAC activity associated with precipitated Myc immunocomplexes from whole-cell extracts of HEK293 cells transfected as described for panel B. Values correspond to one experiment performed in duplicate, expressed as the increase over the activity in mock transfection conditions and corrected by the amount of Myc-CoREST2 mutant found in each precipitation. (D) The reporter plasmid G5S4tkLuc was cotransfected with each Gal4-CoREST2 mutant-expressing plasmid into HEK293T cells. Twenty-four hours after transfection, cells were treated with 5.0 μM SAHA, and they were harvested 24 h later. Values are means ± SEM from 4 independent experiments performed in triplicate and are expressed as the increase over control values (Gal4-DBD empty vector). ***, $P < 0.001$, according to two-way ANOVA.

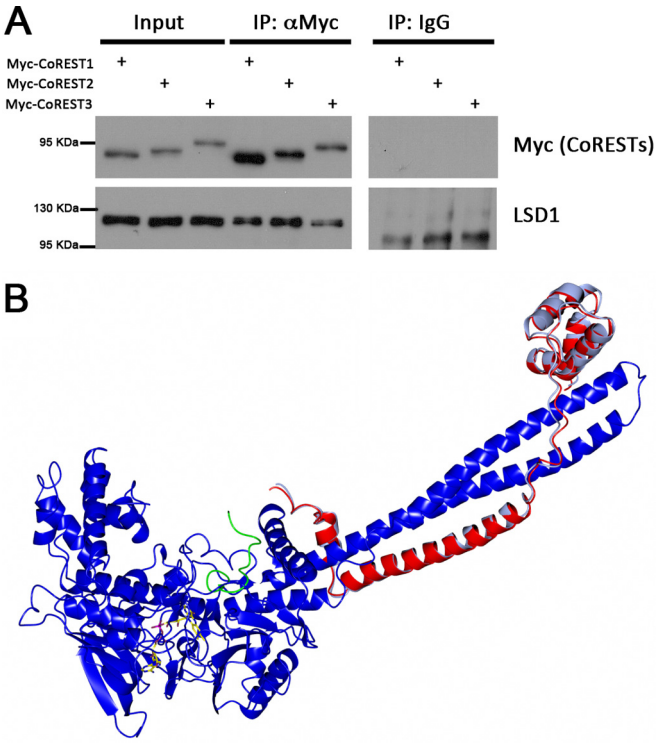

FIG 5 CoREST 2 and CoREST3 interact with LSD1/KDM1A. (A) Whole-cell extracts of HEK293T cells transfected with Myc-CoREST1, Myc-CoREST2, or Myc-CoREST3 were immunoprecipitated with anti-Myc antibody, and complexes were fractionated on SDS-PAGE. Specific antibody was used to detect endogenous LSD1/KDM1A in Western blots. (B) Comparative structural analysis of CoREST1-LSD1/KDM1A (PDB entry 2V1D) and CoREST3-LSD1/KDM1A complexes. LSD1/KDM1A (residues 171 to 836) is in dark blue, CoREST3 (residues 273 to 405) is in red, superimposed CoREST1 (residues 308 to 440) is in light blue, and the histone H3 peptide (residues 1 to 16) is in green. The FAD cofactor is in yellow. The root mean square deviation between 666 Cα atoms of LSD1/KDM1A is 0.4 Å, whereas the root mean square deviation of 133 atoms of CoREST is 0.7 Å.

HDAC activity and it is mediated by a different domain than ELM2.

All CoREST proteins form complexes with LSD1/KDM1A. CoREST1 strongly interacts with LSD1/KDM1A, and it is essential for nucleosome demethylation (14, 15). To see if CoREST2 and CoREST3 similarly interact with LSD1/KDM1A, we carried out coimmunoprecipitation assays. Myc-CoREST2 and Myc-CoREST3 interacted with LSD1/KDM1A with strength similar to that of Myc-CoREST1 (Fig. 5A). Having discarded a defect in the interaction of CoREST2 and CoREST3 with LSD1/KDM1A, we evaluated whether the enzymatic activity of LSD1/KDM1A was affected when the demethylase was in complex with CoREST2 or CoREST3, considering that it is known that LSD1/KDM1A stability and full enzymatic activity depend on CoREST1 interaction (26). Previous studies showed that LSD1/KDM1A-CoREST2 is active on the nucleosomes (29), but we were unable to further investigate the enzymatic properties of this complex, because recombinant CoREST2 proved to be biochemically intractable due to a strong tendency to aggregate. Conversely, the enzymatic properties of LSD1/KDM1A-CoREST3 could be investigated, revealing potentially significant differences compared to LSD1/KDM1A-CoREST1. Specifically, the K_m value for the substrate is >5-fold higher and is associated with a relevant 6-fold-lower k_{cat}/K_m ratio (Table 1). The three-dimensional structural analysis of LSD1/KDM1A-CoREST3 shows that LSD1/KDM1A-CoREST3 adopts a conformation identical to that of a complex with CoREST1 (Fig. 5B; Table 2). All amino acid changes (a total of 34 out of 133 residues visible in electron density) affect side chains on the protein surface, with no overall or local effects on the protein conformation. Nevertheless, these changes might exert some effects on catalysis (as gathered from the decreased k_{cat}/K_m ratio) through long-range electrostatic interactions affecting the binding of the highly charged N-terminal tail to the LSD1/KDM1A active site. Collectively, the data indicate that the lower transcriptional repressive capacity of CoREST3 than of CoREST1 is not due to a diminished interaction with LSD1/KDM1A, but it might arise from the lower catalytic efficiency, although other factors might also contribute to this effect.

CoREST type determines complex composition. The LCH complex exists as a multiprotein entity. Nonetheless, the data presented in this work suggest that the composition could be more dynamic than initially suspected. We wondered whether CoREST proteins determine the constitution of the complexes. First, we overexpressed LSD1/KDM1A with CoREST2 or CoREST3 in HEK293T cells and analyzed the presence of the endogenous HDAC1 and HDAC2 in LSD1/KDM1A-immunoprecipitated complexes. The results showed that when Myc-CoREST2 was overexpressed, LSD1/KDM1A complexes barely harbored HDAC1 and HDAC2 proteins (Fig. 6A). In contrast, when Myc-CoREST3 was overexpressed, LSD1/KDM1A immunoprecipitation yielded substantial amounts of both HDAC1 and HDAC2 (Fig. 6B). Next, we explored the ability of CoREST2 and CoREST3 to interact with CoREST1. Endogenous CoREST1 was strongly precipitated in complexes with Myc-CoREST1, whereas Myc-CoREST2 and Myc-CoREST3 showed a very low interaction with CoREST1 (Fig. 6C and D), confirmed by reverse coimmunoprecipitation (Fig. 6C, bottom). Anti-Myc immunoprecipitates from mock-transfected cells did not show any CoREST1 signal, ruling out unspecific interaction (Fig. 6C). These data confirm that CoREST proteins are the HDAC recruiters in the complex and support the existence of higher-order protein complexes between CoRESTs.

CoREST proteins share common brain territories. CoREST1 plays key roles in the regulation of neuronal gene expression (5). To learn whether CoREST2 and CoREST3 accompany CoREST1 in regulating the expression of genes in mature neurons, we studied the expression of CoREST family in the adult rat brain. We performed different experimental approaches depending on the capacity of each specific antibody. We checked the specificity of each antibody by Western blot and immunofluorescence assays (see Fig. S4 in the supplemental material). Total protein extracts were obtained from different nuclei of adult rat brain, and Western blot assays were performed for the identification of CoREST1, CoREST3, and LSD1/KDM1A. Figure 7A shows that CoREST1, CoREST3, and LSD1/KDM1A are expressed in all tested brain nuclei, indicating ubiquitous expression of these proteins in the adult rat brain. Immunofluorescence assays revealed ample expression of CoREST2, with positive cells all over the cortex, hippocampus, and striatum, which were also positive for CoREST1 and/or CoREST3 (Fig. 7B; also, see Fig. S4 in the supplemental material for antibodies' specificity). Detailed observation of immunofluorescence in brain slices showed cells with no signal, sug-

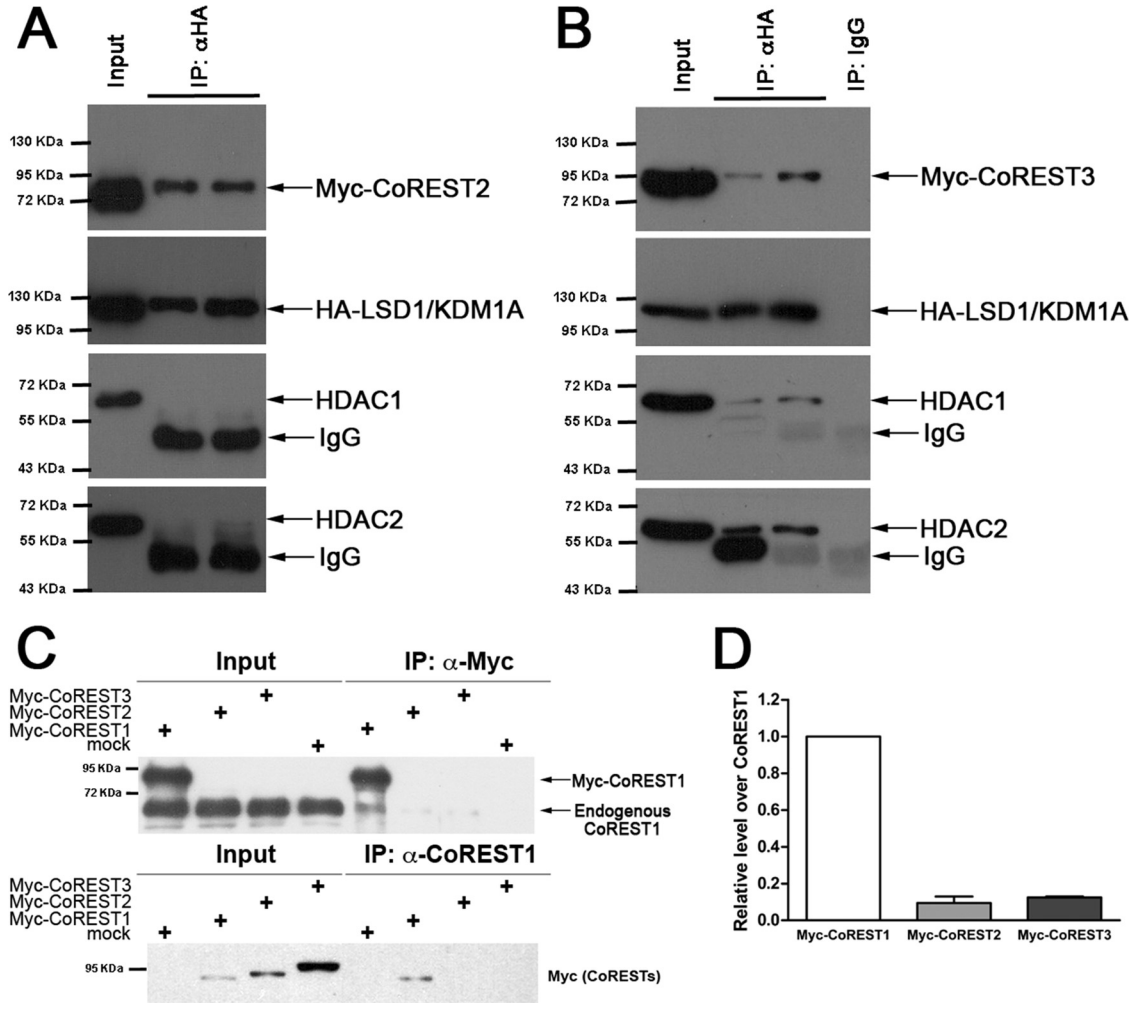

FIG 6 CoREST type determines complex composition. (A and B) CoREST2 impairs LSD1/KDM1A interaction with HDAC1/2. Whole-cell extracts of HEK293T cells cotransfected with HA-LSD1/KDM1A and Myc-CoREST2 (A) or Myc-CoREST3 (B) were immunoprecipitated with anti-HA antibody or control IgG and fractionated on SDS-PAGE. Specific antibodies were used to detect endogenous HDAC1 and HDAC2. (C) Self-association of CoREST1. Whole-cell extracts of HEK293T cells transfected with Myc-CoREST1, Myc-CoREST2, Myc-CoREST3, or Myc empty vector (control) were immunoprecipitated with the indicated antibodies and fractionated on SDS-PAGE. Western blots were developed with anti-Myc or anti-CoREST1 antibodies, as indicated. (D) Densitometry quantification of endogenous CoREST1 precipitated by recombinant Myc-CoRESTs.

gesting that a few cells express little or no CoREST proteins. In addition, the intensity of green (CoREST1/CoREST3) and red (CoREST2) fluorescence in the hippocampus indicates that granular neurons of the dentate gyrus express more CoREST1 (or CoREST3) than CoREST2, in contrast to the hilum, where a more intense signal for CoREST2 is observed, and a few cells show only CoREST2-positive immunofluorescence (Fig. 7B). Together, the data indicate a wide expression of CoREST proteins in the brain, with a variable degree of colocalization in different cells.

DISCUSSION

The presence of 3 *rcor* genes in 5 classes of vertebrates is indicative that these genes were present in a common vertebrate ancestor and have been preserved throughout evolution. As stated by Presgraves (39), duplicate genes survive because they evolve new and essential functions. The data presented here show that transcriptional complexes formed by CoREST proteins have different protein compositions and transcriptional repressor strengths, suggesting that each CoREST-containing complex has evolved different and essential functions.

The data shown indicate that CoREST3 is the most similar to CoREST1. CoREST3 interaction with LSD1/KDM1A and HDAC1/2 resembles that of CoREST1, and its transcriptional repression is abolished by HDAC inhibitors, as happens with CoREST1. Therefore, CoREST3 could be functionally redundant with CoREST1. Nonetheless, CoREST3 exhibited a significantly lower repressive capacity than CoREST1, implying that CoREST3 likely has functions other than a role redundant with that of CoREST1. The small but significant decrease in catalytic efficiency (especially with regard to the higher K_m value) might contribute to a lower transcriptional repressive action of the LSD1/KDM1A-CoREST3 complex.

Three lines of evidence indicate that complexes formed with CoREST2 are different from those formed with CoREST1 or CoREST3: (i) CoREST2-dependent transcriptional repression is not inhibited by SAHA or TSA, (ii) CoREST2 immunocomplexes

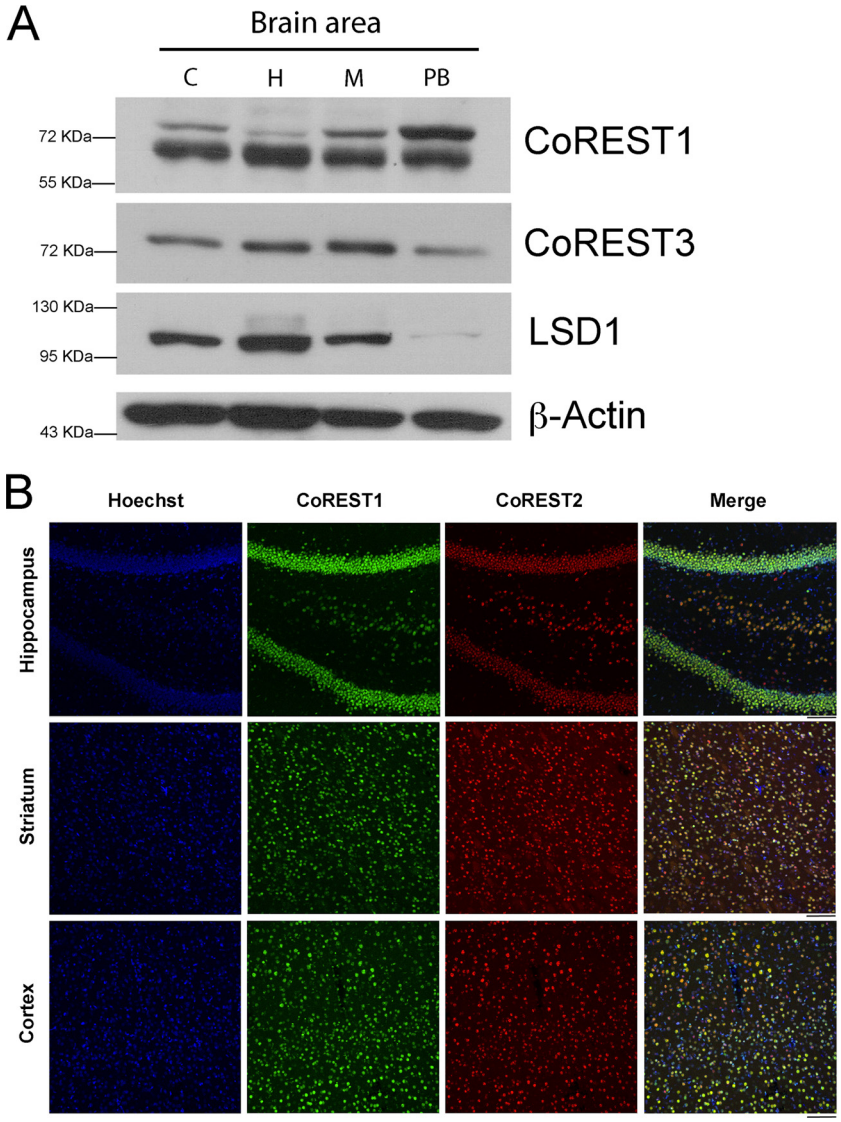

FIG 7 All CoRESTs are expressed in the adult rat brain. (A) Thirty micrograms of protein extracts obtained from different nuclei of adult rat brains was fractionated on SDS-PAGE. Western blot assays were performed for the identification of CoREST1, CoREST3, LSD1/KDM1A, and β-actin (loading control) in the brain cortex (C), hippocampus (H), midbrain (M), and parabrachial nucleus (PB). (B) Thirty-micrometer-thick paraformaldehyde-fixed adult rat brain slices were incubated with rabbit anti-CoREST2 and mouse anti-CoREST1. Twenty-four hours later, slices were incubated with fluorescent secondary antibodies. Hoechst is a nuclear stain. Images were taken with a confocal microscope.

display significantly lower HDAC activity than those constituted by CoREST1 or CoREST3, and (iii) coimmunoprecipitation assays show that the interaction of CoREST2 with HDAC1/2 is significantly lower than those exhibited by CoREST1 or CoREST3. On the other hand, the interaction between CoREST2 and LSD1/KDM1A is similar to that of CoREST1 and CoREST3 with LSD1/KDM1A. Supporting our data, a human complexome study showed strong interactions of LSD1/KDM1A with the 3 CoRESTs and of HDAC1 and HDAC2 with CoREST1 and CoREST3, but to a lesser extent with CoREST2 (40).

Comparative protein sequence analysis among CoRESTs showed that amino acid differences exist, especially in the N-terminal portion of the ELM2 domain of CoREST2. Replacing the ELM2 of CoREST2 with the ELM2 of CoREST1 increased the interaction with HDAC2, and deleting the ELM2 abolished HDAC interaction and HDAC activity associated with CoREST2. Remarkably, we found that leucine 165 in the SANT1 domain of CoREST2 restricts the interaction of the ELM2 with HDACs. In fact, just replacing leucine 165 of CoREST2 with a serine, found in the equivalent position of CoREST1, enhanced both HDAC interaction and activity associated with CoREST2. These findings demonstrate that the ELM2 of CoREST2 is capable of interacting with HDACs. This interaction is controlled by specific features in the sequence of the SANT1 domain, in agreement with the notion that ELM2 and SANT domains are jointly responsible for the selection of and association with deacetylases in corepressors featuring this two-domain module. In this regard, Millard et al. (41) showed that the corepressor SMRT (silencing mediator for retinoid and thyroid hormone receptors) forms complexes with the class I histone deacetylase HDAC3 but not with HDAC1, whereas the core-

pressor MTA1 (metastasis-associated protein 1) forms complexes with HDAC1 but not with HDAC3, despite the high homology between HDAC1 and HDAC3 (57% identical) (41). MTA1, like CoREST proteins, presents an ELM2-SANT organization, whereas SMRT displays an ELM2-like–SANT organization (38, 41), further supporting the significance of these domains in the specificity of the HDAC recruited in the transcriptional complexes. The findings showing that the ELM2-SANT1 region of the CoREST proteins determines the strength of the interaction with HDAC1/2 confirm CoRESTs as the HDAC recruiters in the complex and further highlights how finely tuned class I HDAC recruitment to different corepressor complexes is.

The data demonstrate that, unlike with CoREST1 and CoREST3, the transcriptional repression exerted by CoREST2 is HDAC independent. Actually, the mutant CoREST2ΔELM2, which is unable to recruit HDACs and does not have associated HDAC activity, displayed the same repressor strength as wild-type CoREST2. Moreover, the mutant CoREST2L165S, which has a higher capacity to recruit HDACs, is not a better repressor than wild-type CoREST2, nor was its repression sensitive to HDAC inhibitors, strongly indicating that CoREST2 represses via an HDAC-independent mechanism. The CoREST2 mechanism of transcriptional repression may involve posttranslational modifications and/or specific CoREST2-interacting proteins, possibly including other kinds of deacetylases. Supporting distinctive features among CoRESTs, it was reported that CoREST1 binds directly and noncovalently to SUMO 2/3 peptides in a SUMO interaction motif (SIM)-dependent form; this characteristic is specific to CoREST1, since no SIM motifs were identified in CoREST3 and no association with SUMO was found for CoREST2 (42).

Transcriptional complexes contain multiple interchangeable subunits, giving plasticity to gene expression regulation (43, 44). The fact that the LSD1/KDM1A-CoREST2 complex is poor in HDAC1/2 content suggests that LSD1/KDM1A activity could be diminished in complexes with CoREST2, since LSD1/KDM1A demethylase function on the histone H3 tail is fully efficient only on deacetylated substrates (15, 24, 45, 46). In addition, the fact that the LSD1/KDM1A-CoREST3 complex has a lower demethylase activity than the LSD1/KDM1A-CoREST1 complex suggests that the CoREST homolog controls the associated LSD1/KDM1A activity. Intriguingly, it was reported that CoREST2 could replace CoREST1 for efficient enzymatic reactions catalyzed by LSD1/KDM1A on purified nucleosomes (28, 29). Our data support the existence of higher-order protein complexes between CoRESTs. Coimmunoprecipitation assays indicate that CoREST1 has a strong tendency to interact with itself and to a lesser extent with CoREST2 and CoREST3, supporting a wider range of interactions and regulatory functions.

Increasing the complexity of existing mechanisms enhances the possibility of adapting and better responding to different stimuli. Our findings that CoREST2 and CoREST3 add different levels of control of HDAC1/2 recruitment and LSD1/KDM1A activity plus their abundance in the mature brain suggest that neuronal gene expression regulation by LCH complexes is a dynamic and finely tuned process. The physiological implications of this versatility in the constitution and transcriptional repression of complexes formed by members of the CoREST family remain to be determined.

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REFERENCES

1. **Andres ME, Burger C, Peral-Rubio MJ, Battaglioli E, Anderson ME, Grimes J, Dallman J, Ballas N, Mandel G.** 1999. CoREST: a functional corepressor required for regulation of neural-specific gene expression. Proc. Natl. Acad. Sci. U. S. A. **96:**9873–9878. http://dx.doi.org/10.1073/pnas.96.17.9873.
2. **Humphrey GW, Wang Y, Russanova VR, Hirai T, Qin J, Nakatani Y, Howard BH.** 2001. Stable histone deacetylase complexes distinguished by the presence of SANT domain proteins CoREST/kiaa0071 and Mta-L1. J. Biol. Chem. **276:**6817–6824. http://dx.doi.org/10.1074/jbc.M007372200.
3. **You A, Tong JK, Grozinger CM, Schreiber SL.** 2001. CoREST is an integral component of the CoREST-human histone deacetylase complex. Proc. Natl. Acad. Sci. U. S. A. **98:**1454–1458. http://dx.doi.org/10.1073/pnas.98.4.1454.
4. **Ballas N, Grunseich C, Lu DD, Speh JC, Mandel G.** 2005. REST and its corepressors mediate plasticity of neuronal gene chromatin throughout neurogenesis. Cell **121:**645–657. http://dx.doi.org/10.1016/j.cell.2005.03.013.
5. **Ballas N, Mandel G.** 2005. The many faces of REST oversee epigenetic programming of neuronal genes. Curr. Opin. Neurobiol. **15:**500–506. http://dx.doi.org/10.1016/j.conb.2005.08.015.
6. **Abrajano JJ, Qureshi IA, Gokhan S, Molero AE, Zheng D, Bergman A, Mehler MF.** 2010. Corepressor for element-1-silencing transcription factor preferentially mediates gene networks underlying neural stem cell fate decisions. Proc. Natl. Acad. Sci. U. S. A. **107:**16685–16690. http://dx.doi.org/10.1073/pnas.0906917107.
7. **Abrajano JJ, Qureshi IA, Gokhan S, Zheng D, Bergman A, Mehler MF.** 2009. REST and CoREST modulate neuronal subtype specification, maturation and maintenance. PLoS One **4:**e7936. http://dx.doi.org/10.1371/journal.pone.0007936.
8. **Welcker JE, Hernandez-Miranda LR, Paul FE, Jia S, Ivanov A, Selbach M, Birchmeier C.** 2013. Insm1 controls development of pituitary endocrine cells and requires a SNAG domain for function and for recruitment of histone-modifying factors. Development **140:**4947–4958. http://dx.doi.org/10.1242/dev.097642.
9. **Saleque S, Kim J, Rooke HM, Orkin SH.** 2007. Epigenetic regulation of hematopoietic differentiation by Gfi-1 and Gfi-1b is mediated by the cofactors CoREST and LSD1. Mol. Cell **27:**562–572. http://dx.doi.org/10.1016/j.molcel.2007.06.039.
10. **Yao H, Goldman DC, Nechiporuk T, Kawane S, McWeeney SK, Tyner JW, Fan G, Kerenyi MA, Orkin SH, Fleming WH, Mandel G.** 20 March 2014. The co-repressor Rcor1 is essential for murine erythropoiesis. Blood
11. **Gomez AV, Galleguillos D, Maass JC, Battaglioli E, Kukuljan M, Andres ME.** 2008. CoREST represses the heat shock response mediated by HSF1. Mol. Cell **31:**222–231. http://dx.doi.org/10.1016/j.molcel.2008.06.015.
12. **Saijo K, Winner B, Carson CT, Collier JG, Boyer L, Rosenfeld MG, Gage FH, Glass CK.** 2009. A Nurr1/CoREST pathway in microglia and astrocytes protects dopaminergic neurons from inflammation-induced death. Cell **137:**47–59. http://dx.doi.org/10.1016/j.cell.2009.01.038.
13. **Shi Y, Lan F, Matson C, Mulligan P, Whetstine JR, Cole PA, Casero RA, Shi Y.** 2004. Histone demethylation mediated by the nuclear amine oxidase homolog LSD1. Cell **119:**941–953. http://dx.doi.org/10.1016/j.cell.2004.12.012.
14. **Lee MG, Wynder C, Cooch N, Shiekhattar R.** 2005. An essential role for CoREST in nucleosomal histone 3 lysine 4 demethylation. Nature **437:**432–435. http://dx.doi.org/10.1038/nature04021.
15. **Shi YJ, Matson C, Lan F, Iwase S, Baba T, Shi Y.** 2005. Regulation of LSD1 histone demethylase activity by its associated factors. Mol. Cell **19:**857–864. http://dx.doi.org/10.1016/j.molcel.2005.08.027.
16. **Ballas N, Battaglioli E, Atouf F, Andres ME, Chenoweth J, Anderson ME, Burger C, Moniwa M, Davie JR, Bowers WJ, Federoff HJ, Rose DW, Rosenfeld MG, Brehm P, Mandel G.** 2001. Regulation of neuronal

traits by a novel transcriptional complex. Neuron **31:**353–365. http://dx.doi.org/10.1016/S0896-6273(01)00371-3.
17. **Yang M, Gocke CB, Luo X, Borek D, Tomchick DR, Machius M, Otwinowski Z, Yu H.** 2006. Structural basis for CoREST-dependent demethylation of nucleosomes by the human LSD1 histone demethylase. Mol. Cell **23:**377–387. http://dx.doi.org/10.1016/j.molcel.2006.07.012.
18. **Dallman JE, Allopenna J, Bassett A, Travers A, Mandel G.** 2004. A conserved role but different partners for the transcriptional corepressor CoREST in fly and mammalian nervous system formation. J. Neurosci. **24:**7186–7193. http://dx.doi.org/10.1523/JNEUROSCI.0238-04.2004.
19. **de la Calle-Mustienes E, Modolell J, Gomez-Skarmeta JL.** 2002. The Xiro-repressed gene CoREST is expressed in Xenopus neural territories. Mech. Dev. **110:**209–211. http://dx.doi.org/10.1016/S0925-4773(01)00565-2.
20. **Jarriault S, Greenwald I.** 2002. Suppressors of the egg-laying defective phenotype of sel-12 presenilin mutants implicate the CoREST corepressor complex in LIN-12/Notch signaling in C. elegans. Genes Dev. **16:**2713–2728. http://dx.doi.org/10.1101/gad.1022402.
21. **Domanitskaya E, Schupbach T.** 2012. CoREST acts as a positive regulator of Notch signaling in the follicle cells of Drosophila melanogaster. J. Cell Sci. **125:**399–410. http://dx.doi.org/10.1242/jcs.089797.
22. **Baudet ML, Zivraj KH, Abreu-Goodger C, Muldal A, Armisen J, Blenkiron C, Goldstein LD, Miska EA, Holt CE.** 2012. miR-124 acts through CoREST to control onset of Sema3A sensitivity in navigating retinal growth cones. Nat. Neurosci. **15:**29–38. http://dx.doi.org/10.1038/nn.2979.
23. **Ding Z, Gillespie LL, Paterno GD.** 2003. Human MI-ER1 alpha and beta function as transcriptional repressors by recruitment of histone deacetylase 1 to their conserved ELM2 domain. Mol. Cell. Biol. **23:**250–258. http://dx.doi.org/10.1128/MCB.23.1.250-258.2003.
24. **Lee MG, Wynder C, Bochar DA, Hakimi MA, Cooch N, Shiekhattar R.** 2006. Functional interplay between histone demethylase and deacetylase enzymes. Mol. Cell. Biol. **26:**6395–6402. http://dx.doi.org/10.1128/MCB.00723-06.
25. **Wang L, Charroux B, Kerridge S, Tsai CC.** 2008. Atrophin recruits HDAC1/2 and G9a to modify histone H3K9 and to determine cell fates. EMBO Rep. **9:**555–562. http://dx.doi.org/10.1038/embor.2008.67.
26. **Forneris F, Binda C, Adamo A, Battaglioli E, Mattevi A.** 2007. Structural basis of LSD1-CoREST selectivity in histone H3 recognition. J. Biol. Chem. **282:**20070–20074. http://dx.doi.org/10.1074/jbc.C700100200.
27. **Tontsch S, Zach O, Bauer HC.** 2001. Identification and localization of M-CoREST (1A13), a mouse homologue of the human transcriptional co-repressor CoREST, in the developing mouse CNS. Mech. Dev. **108:**165–169. http://dx.doi.org/10.1016/S0925-4773(01)00477-4.
28. **Zeng W, Kong Q, Li C, Mao B.** 2010. Xenopus RCOR2 (REST corepressor 2) interacts with ZMYND8, which is involved in neural differentiation. Biochem. Biophys. Res. Commun. **394:**1024–1029. http://dx.doi.org/10.1016/j.bbrc.2010.03.115.
29. **Yang P, Wang Y, Chen J, Li H, Kang L, Zhang Y, Chen S, Zhu B, Gao S.** 2011. RCOR2 is a subunit of the LSD1 complex that regulates ESC property and substitutes for SOX2 in reprogramming somatic cells to pluripotency. Stem Cells **29:**791–801. http://dx.doi.org/10.1002/stem.634.
30. **Galleguillos D, Fuentealba JA, Gomez LM, Saver M, Gomez A, Nash K, Burger C, Gysling K, Andres ME.** 2010. Nurr1 regulates RET expression in dopamine neurons of adult rat midbrain. J. Neurochem. **114:**1158–1167. http://dx.doi.org/10.1111/j.1471-4159.2010.06841.x.
31. **Toffolo E, Rusconi F, Paganini L, Tortorici M, Pilotto S, Heise C, Verpelli C, Tedeschi G, Maffioli E, Sala C, Mattevi A, Battaglioli E.** 2014. Phosphorylation of neuronal lysine-specific demethylase 1LSD1/KDM1A impairs transcriptional repression by regulating interaction with CoREST and histone deacetylases HDAC1/2. J. Neurochem. **128:**603–616. http://dx.doi.org/10.1111/jnc.12457.
32. **Campos-Melo D, Quiroz G, Noches V, Gysling K, Forray MI, Andres ME.** 2011. Repeated immobilization stress increases nur77 expression in the bed nucleus of the stria terminalis. Neurotox. Res. **20:**289–300. http://dx.doi.org/10.1007/s12640-011-9243-1.
33. **Forneris F, Binda C, Vanoni MA, Battaglioli E, Mattevi A.** 2005. Human histone demethylase LSD1 reads the histone code. J. Biol. Chem. **280:**41360–41365. http://dx.doi.org/10.1074/jbc.M509549200.
34. **Leslie C, Landree E, Collazo-Davila C, Bengu E, Grozea D, Marks LD.** 1999. Electron crystallography in surface structure analysis. Microsc. Res. Tech.**46:**160–177.http://dx.doi.org/10.1002/(SICI)1097-0029(19990801)46:3<160::AID-JEMT2>3.0.CO;2-#.
35. **Murshudov GN, Vagin AA, Dodson EJ.** 1997. Refinement of macromolecular structures by the maximum-likelihood method. Acta Crystallogr. Sect. D Biol. Crystallogr. **53:**240–255. http://dx.doi.org/10.1107/S0907444996012255.
36. **Emsley P, Cowtan K.** 2004. Coot: model-building tools for molecular graphics. Acta Crystallogr. Sect. D Biol. Crystallogr. **60:**2126–2132. http://dx.doi.org/10.1107/S0907444904019158.
37. **Finnin MS, Donigian JR, Cohen A, Richon VM, Rifkind RA, Marks PA, Breslow R, Pavletich NP.** 1999. Structures of a histone deacetylase homologue bound to the TSA and SAHA inhibitors. Nature **401:**188–193. http://dx.doi.org/10.1038/43710.
38. **Watson PJ, Fairall L, Santos GM, Schwabe JW.** 2012. Structure of HDAC3 bound to co-repressor and inositol tetraphosphate. Nature **481:**335–340.
39. **Presgraves DC.** 2005. Evolutionary genomics: new genes for new jobs. Curr. Biol. **15:**R52–R53. http://dx.doi.org/10.1016/j.cub.2004.12.053.
40. **Malovannaya A, Lanz RB, Jung SY, Bulynko Y, Le NT, Chan DW, Ding C, Shi Y, Yucer N, Krenciute G, Kim BJ, Li C, Chen R, Li W, Wang Y, O'Malley BW, Qin J.** 2011. Analysis of the human endogenous coregulator complexome. Cell **145:**787–799. http://dx.doi.org/10.1016/j.cell.2011.05.006.
41. **Millard CJ, Watson PJ, Celardo I, Gordiyenko Y, Cowley SM, Robinson CV, Fairall L, Schwabe JW.** 2013. Class I HDACs share a common mechanism of regulation by inositol phosphates. Mol. Cell **51:**57–67. http://dx.doi.org/10.1016/j.molcel.2013.05.020.
42. **Ouyang J, Shi Y, Valin A, Xuan Y, Gill G.** 2009. Direct binding of CoREST1 to SUMO-2/3 contributes to gene-specific repression by the LSD1/CoREST1/HDAC complex. Mol. Cell **34:**145–154. http://dx.doi.org/10.1016/j.molcel.2009.03.013.
43. **Malik S, Roeder RG.** 2010. The metazoan Mediator co-activator complex as an integrative hub for transcriptional regulation. Nat. Rev. Genet. **11:**761–772. http://dx.doi.org/10.1038/nrg2901.
44. **Lessard J, Wu JI, Ranish JA, Wan M, Winslow MM, Staahl BT, Wu H, Aebersold R, Graef IA, Crabtree GR.** 2007. An essential switch in subunit composition of a chromatin remodeling complex during neural development. Neuron **55:**201–215. http://dx.doi.org/10.1016/j.neuron.2007.06.019.
45. **Karytinos A, Forneris F, Profumo A, Ciossani G, Battaglioli E, Binda C, Mattevi A.** 2009. A novel mammalian flavin-dependent histone demethylase. J. Biol. Chem. **284:**17775–17782. http://dx.doi.org/10.1074/jbc.M109.003087.
46. **Forneris F, Binda C, Dall'Aglio A, Fraaije MW, Battaglioli E, Mattevi A.** 2006. A highly specific mechanism of histone H3-K4 recognition by histone demethylase LSD1. J. Biol. Chem. **281:**35289–35295. http://dx.doi.org/10.1074/jbc.M607411200.
47. **Forneris F, Binda C, Vanoni MA, Mattevi A, Battaglioli E.** 2005. Histone demethylation catalysed by LSD1 is a flavin-dependent oxidative process. FEBS Lett. **579:**2203–2207. http://dx.doi.org/10.1016/j.febslet.2005.03.015.