A Conserved Motif Common to the Histone Acetyltransferase Esa1 and the Histone Deacetylase Rpd3*

Received for publication, May 13, 2002, and in revised form July 9, 2002
Published, JBC Papers in Press, July 10, 2002, DOI 10.1074/jbc.M204640200

Naruhiko Adachi‡, Akatsuki Kimura¶¶, and Masami Horikoshi¶§

From ‡The Horikoshi Gene Selector Project, Exploratory Research for Advanced Technology (ERATO), Japan Science and Technology Corporation (JST), 5-9-6 Tokodai, Tsukuba, Ibaraki 300-2635 Japan and ¶¶Laboratory of Developmental Biology, Institute of Molecular and Cellular Biosciences, University of Tokyo, 1-1-1 Yayoi, Bunkyo-ku, Tokyo 113-0032 Japan

Post-translational modification of histones enables dynamic regulation of chromatin structure in eukaryotes. Histone acetyltransferase (HAT) and histone deacetylase (HDAC) modify the N-terminal tails of histones by adding or removing acetyl groups to specific lysine residues. A particular pair of HAT (Esa1) and HDAC (Rpd3) is proposed to modify the same lysine residue in vitro and in vivo. Thus, HAT and HDAC might have similar structural and functional motifs. Here we show that HAT (Esa1 family) and HDAC (Rpd3 family) have similar amino acid stretches in the primary structures through evolution. We refer to this region as the “ER (Esa1-Rpd3) motif.” In the tertiary structure of Esa1, the ER motif is located near the active center. In Rpd3, for which the tertiary structure remains unclear, we demonstrate that the ER motif contains the same secondary structure as found in Esa1 by circular dichroism analysis. We did alanine-scanning mutagenesis and found that the ER motif regions of Esa1 or Rpd3 are required for HAT activity of Esa1 or HDAC activity of Rpd3, respectively. Our discovery of the ER motif present in the pair of enzymes (HAT and HDAC) indicates that HAT and HDAC have common structural bases, although they catalyze the reaction with opposite functions.

The eukaryotic genome is compacted in the nucleus as a highly condensed structure called chromatin. For various reactions on chromosomes to occur, the compacted chromatin structure needs to be altered. Histones are well-conserved nuclear basic proteins and function as cores to package eukaryotic DNA into nucleosomes, which are repeating units of chromatin structure (1). Core histones H2A, H2B, H3, and H4 are components of nucleosome core particles (2), whereas linker histones (e.g. H1) are located outside of core particles (3). The N-terminal tails of core histones extend to the accessible surface of the nucleosome core particles (2) and are therefore called “N-tails” and are thought to be important for modulation of chromatin structure. After the discovery of histone acetylation (4), numerous studies have indicated that acetylation of specific lysine residues in the N-tails plays an important role in DNA-mediated reactions, including transcriptional regulation (5–7). Analyses of molecules involved in this modification and relationships between these molecules are necessary to reveal the molecular mechanisms of these reactions.

Histone acetyltransferase (HAT) and histone deacetylase (HDAC) act competitively to bring about a steady state of acetylation of the core histones. In vitro, the deletion of HAT or HDAC causes hypoacetylation or hyperacetylation to core histones, respectively (8, 9). In vivo, HAT and HDAC are classified into certain conserved families, and each family acts on particular lysine residues (10–12). In addition, particular pairs of HATs and HDACs are reported to act as “functional pairs” in vivo. Nuclear hormone receptors change their interaction partners from Sin3 complex (HDAC) to p160 family (HAT) in a ligand-dependent manner to activate the target gene from a repressed state (13). In yeast, Esa1 (HAT) and Rpd3 (HDAC) are reported to function in acetylation and deacetylation, respectively, of lysine 12 of histone H4 at the PHO5 gene promoter (14).

Esa1 (15, 16) and Rpd3 (9, 17) are both conserved throughout the eukaryotes, and the members of both families are known to be involved in transcriptional regulation (Esa1 family (18), Rpd3 family (19–24)), gene silencing (Esa1 family (25), Rpd3 family (9, 26)), X-chromosome dosage compensation (Esa1 family (27), Rpd3 family (28)), DNA repair (Esa1 family (29), Rpd3 family (30)), and apoptosis (Esa1 family (29), Rpd3 family (31)). These reports indicate that Esa1 family members and Rpd3 family members function as pairs of HAT and HDAC in any eukaryote. The strong functional linkage between Esa1 and Rpd3 suggests that both enzymes might have a structural similarity, which can serve as structural bases for these enzymes to act on a common lysine residue of histone in common biological processes throughout the eukaryotes. However, previous studies have not found any structural similarity between Esa1 and Rpd3 families (32, 33).

In this paper, we report the discovery of a conserved amino acid stretch in the primary structures between HAT (Esa1) and HDAC (Rpd3). We refer to this stretch as the “ER (Esa1-Rpd3) motif.” The ER motif resides in the catalytic region responsible for HAT and HDAC activities. Structural and functional analyses revealed that the ER motifs in both enzymes play important roles in the activities of each enzyme.

*This work was supported in part by grants-in-aid for scientific research from the Ministry of Education, Science, Technology, Sports and Culture of Japan, the New Energy and Industrial Technology Development Organization (NEDO), and Exploratory Research for Advanced Technology (ERATO) of the Japan Science and Technology Corporation (JST). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

¶ Research Fellow of the Japan Society for the Promotion of Science.
¶ To whom correspondence should be addressed. Tel.: 81-3-5841-8469; Fax: 81-3-5841-8468; E-mail: horikosh@iam.u-tokyo.ac.jp.

1 The abbreviations used are: HAT, histone acetyltransferase; HDAC, histone deacetylase; ER, Esa1-Rpd3; MYST, MOZ, Ybf2/Sas3, Sas2, Tip60; HDLP, HDAC-like protein of hyperthermophilic bacterium.
A Conserved Motif Common to HAT Esa1 and HDAC Rpd3

MATERIALS AND METHODS

Sequence Analysis—BLAST similarity search against yeast sequences (34) was done (mips.gsf.de/typ/yeast/CYGD/db/index.html) with the following specifications: E value of 10, cutoff score of 100, and no filtering. Multiple-sequence alignment was done using ClustalW (35) followed by manual adjustment (Fig. 1A). Tertiary structure comparisons were done using DALI (36). Figs. 1, B–D, and 4, A and B were generated using Swiss-PdbViewer (37).

CD Analysis—All peptides were synthesized by the Peptide Institute (Osaka, Japan), except histone H4 N-terminal tail peptide, which was purchased from Upstate Biotechnology (Fig. 2B). The CD spectra were acquired with a Jasco J-820 (Jasco, Tokyo, Japan), using the continuous mode with 1-nm bandwidth, 2-s response, a scan speed of 50 nm/min, and a cuvette with 1-mm path length. All spectra were averaged over at least five scans. The samples were prepared using a solution containing 50 mM sodium phosphate buffer (pH 7.0 at 5°C), 1 mM dithiothreitol, 40% trifluoroethanol, and 20 μM peptide.

Preparation of Recombinant Proteins—Oligonucleotide-directed mutagenesis was done by the method of Kunkel (38). Oligonucleotides used to create Esa1 and Rpd3 mutants are shown in Table I. The correct introduction of the various mutations was verified by DNA sequencing. The yeast ESA1 or RPD3 gene and its derivatives were cloned into pMH524 single-copy vector to express FLAG-tagged versions of the proteins under the control of GAL1 promoter (39). Whole-cell extracts were prepared from Saccharomyces cerevisiae W303-1a strains expressing the FLAG-Esa1, FLAG-Rpd3, and their mutants by lysing cells with glass beads in an extraction buffer (40 mM HEPES (pH 7.3 at 25°C), 350 mM NaCl, 10% glycerol, 0.1% Tween 20, 2 μg/ml pepstatin, 2 μg/ml leupeptin, 1 mM phenylmethlysulfonfluoride, 1.72 μg/ml aprotinin). The extracts were incubated overnight at 4°C with anti-FLAG M2-agarose (Sigma). After washing with extraction buffer, proteins were eluted from the beads three times by incubating at 4°C for 20 min with extraction buffer containing 1 mg/ml FLAG peptidase (Sigma). The mock elute was prepared as a control from a yeast clone carrying only pMH524 vector. The amount of each protein is quantified from the density of each band on SDS-PAGE using the public domain NIH image program, respectively (developed at the U.S. National Institutes of Health and available on the Internet at rsb.info.nih.gov/nih-image).

Histone Acetyltransferase Assay—Filter binding assays for HAT activity were done essentially as described elsewhere (11, 39). Reactions were done at 30°C for 30 min in 25 μl of buffer containing 50 mM Tris-HCl (pH 8.0 at 25°C), 50 mM KCl, 1 mM dithiothreitol, 1 mM phenylmethylsulfonfluoride, 10 mM sodium butyrate, 0.1 mM EDTA, 5% glycerol, 0.2 mg/ml bovine serum albumin (Seikagaku, Tokyo, Japan) and [3H]acetyl-CoA (PerkinElmer Life Sciences), spotted on P81 phosphocellulose filter paper (Whatman), washed with 0.2 M sodium carbonate buffer (pH 9.2 at 25°C), and then dried and subjected to liquid scintillation. In the reactions, 50 pmol of histone H4 N-terminal peptides (SGRRGGKGLGKKGAHRRC) (Upstate Biotechnology) and ~1 pmol of Esa1 derivative proteins were used.

Histone Deacetylase Assay—Histone deacetylase assay was assayed using a Histone Deacetylase Assay Kit (Upstate Biotechnology) according to the instructions (2A). Kimura and M. Horikoshi, unpublished data.

motifs (blue), MYST domain (yellow), acetyl-CoA-binding site (purple), chromo-like domain (orange), HDAC domain (green), and zinc-binding site (red). In the bottom panel, identical, chemically similar, and conserved residues between Esa1 and Rpd3 are indicated by red letters. Above the primary structure, the α-helix (green box) and a loop (white box) are shown. Below the primary structure, the residues on the surface of the cleft is in cyan (including ER1, see "Results"), the residues in a hydrophobic core is in blue (including ER2), and the residues on the surface opposite to the active center is in green (including ER3). Abbreviations: Sc, Saccharomyces cerevisiae; Ce, Caenorhabditis elegans; Hs, Homo sapiens. B, position and tertiary structure of the ER motif in Esa1. The ER motif lies within the blue circle. Nine conserved amino acids in the ER motif are indicated. ER1 is in cyan, ER2 is in blue, and ER3 is in green. The active center lies within the red circle; CoA is in magenta. C, enlarged diagram of the ER motif in Esa1. Nine conserved amino acids in the ER motif are indicated and labeled. ER1 is in cyan, ER2 is in blue, and ER3 is in green. D, predicted position of the ER motif in HDLP. The region in HDLP (98–80) corresponding to the position of the ER motif in Rpd3 (see text) lies within the blue circle; trichostatin A is in magenta; zinc is in gray. The active center lies within the red circle. E, existence of the ER motif specifically in Rpd3, not in HDLP. Colors used in boxes are as for panel A.

Fig. 1. A conserved motif common to Esa1 and Rpd3. A, schematic of the primary structure (top) and the sequence alignment of the ER motif (bottom) of Esa1 and Rpd3. The top panel indicates the ER motif (bottom) of Esa1 and Rpd3.
200- 

acetic acid was extracted with 600

Speed-Vac, and the peptides were then dissolved in 250

The buffer was removed from the radiolabeled peptides by using a

tion of 50

mM HCl in 10% methanol, and eluted with 3 M HCl in 50% isopropanol.

loaded into a Microcon-SCX spin column (Millipore), washed with 10

°

C), 150 mM NaCl, 1 mM phenylmeth-

Daspartic acid (2). The peptide of the histone H4 N-tail (black

structure (48). The peptide of the positive control (white box in Fig. 1A),

CD spectra of peptides corresponding to Esa1 residues 242–259 (green) and Rpd3 residues 317–334 (blue).

The peptide of the positive control (red) is known to form an α-helical

structure (8). The peptide of the histone H4 N-tail (black) is known to have a divergent structure (2). B, amino acid sequences of peptides and molecular ellipticity at 222 nm. Colors used in peptides are as described

manufacturer’s instructions. One hundred micrograms of histone H4

N-tail peptides were incubated overnight at room temperature with 1.25 mCi of [3H]acetic acid (PerkinElmer Life Sciences) and 5 μl of BOP

solution (0.24 M benzotriazol-1-yl-oxytris(dimethylamino) phosphoniuim hexafluorophosphate and 0.2 M triethylamine in acetonitrile), loaded into a Microcon-SCX spin column (Millipore), washed with 10

mM HCl in 10% methanol, and eluted with 3 M HCl in 50% isopropanol.

The buffer was removed from the radiolabeled peptides by using a

Speed-Vac, and the peptides were then dissolved in 250 μl of distilled

water. Reactions were done at 37 °C for 4 h in 200 μl of buffer containing

10 mM Tris-HCl (pH 8.0 at 25 °C), 150 mM NaCl, 1 mM phenylmethyl-

sulfonyl fluoride, and 10% glycerol, and then quenched by the addition

of 50 μl of 1 M HC1/0.16 M acetic acid in distilled water. Released

[3H]acetic acid was extracted with 600 μl of ethyl acetate, and two

200-μl aliquots of each ethyl acetate phase were subjected to liquid

scintillation. In the reactions, 20,000 cpm of [3H]acetyl histone H4

N-tail peptides and ~1 pmol of Rpd3 derivative proteins were used.

RESULTS

Esa1 and Rpd3 Share Similar Primary Structure.—To identify a common structure between the Esa1 and Rpd3 families, we performed systematic BLAST searches using the entire or partial sequences of Esa1 and Rpd3 families as queries, and found a previously unrecognized, but unequivocal sequence similarity between Esa1 and Rpd3 over 21 amino acids ((R/K)/X/W/Y/X/N/E/L/C/G/A/L/I/V)/(L/A)XXXX(L/V/I)XXXLYXY in Fig. 1A). Nine sites were occupied by identical or similar residues among Esa1 family members (15, 16) and Rpd3 family members (9, 17) through evolution. We refer to this set of residues as the “ER (Esa1-Rpd3) motif.”

To characterize this motif, we examined the structural features of these residues. The central residues of the ER motif are composed of hydrophobic amino acids (i.e. Cys252, Leu253, Leu254, Leu259, Leu264 of Esa1), whereas both ends of the motif are composed of large hydrophilic amino acids (i.e. Arg245, Trp247, Asn250, Tyr266 of Esa1). Because small hydrophobic residues tend to form turns (40), Cys and Leu residues at the central part of the ER motif might form a turn. This notion is consistent with the tertiary structure of the ER motif in Esa1, because the residues (Leu257 and Leu259) of the ER motif make a turn (Fig. 1, B and C) (32). The corresponding residues of the ER motif in Rpd3 are thus expected to make a similar turn, although the tertiary structure of Rpd3 has still not been clarified.

We then examined the positions of the ER motif on the domain structures of Esa1 and Rpd3. The ER motif of Esa1 is located within the MYST (MOZ, Ybf2/Sas3, Sas2, Tip60) domain (blue and yellow boxes of Esa1 in Fig. 1A). Because the MYST domain is responsible for HAT activity (15), the ER motif in the MYST domain might be involved in HAT activity of Esa1. Because the ER motif is not located within the acetyl-CoA binding site in the MYST domain (purple box of Esa1 in Fig. 1A) (25, 41), it might be involved in processes other than acetyl-CoA binding. The ER motif of Rpd3 is located within the HDAC domain (blue and light green boxes of Rpd3 in Fig. 1A), indicating that the ER motif might also be involved in HDAC activity of Rpd3. Because the motif is not located at the zinc-binding site in Rpd3 (red box of Rpd3 in Fig. 1A; the zinc ion functions as a metal cofactor of HDAC activity) (33), it might be involved in processes other than zinc recognition.

The ER Motif of Esa1 Is Located Close to the Active Center.—To estimate the functional role of the ER motif, we mapped the position of the ER motif in the tertiary structure of Esa1-CoA complex (32) (Fig. 1B). The tertiary structure of the MYST domain of Esa1 is composed mainly of three regions: (i) an N-terminal region (a1,a2, β1–β6 in Fig. 1B) which contains a zinc finger; (ii) a central core region (a3, β7–β9 in Fig. 1B), which contains an acetyl-CoA binding site; and (iii) a C-terminal region (a4–a7, β10, β11 in Fig. 1B). These three regions form a cleft surrounding the active center (red circle in Fig. 1B). The ER motif is located within the N-terminal region of the MYST domain (blue circle in Fig. 1B). In addition, the ER motif forms a surface of the cleft that, based on the tertiary structures of other HATs (Gcn5 (42–44), Hat1 (45)), is predicted to be important for the catalytic activity of Esa1.

The ER motif contains an α-helix (a2: from Arg245 to Lys256) and a loop (from Leu257 to Tyr266) (blue circle in Fig. 1B). As mentioned above in the preceding subsection, the residues (Leu257 and Leu259) of the ER motif in Esa1 make a turn and the ER motif forms an oval architecture (32). Because the start and end points of the ER motif region are closely positioned in the tertiary structure, the ER motif seems to be a structural module in Esa1. The ER motif is located within ~20 Å of the active center, which is a distance predicted from the tertiary structure of Esa1-CoA (red circle in Fig. 1B). Such a close proximity suggests that the motif plays an important role in the HAT activity of Esa1 as we speculated in the previous subsection.

The tertiary structure of the ER motif in Esa1 (32) indicates that nine residues of the ER motif are divided into three regions (i.e. ER1, ER2, and ER3) according to structural roles: (i) the residues of ER1 are located on the surface of the cleft and face the active center (Cys252 and Leu259 in Esa1; cyan in Fig. 1C (Gly327 and Leu334 in Rpd3)), (ii) the residues of ER2 are buried in a hydrophobic core and maintain the architecture of the cleft surrounding the active center (Trp247, Asn250, Leu253,
The newly created restriction enzyme sites are indicated in parentheses. The sequence of each mutant was confirmed by nucleotide sequence analysis.

| Mutant     | Oligonucleotide                  |
|------------|----------------------------------|
| Esa1-R245A | TCA TTT TTT GAA ATT GAC GAC GGG ACT GGG TGG TGT CAA AAC (BsiEI) |
| Esa1-T246A | ACT GAT GGT AGA ACG CAC GCT TGG TGG CAA AAC TGG (BsrHI) |
| Esa1-W247A | AGA AAG CAA AGG ACT GCA TGC CAA AAC TGG TGT TTA (SphiI) |
| Esa1-C248A | AGA CAA AGG ACT TGG GCC CAA AAT TGG TTA (Apal) |
| Esa1-R249A | AAG CAA AGG ACT TGG TGC CAA ACG GCG CTT TGG TTA (Eco47I) |
| Esa1-L251A | ACT TGG TGG CAA ACG TGG TTA (ApaI) |
| Esa1-C252A | ACT TGG TGT CAA AAG TGG TTA (ApaI) |
| Esa1-L253A | TGG TGT CGA AAC TGG TCA GCA TTT CAC AAA TTT TGG (Eco47I) |
| Esa1-L254A | CGA AAC TGG TGT TTA GTC AAG CTT CTC CTA GAT CAC (HindIII) |
| Esa1-S255A | AAC CTG TGT TTA CTC GGG CTT TTT CCA CTA GAT CAC (HindIII) |
| Esa1-R256A | AAC TTG TGT TTA CTT CCA GCT CTT TCT CTA GAT CAC (EcoI) |
| Esa1-L257A | TGT TTA CTT CCA AAA GCT CTT CTA GAT CAC AAA (HindIII) |
| Esa1-L259A | CCT TCT AAA AAT TCT TGT GTC CAC AAA ACA TTA CAC (BsaI) |
| Esa1-D260A | CTT CAC AAA ATT TCT TGG GTC AAC AAA ACA TTA TAC (XcmI) |
| Esa1-K262A | CCT TCC AAA ATT TCT CTT GAT CAC AAA ACA TTA Tactical (BgiI) |
| Esa1-T263A | TCT CTA GAT CAC AAA GCT TTA TAT GCT TAT GAC (HindIII) |
| Esa1-L264A | CTA GAT CAC AAA ACA GCA TAC TAT GCT GAT CCC TTT TGT TAT (SalI) |
| Esa1-Y266A | CAT CAC AAC AAT TGG TCA GAT CAC CTT TTT CTA CGG (NdeI) |
| Esa1-Y262A | CAT CAC AAC AAT TGG TCA GAT CAC TTA CAC ACA AAC (NdeI) |
| Esa1-T289A | GGA GGC TAT CAT CTT CAC AAC GAT GTT GCA GCC ACA TGG TGT TTG GAA (FspI) |
| Rd-Rd3-T231A | ATC AGA ATT GGT ACT CAA GGC TGG TGG AAC TAT (Stul) |
| Rd-Rd3-W322A | AAT GAT GCA AGG ACA GCA TGG TTT GAA ACA AGT (SphiI) |
| Rd-Rd3-C323A | AGA AAT GAT GCA AGG ACC TGG CCC TTT GCA ACA GGT CTA CTA (FspI) |
| Rd-Rd3-E325A | AGA CCC TTT GCA ACA GAT GCA CTA CTA (Stul) |
| Rd-Rd3-F324A | AGA CCC TTT GCA ACA GAT GCA CTA CTA (Stul) |
| Rd-Rd3-C323A | AGA AAT GAT GCA AGG ACC TGG CCC TTT GCA ACA GGT CTA CTA (FspI) |
| Rd-Rd3-W322A | AAT GAT GCA AGG ACA GCA TGG TTT GAA ACA AGT (SphiI) |
| Rd-Rd3-C323A | AGA AAT GAT GCA AGG ACC TGG CCC TTT GCA ACA GGT CTA CTA (FspI) |
| Rd-Rd3-E325A | AGA CCC TTT GCA ACA GAT GCA CTA CTA (Stul) |
| Rd-Rd3-F324A | AGA CCC TTT GCA ACA GAT GCA CTA CTA (Stul) |
| Rd-Rd3-C323A | AGA AAT GAT GCA AGG ACC TGG CCC TTT GCA ACA GGT CTA CTA (FspI) |
| Rd-Rd3-W322A | AAT GAT GCA AGG ACA GCA TGG TTT GAA ACA AGT (SphiI) |
| Rd-Rd3-E325A | AGA CCC TTT GCA ACA GAT GCA CTA CTA (Stul) |
| Rd-Rd3-F324A | AGA CCC TTT GCA ACA GAT GCA CTA CTA (Stul) |

and Leu<sup>254</sup> in Esa1; blue in Fig. 1C (Trp<sup>322</sup>, Glu<sup>325</sup>, Leu<sup>328</sup>, and Leu<sup>329</sup> in Rpd3), and (iii) the residues of ER3 are located on the surface opposite to the active center (Arg<sup>245</sup>, Leu<sup>246</sup>, and Tyr<sup>246</sup> in Esa1; green in Fig. 1C (Arg<sup>320</sup>, Leu<sup>323</sup>, and Tyr<sup>326</sup> in Rpd3)). From their structural positions, the residues of ER1 and ER2 seem to be especially important for the HAT activity of Esa1, whereas those of ER3 do not. A residue of ER2 (Leu<sup>244</sup>) is reported to be critical for HAT activity of Esa1<sup>in vitro</sup> and cell-cycle progression<sup>in vivo</sup> (46). These observations suggest that the ER motif of Esa1 is located close to the active center and that the residues of ER1 and ER2 are especially important for the HAT activity of Esa1.

The ER Motif of Rpd3 Is Predicted To Be Near the Active Center—Because the tertiary structure of Rpd3 has not yet been clarified, the precise structure and position of the ER motif in Rpd3 are not clear. However, the tertiary structure of HDAC-like protein of hyperthermophilic bacterium (HDLP) has been clarified, which is 37% identical and 55% similar to yeast HDAC (6). It has over 300 residues containing the catalytic core of HDAC activity (33). HDLP has a single globular domain structure belonging to the open α/β class of folds (Fig. 1D). The active center predicted from the binding site of trichostatin A (47), which is the inhibitor of HDAC activity, forms a pocket surrounded by eight repeating units consisting of a β-sheet loop-α-helix (red circle in Fig. 1D). The zinc ion is near the bottom of the active center (gray sphere in Fig. 1D). This tertiary structure indicates that HDLP and HDAC cause (i) HDAC reaction through a mechanism of binding the substrate lysine residue by the pocket and (ii) HDAC reaction using the zinc ion as a metal cofactor (33).

To estimate the position and structure of the ER motif in Rpd3, we aligned the sequence of Rpd3 with that of HDLP (Fig. 1E). In the alignment, HDLP lacked the region corresponding to the ER motif of Rpd3, and, accordingly, we could not estimate the structure of the ER motif in Rpd3. However, because the region adjacent to the ER motif in Rpd3 corresponds to the region near the binding site of trichostatin A in HDLP, the position of the ER motif in Rpd3 was estimated to be between β8 and α8 (blue circle in Fig. 1D). Because trichostatin A is known to mimic the substrate (lysine side chain) (33), the ER motif in Rpd3 is predicted to be near the active center. The lack of the ER motif in HDLP might be one reason why HDLP has a specific activity of HDAC of only about 7.5%<sup>in vitro</sup> (33). From these observations, we expect the ER motif to play a functional role in the enzymatic reactions of Rpd3.

The ER Motifs of Esa1 and Rpd3 Are Similar in Their Secondary Structures—The close proximities between the active center and the ER motif of Esa1 and Rpd3 suggest that the ER motifs of Esa1 and Rpd3 might have similar tertiary structures, although the tertiary structure of Rpd3 has not yet been clarified. We examined whether the ER motif of Rpd3 is similar in secondary structure to that of Esa1 by analyzing the CD spectra of synthetic peptides. We found that the ER motifs of Esa1 showed contents typical of an α-helical structure, which
Values of activity per amount of protein. The residues of the ER motif are in mutations in the ER motif on HAT activ-type protein of Esa1. The mutants of the ER motif are in red (lanes 3, 5, 8, 10–12, 16, 20, and 22). The arrowhead indicates the position of Esa1. Protein size standards are shown on the left. B, in vitro HAT activity of each mutant. Incorporation of the 3H-labeled acetyl group into histone H4 N-tail peptides was measured. The mutants of the ER motif are in red (lanes 3, 5, 8, 10–12, 16, 20, and 22). Values of activity per amount of protein relative to the wild-type protein (100%) are shown. C, summary of the effects of mutations in the ER motif on HAT activity. The residues of the ER motif are in red. Solid circles indicate that the mutations of the residues decreased (over 50%) the enzymatic activity of Esa1. Open circles indicate that the mutant proteins retained activity comparable with the wild-type protein of Esa1.

Two observations attest to the specificity of the CD analysis. First, a positive control peptide (48) showed contents typical of an α-helical structure (blue in Fig. 2A). Second, the histone H4 N-tail peptide, which is known to have a divergent structure according to x-ray structure analysis (2), did not form any ordered structure (black in Fig. 2A). The ellipticity at 222 nm corresponds to the α-helical contents. The ellipticities at 222 nm of these peptides also indicate that the ER motif of Esa1 and Rpd3 form an α-helix (Fig. 2B). These data suggest that the secondary structures of the ER motifs of Esa1 and Rpd3 also are similar.

The ER Motif of Esa1 Is Required for HAT Activity—Our results show that the ER motif of Esa1 is near the active center and that the residues of ER1 and ER2 face the furrow involved in catalytic reaction. The ER motif of Rpd3 is also predicted to be near the active center. Moreover, the ER motif of Esa1 and Rpd3 share common features in their secondary structure. Therefore, the ER motif, especially the residues of ER1 and ER2, might play important roles common to HAT and HDAC activities. To determine whether the ER motif of Esa1 is required for HAT activity, we introduced alanine-scanning mutations in the ER motif by site-directed mutagenesis. Alanine was chosen because alanine mutations change the properties of individual residues with minimum effects on the overall structure of proteins (49). In addition, the ER motif of Esa1 binds with the rest of domain of Esa1 through the 11 hydrogen bonds (5 main-chain interactions and 6 side-chain interactions) (32). Collectively, these data suggest that alanine-scanning mutations do not disrupt overall structure radically.

Wild-type and mutant proteins of Esa1 were expressed and purified from yeast (Fig. 3A) and then examined for HAT activity using H4 peptide as the substrate (Fig. 3B). The results show that 11 mutations among 20 residues in the ER motif region reduced the HAT activity, indicating that the region of the ER motif plays a role in the catalytic activity (Fig. 3B). Among the nine conserved residues of the ER motif, all six residues having mutations that severely reduce the catalytic activity (solid circles in Fig. 3C) are the residues of ER1 and ER2. This result is reasonable because the residues of ER1 face the active center and the residues of ER2 maintain the architecture of the cleft (cyan and blue in Fig. 4A, respectively). On the other hand, all three mutations that do not severely reduce catalytic activity (open circles in Fig. 3C) are the residues of ER3. This result also is reasonable because the residues of ER3 form a surface opposite to the active site (green in Fig. 4B). These results are consistent with the classification predicted from the tertiary structure of Esa1. We thus conclude that the residues of ER1 and ER2 are functionally required for the HAT activity of Esa1 in vitro, whereas the residues of ER3 are not. Because the three residues of ER3 are conserved among HAT and HDAC, the residues of ER3 might also be involved in a conserved function other than the catalytic activity.

The ER Motif of Rpd3 Is Also Required for HDAC Activity—Wild-type and mutants of Rpd3 were also expressed and puri-
A Conserved Motif Common to HAT Esa1 and HDAC Rpd3

The ER motif are labeled. ER1 is in cyan, ER2 in blue, ER3 in green, and CoA in magenta. B, positions and their effects on HAT activity of the ER motif mutations in Esa1 (ribbon diagram). The residues with mutant protein that had activity less than 50% of the wild-type protein are shown in blue, and the other residues examined are shown in orange. Nine residues in the ER motif are labeled. ER1 is in cyan, ER2 in blue, ER3 in green, and CoA in magenta. B, positions and their effects on HAT activity of the ER motif mutations in Esa1 (surface distribution). The residues with mutant protein that had activity less than 50% of the wild-type protein are shown in blue, and the other residues examined are shown in orange. Five residues in the ER motif located on the molecular surface are labeled. ER1 is in cyan, and ER3 in green.

fied from yeast (Fig. 5A) and examined for HDAC activity using acetylated H4 peptide (Fig. 5B). The results show that 9 mutations among 20 residues in the ER motif region reduced the HDAC activity, indicating that the region of the ER motif also plays a role in the catalytic activity (Fig. 5B). Among nine residues of the ER motif, alanine substitution mutations in seven residues severely inhibited the HDAC activity (solid squares in Fig. 5C). Similar to the results for Esa1, all six residues of ER1 and ER2 were involved in the reduction of HDAC activity. In contrast, two of three residues of ER3 did not severely reduce catalytic activity (open squares in Fig. 5C). These results are consistent with the classification predicted from the tertiary structure of Esa1, except for a residue of ER3 (Leu338) (Fig. 5C). We thus conclude that the residues of ER1 and ER2 are functionally required for the HDAC activity of Rpd3 as well as Esa1. Based on these observations, as well as on the amino acid identities and secondary structure similarities, the ER motif of Rpd3 is likely to have similar tertiary structure and functional role to the ER motif of Esa1.

Leu338 in Rpd3, which is a corresponding residue located on the surface opposite to the cleft in Esa1, is required for HDAC activity. This differs from the result for the corresponding residue (Leu264) in Esa1 (Fig. 5C). The residue located at the C-terminal side to Leu264 in Esa1 or Leu338 in Rpd3 is tyrosine (Tyr265) or proline (Pro339), respectively, both of which were perfectly conserved from yeast to human (Fig. 1A). The conformation of proline residue is known to have limited flexibility, because the side chain of the proline residue is fixed in the main chain. Therefore, the alanine-substitution mutation of Leu338 in Rpd3 probably creates a larger space around the corresponding residue than does wild-type protein, and increases the flexibility of the main chain of Leu338. Therefore, the main chain of Leu338 is significantly affected by the conformation of the next proline residue. These observations strongly suggest that the ER motif of Rpd3 plays similar functional roles as that of Esa1 in the catalytic reaction.

FIG. 4. Structural roles of the amino acids in the ER motif. A, positions and their effects on HAT activity of the ER motif mutations in Esa1 (ribbon diagram). The residues with mutant protein that had activity less than 50% of the wild-type protein are shown in blue, and the other residues examined are shown in orange. Nine residues in the ER motif are labeled. ER1 is in cyan, ER2 in blue, ER3 in green, and CoA in magenta. B, positions and their effects on HAT activity of the ER motif mutations in Esa1 (surface distribution). The residues with mutant protein that had activity less than 50% of the wild-type protein are shown in blue, and the other residues examined are shown in orange. Five residues in the ER motif located on the molecular surface are labeled. ER1 is in cyan, and ER3 in green.

DISCUSSION

Discovery of the ER Motif as a Structural and Functional Motif—Esa1 and Rpd3 act as a pair of enzymes that both function at the same PHO5 promoter through the acetylation or deacetylation of lysine 12 of histone H4 in vivo (14). None of the past studies has succeeded in discovering the structural similarity between two enzymes, because both enzymes catalyze opposite directions of histone acetylation. This study revealed that Esa1 and Rpd3 both have a common structural motif (the ER motif) (Fig. 1). Our CD analysis indicates that the secondary as well as the primary structures (Fig. 2) of the ER motifs of Esa1 and Rpd3 are similar. This study also revealed that the ER motif is near the active center (Fig. 1, B and D). The residues of ER1 and ER2 are required for HAT and HDAC activities, and the results of mutant analyses of both enzymes are consistent with each other (Figs. 3 and 5). These observations suggest that the ER motifs of Esa1 and Rpd3 have similar functional roles as well as similar structure. Therefore, our present data suggest for the first time that there is a structural base between a specific functional pair of HAT and HDAC.

Role of the ER Motif—The action of HAT activity of Esa1 includes five steps: (i) binding to an acetyl-CoA, (ii) binding to a histone, (iii) acetylating a histone, (iv) dissociating from an acetylated histone, and (v) dissociating from a CoA (32). The HDAC activity of Rpd3 includes three steps: (i) binding to an acetylated histone, (ii) deacetylating an acetylated histone, and (iii) dissociating from a histone (33). Because both sub-reactions contain an acetyl group recognition and a histone-binding step in common, one of the common roles of the ER motifs in Esa1 and Rpd3 might be to bind acetyl groups or histones. It is unlikely that the motif is involved in binding to an acetyl group because the ER motif in Esa1 is not located within the acetyl-CoA binding site in the MYST domain. On the other hand, histone binding by the ER motif in Esa1 is likely. The tertiary structure of the central region of Gen5 (44) is solved with histone H3 and shows structural homology to Esa1 (32), despite limited sequence homology (50). Comparison between the tertiary structure of Esa1 and that of Gen5-histone H3 complex indicates that the region containing the ER motif in Esa1 forms a surface for histone binding. The above observations collectively suggest that the ER motif is involved in a substrate (histone)-binding process. This issue will be confirmed by tertiary structure analysis of Esa1-histone complex.
A homologous protein from HDLP hints at the roles of the ER motif in Rpd3. As we discussed previously, HDLP lacks the region corresponding to the ER motif of Rpd3, despite the overall similarity between the two molecules (Fig. 1E). The sequence comparison shows that the ER motif in Rpd3 is estimated to be located between β8 and α8 (blue circle in Fig. 1D). In the open α/β class of folds, insertions can occur commonly after one or more of the β-sheets (51), suggesting that the ER motif is inserted into the corresponding region of an ancestor of Rpd3 without disrupting the overall topology. The inserted motif might be involved in histone recognition by the eukaryotic histone deacetylases, because HDLP, which lacks this insertion, is a protein from hyperthermophilic bacterium, which lacks apparent counterparts of histones. This notion agrees with the previous experiment showing that HDLP deacetylates histones in vitro with a specific activity only about 7.5% of that of HDAC1 (33), a human counterpart of yeast Rpd3. Our study also shows that the residues of ER1 and ER2 in the ER motif of Rpd3 are required for HDAC activity of Rpd3. These experimental results support the notion that the region containing the ER motif is inserted into an ancient HDLP as a histone-binding module and that the residues of ER1 and ER2 form a surface for the binding. Therefore, we propose that the residues of ER1 and ER2 function in common as a histone-binding surface in both Esal (HAT) and Rpd3 (HDAC).

The ER motif is divided into three regions: ER1, ER2, and ER3. The residues of ER1 and ER2 are involved in catalytic reactions, whereas the residues of ER3 are apparently involved in roles other than catalytic reactions. In Esal, the position of the residues of ER3 at the surface opposite to the cleft and the composition of the residues of ER3 including basic (Arg245) and aromatic (Tyr266) residues are reminiscent of similar features found in DNA-binding protein. We therefore predict that the residues of ER3 interact with DNA and play a role when the enzymes act on chromatin. If this is true, our approach will help reveal unknown activities of proteins.

Implications of the Evolutionary Origin of a Functional Pair of Enzymes—A pair of enzymes with opposite functions is considered a product of convergent evolution. However, Esal1 and Rpd3 are a pair of enzymes that regulate the gene expression from the PHO5 promoter through the acetylation or deacetylation of the same Lys12 of histone H4 (14). Existence of a similar structural and functional motif (the ER motif) between a functional pair of enzymes (HAT and HDAC) suggests that the pair of enzymes partially originated from a common ancestor. Structural motifs have been found among a variety of proteins (for example, the bromodomain is found in various chromatin factors, such as HAT and ATPase (52–54)). However, our finding of a motif between a pair of enzymes with opposite functions near the active center opens a novel way to characterize the enzymes of opposite functions and thus enables the prediction of a functional pair between various enzymes based on a common motif. Although no common structural motifs between other HATs and HDACs have yet been found, the...
possibility that these HATs and HDACs have a common structure on the tertiary structural motif cannot be denied. For example, the β3, β4, and α1 of Esa1 form a zinc-finger fold despite the absence of an apparent zinc-finger motif in the primary structure of Esa1 (32). Therefore, to find a common motif other than the ER motif between Esa1 and Rpd3, analysis of the tertiary structure of the remaining HATs and HDACs is needed. Our findings might also help in finding functional pairs between various enzymes other than HAT and HDAC.

Acknowledgments—We thank Drs. T. Takakawa and K. Kawaguchi at JASCO Corporation for help with the CD analysis and Drs. F. Inagaki, H. Kurumizaka, and R. Shimada for helpful discussions and information. We also thank Drs. T. Suzuki, R. Weisburd, and N. Whitby for comments on the manuscript.

REFERENCES

1. Kornberg, R. D., and Lorch, Y. (1999) Cell 98, 285–294
2. Luger, K., Mader, A. W., Richmond, R. K., Sargent, D. F., and Richmond, T. J. (1997) Nature 389, 251–260
3. Ramakrishnan, V., Finch, J. T., Graziano, V., Lee, P. L., and Sweet, R. M. (1995) Nature 376, 219–223
4. Allfrey, V., Faulkner, R. M., and Miersky, A. E. (1964) Proc. Natl. Acad. Sci. U. S. A. 51, 786–794
5. Grunstein, M. (1997) Nature 389, 349–352
6. Struhl, K. (1998) Genes Dev. 12, 599–606
7. Strahl, B. D., and Allis, C. D. (2000) Nature 403, 41–45
8. Kao, M. H., Zhou, J., Jambeck, P., Churchill, M. E., and Allis, C. D. (1998) Genes Dev. 12, 627–639
9. Rundlett, S. E., Carmen, A. A., Kobayashi, R., Bavykin, S., Turner, B. M., and Grunstein, M. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 14503–14508
10. Kimura, A., and Horikoshi, M. (1998) FEBS Lett. 431, 131–133
11. Kimura, A., and Horikoshi, M. (1998) Genes Cells 3, 789–800
12. Carmen, A. A., Griffin, P. R., Calaycay, J. R., Rundlett, S. E., Suka, Y., and Grunstein, M. (1999) Proc. Natl. Acad. Sci. U. S. A. 96, 6156–6161
13. Wade, P. A., Pruss, D., and Wolfe, A. P. (1997) Trends Biochem. Sci. 22, 128–132
14. Vogelauer, M., Wu, J., Suka, N., and Grunstein, M. (2000) Nature 406, 495–498
15. Yamamoto, T., and Horikoshi, M. (1997) J. Biol. Chem. 272, 30595–30598
16. Smith, E. R., Eisen, A., Gu, W., Sattah, M., Panmutti, A., Zhou, J., Cook, R. G., Luecchis, J., and Allis, C. D. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 3561–3565
17. Taunton, J., Hassig, C. A., and Schreiber, S. L. (1996) Science 272, 408–411
18. Allard, S., Uley, R. T., Savard, J., Clarke, A., Grant, P., Brandl, C. J., Pillus, L., Workman, J. L., and Cote, J. (1999) EMBO J. 18, 5108–5119
19. Alland, L., Muhle, R., Hou, H. Jr., Potes, J., Chin, L., Schreiber-Agus, N., and DePinho, R. A. (1997) Nature 387, 49–55
20. Hassig, C. A., Fleischer, T. C., Billin, A. N., Schreiber, S. L., and Ayer, D. E. (1997) Cell 89, 341–347
21. Heinzel, T., Lavinsky, R. M., Mullen, T. M., Soderstrom, M., Laherty, C. D., Torchia, J., Yang, W. M., Brard, G., Ngi, S. D., Dave, J. R., Setsu, E., Eisenman, R. N., Rose, D. W., Glass, C. K., and Rosenfeld, M. G. (1997) Nature 387, 43–48
22. Kadosh, D., and Struhl, K. (1997) Cell 98, 365–371
23. Laherty, C. D., Yang, W. M., Sun, J. M., Davie, J. R., Seto, E., and Eisenman, R. N. (1997) Cell 89, 349–356
24. Nagy, L., Kao, H. Y., Chakravartii, D., Lin, R. J., Hassig, C. A., Ayer, D. E., Schreiber, S. L., and Evans R. M. (1997) Cell 89, 373–380
25. Reifsnyder, C., Lowell, J., Clarke, A., and Pillus, L. (1996) Nat. Genet. 14, 29–41
26. De Ruberti, F., Kadosh, D., Henchoz, S., Pauli, D., Reuter, G., Struhl, K., and Spierer, P. (1996) Nature 384, 589–591
27. Hilfiker, A., Hilfiker-Kleiner, D., Panmutti, A., and Lucchesi, J. C. (1997) EMBO J. 16, 2054–2060
28. O’Neill, L. P., Reobane, A. M., Lavender, J. S. McCabe, V., Heard, E., Avner, P., Brockdorff, N., and Turner, B. M. (1999) EMBO J. 18, 2897–2907
29. Ikura, T., Ogryzko, V. V., Grigoriev, M., Groisman, R., Wang, J., Horikoshi, M., Scully, R., Qin, J., and Nakatani, Y. (2000) Cell 102, 463–473
30. Yan, Y., Barley, N. A., Haley, R. H., Berger, S. L., and Marmorstein, R. (2000) Mol. Cell 6, 1195–1205
31. Finnin, M. S., Donigian, J. R., Cohen, A., Richon, V. M., Rifkind, R. A., Marks, P. A., Breslow, R., and Pavletich, N. P. (1999) Nature 401, 188–193
32. Altschul, S. F., and Gish, W. (1996) Methods Enzymol. 266, 460–485
33. Thompson, J. D., Higgins, D. G., and Gibson, T. J. (1994) J. Mol. Biol. 232, 401–408
34. Wilmot, C. M., and Thornton, J. M. (1988) J. Mol. Biol. 205, 427–438
35. Borrow, J., Stanton, V. P., Jr., Andresen, J. M., Becher, R., Behm, F. G., Chaganti, R. S., Civin, C. I., Disteche, C., Dube, I., Fruchhauf, A. M., Horman, D., Mitelman, F., Voinia, S., Watmore, A. E., and Housman, D. E. (1996) Nat. Genet. 14, 33–41
36. Clarke, A. S., Lowell, J. E., Jacobson, S. J., and Wagner, G. (1999) Nature 400, 86–89
37. Gausk, N., and Peitsch, M. C. (1998) Electrophoresis 19, 2714–2723
38. Munoz, V., Blanco, F. J., and Serrano, L. (1995) Trends Biochem. Sci. 20, 154–155
39. Heinkein, P., Goldman, A., Jeffries, C., and Oliss, D. L. (1999) Struct. Fold. Des. 7, R141–144
40. Georgakopoulos, T., and Thireos, G. (1992) EMBO J. 11, 4145–4152
41. Haynes, E. R., Dallard, C., Winston, F., Beck, S., Trowdale, J., and Dawid, I. B. (1995) Nucleic Acids Res. 20, 2693
42. Tamkun, J. W., Deuring, R., Scott, M. P., Kissing, M., Pattatucci, A. M., Kaufman, T. C., and Kennison, J. A. (1992) Cell 68, 561–572