The human monocytic leukemia zinc finger (MOZ) protein is an essential transcriptional coactivator and histone acetyltransferase (HAT) that plays a primary role in the differentiation of erythroid and myeloid cells and is required to maintain hematopoietic stem cells. Chromosomal translocations involving the HAT-encoded region are also associated with acute myeloid leukemia. Here we present the x-ray crystal structure of the MOZ HAT domain and related biochemical studies. We find that the HAT domain contains a central region that is structurally and functionally conserved with the yeast MYST HAT protein Esa1, but contains more divergent N- and C-terminal regions harboring a TFIHIA-type zinc finger and helix-turn-helix DNA-binding motifs. Solution DNA-binding and acetyltransferase activity assays, in concert with mutagenesis, confirm that the MOZ HAT domain binds strongly to DNA through the zinc finger and helix-turn-helix motifs and that DNA binding and catalysis are not mutually exclusive. Consistent with the DNA-binding properties of MOZ, we also show that MOZ is able to acetylate nucleosomes and free histones equally well, whereas other HATs prefer free histones. Our results reveal, for the first time, that enzymatic and DNA-targeting activities can be contained within the same chromatin regulatory domain.

The eukaryotic genome is packaged into chromatin, the highly organized DNA-protein complex that not only serves as a structural element in preserving genetic information but also as a dynamic scaffold from which nuclear processes occur such as transcription, replication, DNA repair, mitosis, and apoptosis (1, 2). The fundamental unit of chromatin is the nucleosome, consisting of 145–147 bp of DNA wrapped around an octameric histone core containing two molecules each of histone proteins H2A, H2B, H3, and H4. There are at least four types of protein domains that regulate DNA processes through chromatin modification. These include (a) enzymatic domains that either use ATP to translocate the DNA relative to the histone core proteins (3) or post-translationally modify the histone proteins (4, 5) and (b) non-enzymatic domains that recognize chromatin, either through interactions with unmodified or modified N-terminal histone tails or the DNA, or histone chaperone proteins that deposit histones or replace variant histones into chromatin. Many chromatin regulatory proteins often contain both an enzymatic and chromatin recognition domain, although, to date, there have been no reports of a single domain harboring both activities.

Chromatin recognition domains that target histones include bromodomains (6, 7), which recognize specific acetyllysine modifications, chromodomains (8, 9), and tudor (10) domains, which bind specific methyllysine modifications, 14-3-3 domains, which recognize phosphoserine modifications (11), and SANT domains (12), which recognize unmodified histones. The SLIDE (12) and SWIRM (13, 14) domains are chromatin recognition modules that can target the DNA within nucleosomes. Among the enzymes that mediate post-translational modification of the histone tails, the histone acetyltransferases (HATs) are among the best characterized. On the basis of their sequence similarity, several subfamilies of HAT enzymes have been identified, including the GCN5/PCAF (15), CREB-binding protein/P300 (16, 17), and MYST subfamilies (18). The acronym MYST is from its four founding members: human MOZ (monocytic leukemia zinc finger protein) (19), yeast Ybf2 (renamed Sas3, for something about silencing 3) (20), yeast Sas2 (20), and mammalian Tip60 (human immunodeficiency virus Tat-interacting 60-kDa protein) (21). Other MYST members include MORF (a human MOZ homologue) (22) and HBO1 (23). Each HAT family has been shown to have substrate preference. For example, members of the GCN5/PCAF family prefer lysine 14 of histone H3, and most members of the MYST family of HATs prefer lysine residues on histone H4. In vivo, many HAT enzymes carry out their catalytic function in the context of large multiprotein complexes (24).

The structure of HAT domains from representative members of the GCN5/PCAF and MYST subfamilies provides...
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insights into their mechanism of action (25–28). In particular, structures of tetrahymena and yeast Gcn5 (25–27, 29) and human PCAF (28) in various liganded forms reveal that the HAT domain contains three regions: a central region associated with acetyl-CoA cofactor binding and catalysis in addition to flanking N- and C-terminal regions involved in histone H3 binding. A structure of the yeast Esa1 member of the MYST HAT family bound to cofactor (30) reveals a structurally and functionally homologous central region but divergent N- and C-terminal regions, presumably also important for substrate binding specificity. Interestingly, all members of the MYST family, except for Esa1, contain a cysteine/histidine-rich region with conserved spacing that is predicted to form a C2HC-type zinc finger, first characterized in the DNA-binding transcription factor TFIIIA (31). Surprisingly, the structure of the Esa1 HAT domains shows a TFIIIA type zinc finger fold, despite the absence of a bound zinc ion (30).

The gene encoding the human MYST HAT, MOZ (monocytic leukemia zinc finger protein) was first identified through its presence in reciprocal chromosomal translocations associated with a subset of acute myeloid leukemias (AMLs) (32). These chromosomal translocation products contain the HAT encoding region of MOZ, fused 5’ to the HAT-encoding region of either CREB-binding protein, p300, or a region of TIF2 that mediates the recruitment of the HAT domain of CREB-binding protein. It has been reported that MOZ-TIF2 translocations immortalize myeloid progenitors in vitro and induces AML in mice (33). The authors of this study also show that animals transplanted with MOZ-TIF2-transduced cells developed AML with 100% penetrance and that transformation is strictly dependent on the integrity of the zinc finger region of the MOZ HAT domain, but only partially dependant on the enzymatic activity of the HAT domain. These results suggest that an activity of the MOZ HAT domain, other than its enzymatic activity, is important for the etiology of AML. More recently, MOZ was shown to be essential for maintenance of hematopoietic stem cells and to play a role in the differentiation of erythroid and myeloid cells (34, 35).

To obtain structural and mechanistic insights into the MOZ HAT domain, the zinc finger region harbored within it, and possibly the underlying basis for its association with chromosomal translocations in AML, we determined the x-ray crystal structure of the MOZ HAT domain-containing plasmids were transformed into E. coli strain BL21(DE3) LysS+ cells for overexpression of an N-terminal His6-tagged fusion protein (His-hMOZ), initially grown at 37 °C to exponential phase and induced with 0.5 mM isopropyl-β-D-galactopyranoside at 15 °C overnight. Cells harboring hMOZ were disrupted by sonication in 50 mM sodium phosphate, pH 8.0, 500 mM NaCl, 5 mM imidazole, pH 8.0, 5% glycerol, 0.01% Triton X-100, 10 mM 2-mercaptoethanol, and 1 mM phenylmethylsulfonyl fluoride, and the protein was partially purified on nickel-nitrotriacetate acid resin with the His6 tag cleaved off essentially as described by the manufacturer (Qiagen). The protein was further purified to homogeneity using a combination of SP-Sepharose (Amersham Biosciences) cation exchange and Superdex 75 (Amersham Biosciences) gel-filtration chromatography, essentially as described (25, 36). Purified protein was concentrated to ~5.5 mg/ml using an Amicon Ultra-15 concentrator, in a buffer containing 20 mM sodium citrate, pH 5.5, 100 mM NaCl, and 1 mM dithiothreitol, and flash frozen for storage at −70 °C.

All MOZ HAT domain mutants were generated from the pRSET A-based expression vector template encoding the wild-type domain using site-directed mutagenesis (QuikChange, Stratagene) and confirmed by DNA sequencing. The mutant MOZ HAT domain-containing plasmids were transformed into E. coli strain BL21(DE3) LysS+ cells for overexpression of a His6-tagged fusion protein (His-hMOZ), as described above. The proteins were purified using nickel affinity, SP-Sepharose cation exchange, and Superdex 75 gel-filtration chromatography as described above, concentrated to 5–8 mg/ml, and flash frozen for storage at −70 °C until further use.

**HAT Assays**—The histone acetyltransferase activity of the recombinant MYST domain of MOZ was determined using a 3H-based radioactive HAT assay as described elsewhere (37) using as substrates H3p19 (5QTARKSTGGKAPRKQLASK23) and H4p19 (5SRGKGKGLGKGGAKRR19) and free core histones (Sigma, taken from calf thymus) essentially as described. The following reaction protocol was utilized for 30-μl reaction volumes: two concentrations of MOZ, representative of the linear range of the enzyme (50 or 100 nm), were incubated with either 100 μM histone peptide(s) or 10 μg of calf histones, and 0.25 μCi (3.9 Ci mmol−1 3H) of Ac-CoA in HAT buffer (20 mM Tris-HCl, pH 7.6, 50 mM sodium chloride, 5% (v/v) glycerol, 10 mM sodium butyrate, 1 mM phenylmethylsulfonyl fluoride, and 10 μg bovine serum albumin) for 25 min at 30 °C. Background control reactions did not include enzyme. Negative control experiments to check for self-acetylation and any false-positives due to the reaction conditions were also
assayed (the core domain of p53 was used as a negative control). All reactions were performed in triplicate.

The radioactive HAT assay in the absence or presence of saturating concentrations of cruciform DNA (2.0 μM) was carried out by incubating 80 nM of the MOZ HAT domain on ice for 45 min with increasing concentrations of H4p19 within the linear range of the enzyme (0–200 μM). All reactions were performed in duplicate.

The HAT activity of the MOZ HAT domain with oligonucleosomes as substrate was compared with its activity with free histones as substrate using the 3H-based radioactive HAT assay (37). Histone and oligonucleosome substrates were purified from HeLa cells as previously described (38). Substrate concentrations were determined by the generation of a standardized BCA curve (BCA Protein Determination Kit purchased from Pierce). Acetylation assays were carried out as outlined above, using equimolar concentrations of either oligonucleosomes or free histones (3.0 μg per reaction, with the only difference being the presence of DNA in the oligonucleosomes) substituted into each reaction mixture as the substrate. The background was determined by measuring the acetylation rate with no enzyme present in the reaction mixture, and enzyme self-acetylation was determined to be negligible by measuring acetylation levels in reaction mixtures with no substrate present. The protein concentration used (500 nM) was within an experimentally determined linear-dependent range based on the substrate concentration (data not shown). All reactions were performed in triplicate.

**DNA Binding Assay** — The DNA-binding activity of the MOZ HAT domain was investigated using fluorescence polarization. Four-way junction cruciform DNA and double-stranded DNA duplex samples were prepared as described by Bianchi et al. (39), and a sequence compatible with the formation of Z-form DNA (two strands of ZDNA-1, CGCGCGCGCGCGCGCGCGGCGCGCGGCGGCGCGG) was used. 2-fold serial dilutions of the recombinant HAT domains were prepared from stock solutions in a protein dilution buffer (20 mM Hepes, pH 7.5, 100 mM NaCl, 100 μg/ml bovine serum albumin), and then incubated with 1 nM of fluorescein-labeled DNA substrate in a buffer containing 20 mM Hepes, pH 7.5, and 200 mM NaCl in a total volume of 100 μl in borosilicate glass cuvettes. A negative control contained no protein. Each binding reaction was allowed to equilibrate for 30 min before fluorescence measurements were taken using a BEACON 2000 variable temperature fluorescence polarization system. Each experiment was performed at least in duplicate, and the average millipolarization value was plotted against a log scale of the protein concentration. The data were fit to a non-linear dose-response logistical transition, \( y = a_0 + a_1/(1 + (x/a_2)^m) \), using the Levenberg-Marquardt algorithm within the SLIDEWRITE software package, where the \( a_2 \) coefficient is the dissociation constant \( K_p \).

The DNA-binding activity of the MOZ HAT domain was compared with other HAT domain and MOZ HAT domain mutants using the same protocol outlined above with only cruciform DNA. The other recombinant HAT domains used were from yeast Esa1 (residues 160–435), Drosophila MOF (residues 533–819), human PCAF (residues 493–658), and yeast GCN5 (residues 99–262).

**Nucleosome Displacement Assay** — Recombinant Xenopus Laevis nucleosome core particles were purified essentially as described from recombinant full-length histones and 146-bp DNA (40), except that 20 mM Tris, pH 7.5, instead of 20 mM sodium acetate, pH 5.2, was used during histone refolding and nucleosome assembly with DNA. Gel electrophoresis of mono-nucleosomes in the presence or absence of wild-type or mutant His\(_6\)-tagged HAT domains of hMOZ, and wild-type yGcn5 were carried out essentially as described (38). Briefly, serial dilutions of respective HAT domain (ranging from 2.5 to 10 μM) was incubated with 50 nM of recombinant nucleosome core particle in a reaction buffer containing 50 mM Hepes, 7.5 pH, 50 mM NaCl, and 10% glycerol in a total volume of 10 μl. After equilibrating at room temperature for 20 min, 10 μl of 2× Native Gel Loading Buffer was added to the samples to 5% v/v sucrose and 0.15% SDS (to prevent smearing of nucleosome bands) and analyzed by electrophoresis on gels containing 5% acrylamide in 0.2× TBE (0.89 M Tris, 0.89 M boric acid, 0.02 M EDTA, Na\(_2\) salt) at 150 V for 150 min. Nucleosomes were detected by staining with Cyber Green (Cambrex). Nucleosome displacement assays using wild-type MOZ and Gcn5 and the MOZ H732D mutant were carried out in three independent experiments producing the same result.

**Structure Determination** — For co-crystallization, the protein was premixed with Ac-CoA and peptide (H4p19 or H3p19) to a final ratio of 0.065 mM (protein): 0.145 mM (Ac-CoA): 0.23 mM peptide, and equilibrated for 30 min at room temperature to allow complex formation. Crystals were obtained at 22–23 °C using hanging-drop, vapor diffusion by equilibrating 2–4 μl of the premixed protein complex solution against 1 ml of reservoir solution containing 90 mM sodium cacodylate, pH 6.5, and 15–20% polyethylene glycol 3350. Crystals grew to a maximum size of ~0.2 mm × 0.15 mm × 0.15 mm over 2 weeks and formed in the space group I422 with one molecule per asymmetric unit. Crystals were transferred to harvest solution containing 90 mM sodium cacodylate, pH 6.5, 10 mM sodium citrate, pH 5.5, 50 mM NaCl, and 15% polyethylene glycol 3350, followed by a gradual transfer to harvest solution supplemented with 5, 10, 15, and 20% (v/v) glycerol before crystals were flash-frozen in liquid nitrogen-condensed propane. The structure was determined by multiple anomalous dispersion. Native and three-wavelength (peak, inflection, and remote) multiple anomalous dispersion data using the zinc ion as the anomalous scattering source were collected on beamline 19BM at the Advanced Photon Source (Argonne, IL) using an ADSC Quantum-4 charge-coupled device detector at 100 K, and data were processed with the HKL 2000 suite (HKL Research) with the relevant statistics summarized in Table 1.

The structure of the MOZ HAT domain complex was determined by multiple anomalous dispersion. A single zinc site, Zn\(^{2+}\), was identified in the I422 crystal form using CNS (41) and SOLVE (42) and confirmed with cross-difference Fourier maps. The site was further refined using CNS and SOLVE. The resulting, initial experimental electron density map was improved by solvent flattening density modification. The program O (43) was used to build residues 507 to 779 of human MOZ (excluding residues 739–747) using the single zinc site and a partially generated backbone from the program.
RESOLVE (44) as a guide and template. Model refinement was performed with simulated annealing and torsion angle dynamic refinement protocols in CNS with iterative manual adjustments of the model using O. Toward the later stages of refinement, individual atomic B-factors were adjusted, and Ac-CoA and solvent molecules were modeled into the electron density map. There was no electron density observed corresponding to the zinc ions, and solvent molecules were modeled into the electron density map. There was no electron density observed corresponding to the zinc ions, and solvent molecules were modeled into the electron density map.

**RESULTS**

Overall Structure of the MOZ:Ac-CoA Complex and Comparison with Other HATs—Crystals of the HAT domain of MOZ (residues 501–784) in complex with acetyl-CoA are formed in space group I422 with one molecule per asymmetric unit. The structure was determined using multiple anomalous dispersion from a single protein bound zinc ion and refined to 3.0-Å resolution. The MOZ:Ac-CoA complex adopts a globular fold with approximate dimensions of 35 Å × 35 Å × 70 Å, characterized by a central core region flanked on opposite sides by N- and C-terminal protein segments (Fig. 1a).

The HAT domain of MOZ is architecturally similar to the previously reported structure of the MYST family member from yeast, Esa1 (30, 36), as well as the recently reported MYST family member from human MOF (MYST1, PDB no. 2GIV). The central core region shows structural homology to yEsa1/hMOF as well as a diverse group of Gcn5-related N-acetyltransferases, despite very limited sequence homology (47) (Fig. 1b). In addition, like yEsa1, the central core region of the MOZ HAT domain participates in cofactor binding and harbors the catalytic glutamate (residue 680) and cysteine (residue 646) residues in analogous positions (36). The pantothenate arm of the Ac-CoA cofactor also adopts a similar position within MOZ and Esa1, although the 3′-phosphate ADP moiety is in a different position for the two proteins, probably reflecting the paucity of protein interaction to this region of the cofactor in both structures.

The N- and C-terminal protein segments of the MOZ HAT domain display structural divergence compared with other N-acetyltransferases but shows a higher degree of similarity to Esa1 with an r.m.s.d. of 1.229 Å (Fig. 1b). Like yEsa1, the N-terminal segment is β-rich, and the C-terminal segment is α-helix-rich with the greatest divergence occurring in the positioning of the connecting loops between the β-sheets and α-helices and an insertion of an α1 N-terminal element in MOZ that is not present in Esa1. A presumed loop region connecting residues 738 to 748 is not modeled into the crystal structure of the MOZ HAT domain, because no electron density is present for this region, probably reflecting an inherent conformational flexibility of this region of the MOZ HAT domain.

Surprisingly, a closer examination of the N- and C-terminal protein segments in the MOZ HAT domain structure reveals two common structural motifs found in DNA-binding proteins. In particular, a β3-turn-β4-α2-loop region of the N-terminal segment forms a classic TFIIIA type zinc finger fold around the one bound zinc ion (Fig. 1c). A common feature to all proteins of the MYST family, except for Esa1, is a CXXCX_{1}HXXXC region that makes up this fold has been previously proposed to ligand a zinc ion (48). Three cysteines and a single histidine residue bind the structural zinc ion; the first two cysteine residues are located in the β3-turn region, the histidine residue is found in the α2 helix, and the final cysteine residue is in the loop succeeding the α2 helix. The importance of this region for HAT function is consistent with deletion studies of the Sas3 protein that reveals that the putative zinc-binding region is required for HAT activity (48).

Inspection of the C-terminal segment of the MOZ HAT domain reveals another DNA binding structural motif consisting of a three-helical bundle (α5-α7) containing an α6 helix-turn-α7 helix motif (Fig. 1c). The helix-turn-helix motif is the archetypical evolutionary conserved DNA-binding motif present in both prokaryotes, illustrated by the seminal structures of lambda Cro and repressor proteins (49), and eukaryotes, including the homeodomain proteins and other families of DNA-binding proteins.

**TABLE 1**

| Data collection, phasing, and refinement statistics for the MOZ HAT domain |
|---------------------------------------------------------------|
| Data for the outer resolution shell for the native MOZ crystal are in parentheses. |
| Crystal parameters |
| Space group | I422 |
| Unit cell |
| a = b (Å) | 109.2 |
| c (Å) | 144.78 |
| Data collection |
| Wavelength (Å) | 1.2829 |
| Resolution (Å) | 50-3.2 |
| Total reflections | 284,344 |
| Unique reflections | 13,757 |
| Completeness (%) | 99.9 |
| I/σ | 30.6 |
| Rmerge (%) | 6.7 |
| Refinement |
| Resolution range | 50–3.0 |
| Rwork (%) | 27.6 |
| Rfree (%) | 28.4 |
| r.m.s.d. Bond lengths (Å) | 0.009 |
| Bond angle (°) | 1.5 |
| Number of atoms | 2,095 |
| Protein | 74.67 |
| Water | 60.79 |
| Ac-CoA | 55.35 |
| Zinc ions | 83.09 |
| Ramachandran plot |
| Residues in most favored regions | 170 |
| Residues in additional allowed regions | 50 |
| Residues allowed in generously allowed region | 13 |
| Residues in disallowed regions | 2 |

**Phasing (SOLVE)**

| Figure of merit | 0.57 |
| Z-score | 8.9 |

| Phasing (SOLVE) |
|------------------|
| Figure of merit | 0.57 |
| Z-score | 8.9 |

The table provides data collection, phasing, and refinement statistics for the MOZ HAT domain. The data include space group information, unit cell parameters, and crystallographic statistics. The table also details the resolution range, completeness, and other relevant parameters for the MOZ HAT domain structure.
transcription factors (50). Taken together, the structure of the MOZ HAT domain suggested that it may harbor DNA-binding activity. Consistent with this hypothesis, an electrostatic potential surface calculation of the MOZ HAT domain reveals positive electrostatic potential on the surface of the zinc finger and helix-turn-helix motifs (Fig. 1d).

**DNA-binding Properties of the MYST HAT Domain of MOZ—** To address whether the HAT domain of MOZ was capable of binding DNA, we employed a fluorescence-based DNA polarization assay to measure its DNA-binding activity against DNA targets of different architecture. DNA targets included biologically ubiquitous B-form double-stranded DNA, single-stranded DNA, Z-form DNA, and cruciform DNA. A plot of polarization values against protein concentration yielded a DNA-binding isotherm for the HAT domain of MOZ (Fig. 2a) against each of the DNA targets used with calculated dissociation constants ranging from ~100 to 900 nM. The observation that the MOZ HAT domain binds DNA with an affinity comparable to dissociation constants that sequence-specific DNA-binding proteins exhibit for nonspecific DNA sites (49) and to proteins that are known to bind DNA nonspecifically (51, 52) suggested that the DNA-binding properties of the HAT domain of MOZ might be biologically significant.

Interestingly, the MOZ HAT domain had the greatest affinity for cruciform DNA with a dissociation constant of 110 nM. This observation is consistent with the hypothesis that both DNA-binding domains of the MOZ HAT domain cannot engage the same DNA duplex simultaneously without a significant DNA bend, a proposal that is supported by modeling B-form DNA on the DNA-binding domains of the MOZ HAT domain (data not shown). Cruciform DNA is the most bendable of the DNA substrates used (39, 53, 54) in this assay and is likely able to simultaneously engage both DNA-binding domains, and single-stranded DNA, which is also bendable but does not form a duplex also yields a dissociation constant (354 nM) higher than cruciform DNA but lower than the duplex DNA sites (483–894 nM).

Given our finding that the HAT domain of MOZ has DNA-binding activity, we asked whether other HAT domains might also harbor this activity. To address this issue, the HAT domains of the MYST proteins yeast Esa1 and Drosophila MOF and the GCN5/PCAF proteins, yeast Gcn5 and human PCAF, were assayed for DNA binding with cruciform DNA (Fig. 2b). The results of these studies reveal that the MYST protein yEsa1 binds cruciform DNA ~4-fold more weakly than MOZ, whereas dMOF binds with significantly less affinity (by at least 50-fold) than MOZ. HAT domains from members of the GCN5/PCAF family of HAT proteins. hPCAF and yGcn5, displayed negligible affinity for cruciform DNA despite having similar basic pI values to the other HAT domains tested. These data illustrate that the DNA-binding properties of the MOZ HAT domain are not a conserved function of HATs, although it is possible that other MYST proteins such as yEsa1 might also exhibit some DNA-binding activity.

To confirm that the DNA-binding activity of MOZ was indeed mediated by the zinc finger and helix-turn-helix motifs, we carried out site-directed mutagenesis of these motifs. To guide the mutagenesis, we overlaid the putative MOZ DNA binding motifs with their corresponding homologous domains bound to DNA to help identify MOZ residues in conserved positions with residues in the corresponding DNA-binding proteins that mediate DNA contacts. The helix-turn-helix motif of MOZ, defined from residues 710 to 735, was used as a search model using the DALI server, which identifies similar structures to an inputed query model based on three-dimensional comparison modeling with structures contained in the Protein Data Bank. The first two non-HAT proteins, identified by the DALI server, to show the strongest structural homology were the bacterial multiple antibiotic resistance protein, MarR, and the human double-stranded RNA-specific adenosine deaminase protein, ADAR1. Utilizing a least squares fit algorithm, the structure of MarR (beginning at residue 54) was superimposed on the helix-turn-helix motif in MOZ (encompassing the a6 and a7 helices) with a r.m.s.d. value of 1.961 Å for the Co backbone residues (Fig. 3a). In the crystal structure of MarR, a winged helix fold is identified that displays similar topology to other winged-helix DNA-binding proteins (55). The "recognition helix (a4)" in MarR, which is implicated in the DNA-binding activity of MarR and other members of its family,
corresponds to the α7 helix in MOZ (55). The other helix-turn-helix proteins identified with a high degree of structure homology with MOZ were CAP and HNF3γ (56).

An overlay of each of the homologue helix-turn-helix motifs with MOZ yielded two possible targets for mutagenesis: a histidine at position 732 in MOZ and an isoleucine at position 727.
Structure and DNA-binding Activity of MOZ

(Fig. 3, b and d). Residues in the homologous helix-turn-helix domains revealed that a basic residue corresponding to His732 of MOZ mediates electrostatic interactions with the DNA backbone and/or specific contacts to the DNA bases (Fig. 3d). His 732 corresponds to a lysine residue in Esa1, lending further support to the proposed hypothesis. Given this correlation, we mutated this histidine to an aspartic acid at residue 732 in MOZ to disrupt possible DNA contacts by this residue. The second residue targeted for mutation was an isoleucine residue at position 727 of MOZ, which corresponded to a conserved hydrophobic residue that mediated the stability of the core region of other helix-turn-helix proteins (Fig. 3d). In light of this observation, we mutated this isoleucine residue to a glutamate residue to target disruption of the core.

To probe the contribution to DNA binding of the zinc finger region of MOZ, we used the DALI server to identify zinc finger proteins with the greatest structural homology to the zinc finger region of MOZ, defined from residues 535–561. The first two proteins identified were two zinc fingers from the U-shaped transcription factor protein, a member of the FOG family of proteins characterized by F-box motifs implicated in protein-protein interactions, and two zinc fingers from the yeast Swi5 protein. The DALI server also indicated homology to the mouse protein Zif268, whose crystal structure was determined bound to DNA (57). Utilizing a least squares fit algorithm, the Zif268-DNA complex (beginning at residue 3) was superimposed on the C2HC zinc finger of MOZ (encompassing the β3-turn-β4-α2-loop region) with an r.m.s.d. of 1.160 Å for the Ca backbone residues (Fig. 3a). Based on this superposition, we identified two possible targets for point mutations (Fig. 3, c and e). The first was a lysine at position 545 in MOZ that corresponded to an arginine (Arg14) in Zif268 that contacted a phosphodiester oxygen in the DNA backbone of the crystal structure of Zif268 (57). The second targeted site for mutation was a glutamine at position 555 in MOZ, which corresponded to another arginine (Arg24) in Zif268, which made base contacts with the DNA (57). Single alanine point mutants in MOZ were constructed to probe the contribution of these residues to DNA binding.

The fluorescence-based DNA polarization assay was utilized to measure the DNA-binding affinity of the MOZ mutants for cruciform DNA for comparison with the wild-type protein (Fig. 2, c and d). Within the helix-turn-helix motif of the MOZ HAT domain, the H732D point mutation bound 5-fold less tightly than the wild-type protein with a dissociation constant of 530 nM (Fig. 2c). The I727E point mutation also decreased the DNA-binding affinity of MOZ but to a lesser extent than the H732D point mutation (Fig. 2c). Within the zinc finger motif of the MOZ HAT domain, the K545A point mutant displayed a 4-fold decrease in DNA-binding affinity with a calculated dissociation constant of 460 nM. The glutamine to alanine mutant, Q555A bound to cruciform DNA with a measured dissociation constant of 55 nM, indicating that this mutant bound DNA with a 2-fold greater affinity than wild-type MOZ. The double mutant, K545A/Q555A, was also constructed, and measurement of cruciform DNA-binding affinity resulted in a 5-fold decrease in binding affinity ($K_D = 570$ nM, Fig. 2d). All the MOZ mutants used for the DNA binding studies displayed similar HAT activity when compared with the wild-type protein (Fig. 2e) indicating that the mutants are properly folded.

Acetyltransferase Activity of the MOZ HAT Domain—Previous in vitro studies demonstrate that MOZ mediates the acetylation of Lys16 of histone H3 and Lys9, Lys8, Lys12, and Lys16 of histone H4 (58). As a baseline to investigate the effect of DNA binding on the HAT activity of the MOZ HAT domain we first characterized the HAT activity of the recombinant MOZ HAT domain in the absence of DNA. Initial acetylation studies were performed with three histone substrates in the form of two 19-amino acid peptides representative of the N-terminal histone tails, H3p19 (9QARKSTGGKPKQLASK19) and H4p19 (9SGRGKGGKGLKGKAKRH19), in addition to free core histones (Sigma, obtained from calf thymus). The results of the acetylation assay reveal that the recombinant MYST domain of MOZ acetylates the histone H3 and H4 peptides and the free core histones in a linear dose-dependent manner (Fig. 4a). These acetylation studies also show that the MYST domain of MOZ acetylates histones H4p19 and H3p19 equally well (Fig. 4a), supporting the previously reported histone substrate specificity studies for the MYST domain of MOZ (58).

Nucleosome-binding Activity of MOZ—To establish if the MOZ HAT domain could interact with DNA in the context of assembled nucleosomes, we prepared nucleosomes from recombinant Xenopus laevis H2A, H2B, H3, and H4 histones and 146 bp of DNA and carried out a nucleosomal displacement experiment based on the principle of an electrophoretic mobility shift assay. As can be seen in Fig. 2f, titrating wild-type MOZ HAT domain into nucleosomes resulted in a reduction in the amount of free nucleosomes that migrate into the gel in a dose-dependent manner. In contrast, the titration of wild-type Gcn5 HAT domain at comparable concentrations, a HAT domain that we showed does not associate with free DNA (Fig. 2b), did not result in a significant reduction in the amount of nucleosomes that migrate into the gel. We interpret this result to suggest that, although wild-type MOZ HAT domain associates with DNA in the context of nucleosomes, Gcn5 does not at comparable concentrations. Although we were surprised to find that a shifted MOZ/nucleosome band was not observed on the gel, as normally seen for a typical electrophoretic mobility

FIGURE 2. DNA binding by the MOZ HAT domain. a, DNA-binding activity of MOZ to different DNA conformers. Fluorescence polarization on the y-axis is plotted against MOZ protein concentration on the x-axis (displayed as a logarithmic scale). The data points are the mean of two separate experiments with background values (fluorescein-tagged DNA alone) subtracted and normalized to 1. The data were fit to the following dose response equation, $y = a_0 + a_1/(1 + (x/a_2)^{a_3})$, where the $a_1$ coefficient is the dissociation constant $K_D$, and the calculated binding isotherm is shown as a solid line. The binding constants are summarized below the graph. b, DNA-binding activity of HAT domains (human MOZ, yeast ESA1, Drosophila MOF, human PCAF, and yeast GCN5) for cruciform DNA. c, DNA-binding activity of MOZ helix-turn-helix mutants. d, DNA-binding activity of MOZ zinc finger mutants. e, the HAT activity levels are shown for MOZ and the DNA-binding mutations. Calf histones (Sigma) were used as the substrate (334 ng of histones/reaction), and all protein concentrations were 200 nM (within the linear range of activity). f, nucleosome displacement of wild-type and mutant human MOZ HAT domains and yeast GCN5. Protein titrations from 10 to 2.5 μM are indicated above the respective lanes. NCPs denote nucleosome core particles with no protein present, and “Double” indicates the MOZ double mutant, K545A/Q555A.
shift assay, we propose that this might be the case for one of two reasons: 1) it is possible that multiple MOZ protein molecules bind the nucleosome particle resulting in a large complex species that might precipitate or aggregate and thus be unable to enter the gel, or 2) MOZ/nucleosome complexes may be heterogeneous in terms of the number of MOZ proteins bound per nucleosome resulting in a mixture of species that is not visible as a distinct band on the gel. Consistent with our MOZ HAT domain binding studies with free DNA (Fig. 2), several of the DNA-binding mutants within the MOZ HAT domain showed a reduction in their ability to associate with nucleosomes (Fig. 2f).

Affect of DNA Binding on the HAT Activity of MOZ—To investigate the biochemical effects of DNA binding on catalysis by the MOZ HAT domain, we carried out HAT assays with the MOZ HAT domain and the H4p19 substrate in the presence or absence of saturating concentrations of cruciform DNA. The concentration of cruciform DNA used was 2 μM, which is ~20-fold higher than the measured K_d. The results of this assay are shown in Fig. 4b and illustrate that at low peptide concentration (0–25 μM), DNA binding did not have a significant effect on acetylation activity, whereas, at higher peptide concentration (50–100 μM), DNA binding had a modest stimulatory effect (~2-fold) on acetylation activity. At higher saturating concentrations of peptide (>100 μM), there was again no measurable difference in histone acetylation levels between reactions in the presence or absence of DNA. Taking these results together, we conclude that the DNA-binding and acetylation activities of the MOZ HAT domain can occur simultaneously. In addition, DNA binding by the MOZ HAT domain does not inhibit its acetyltransferase activity and may exhibit a small stimulatory effect on acetyltransferase activity under certain conditions. These kinetic data are indicative of a mutually exclusive surface for DNA binding that is separate and distinct from the site of catalysis and support the hypothesis that the DNA-binding and acetyltransferase activities of MOZ are not mutually exclusive.

We next wanted to assess the effect of nucleosomal DNA on histone acetylation by the MOZ HAT domain. For these studies we prepared oligonucleosome substrates purified from HeLa cells. We also prepared free histones from these oligonucleosomes as substrates by passing them through a hydroxyapatite column to strip off the associated DNA. We then directly com-

FIGURE 3. Structure of the DNA binding motifs of MOZ. a, the first zinc finger from Zif268 (residues 3-30, magenta, PDB accession number 1ZAA) and helix-turn-helix domain from MarR (residues 40–83, red, PDB accession number 1JGS) are superimposed on the MOZ-Ac-CoA structure. b, close-up view of the MarR/MOZ (red/cyan) superposition with mutagenized MOZ side chains in yellow. c, close-up view of the Zif268/MOZ (magenta/cyan) superposition. d, sequence alignment of the helix-turn-helix domain of MOZ with other structurally homologous helix-turn-helix proteins. MOZ residues selected for mutagenesis are boxed in red. e, sequence alignment of the zinc finger of MOZ with other structurally homologous zinc finger proteins. MOZ residues selected for mutagenesis are boxed.
To directly test if the DNA-binding activity of the MOZ HAT domain contributes to nucleosomal acetylation, we carried out the same experiments described above but using the MOZ HAT domains harboring the DNA-binding mutants we characterized earlier. As can be seen in Fig. 4 (c and d), whereas the K545A/Q555A and I727E mutants did not show a statistically significant difference in their abilities to acetylate free histones and nucleosomes, the DNA-binding mutants, K545A/Q555A and H732D had a statistically significant preference for nucleosomes over free core histones (p < 0.05). These data correlate well with the earlier anisotropy experiments in that the double K545A/Q555A and H732D point mutants displayed the greatest decrease in DNA binding as measured by their dissociation constants when compared with the WT protein. Notably, the H732D and K545A/Q555A MOZ HAT domain mutants exhibit substrate preferences that are comparable to the HAT domains of hPCAF and dMOF (Fig. 4d), two HAT domains that we showed in Fig. 2b, had relatively poor affinity for DNA. Taken together with our earlier results, we propose a DNA-targeting function of the MOZ HAT domain that is mediated by the embedded helix-turn-helix and zinc finger motifs and that facilitates nucleosomal acetylation by MOZ.

DISCUSSION

The crystal structure of the MOZ HAT domain in concert with the biochemical data illustrates some structural and functional similarities and differences with its yeast Esa1 homologue. In particular, the two HAT domains show a high degree of structural homology with the greatest homology mapping to the central core domain that is associated with cofactor binding and catalysis in both proteins (30, 36). Regions N- and C-terminal to the central core domain of MOZ also show structural superposition with the corresponding regions of yEsa1, and we infer that these regions play a role in histone substrate-binding specificity, like the role these regions play in the GCN5/PCAF family of histone acetyltransferases (27, 28, 59). The greatest degree of structural deviation between the HAT domains of yEsa1 and MOZ maps to an N-terminal zinc finger and C-terminal helix-turn-helix motif in MOZ. Although yEsa1 contain both folds, they show...
some deviations, and the zinc finger in yEsa1 does not contain a bound zinc atom. Taken together, this comparison suggests that the MYST HAT domains will harbor the same overall fold.

Despite the conservation in overall structure between yEsa1 and hMOZ and presumably other MYST proteins, we show that the MOZ HAT domain harbors DNA-binding activity that is not shared by other MYST HAT proteins, or members of the GCN5/PCAF family of HAT proteins. Through mutagenesis, we also show that this DNA-binding activity is mediated by the zinc finger and helix-turn helix motifs within the MOZ HAT domain. We also show that the DNA-binding and acetyltransferase activities of hMOZ are not mutually exclusive, and under certain conditions DNA binding can provide an enhancement of acetylation. The activity assays in the presence of saturating concentrations of DNA illustrate that the one or more DNA-binding sites of MOZ are separate and distinct from the sub-strate and CoA-binding surfaces, indicative of a novel, and previously undiscovered DNA-binding function for the MOZ protein. Finally, we show that, unlike other HAT domains, MOZ is able to acetylate nucleosomal and free histone substrates equally well, presumably due to its DNA-binding properties. Taken together, we propose that the DNA-binding properties of the MOZ HAT domain facilitate nucleosomal targeting for histone acetylation by MOZ. To our knowledge, the MOZ HAT domain is the first characterized protein domain with both enzymatic and chromatin-targeting activity.

We note that several non-catalytic subunits of several multi-protein HAT complexes have been shown to function, at least in part, to target the catalytic HAT subunits to nucleosomes for histone acetylation. This includes the SWIRM domain within the Ada2 subunit of the yeast GCN5 HAT complex and one of the subunits of the piccolo NuA4 complex. Although the SWIRM domain has been shown to target the DNA component (13), the piccolo NuA4 complex has been shown to target nucleosomes through interaction with the histone core domain (60). Consistent with our finding that the MOZ domain binds DNA and mediates catalysis in a non-mutually exclusive manner, Berndsen et al. (60) showed that piccolo NuA4 recognition of nucleosomes occurs independently of the histone tail regions. Although we have not addressed whether or not the MOZ domain binds DNA with sequence specificity, this intriguing possibility also exists, possibly to further contribute to the targeting of MOZ-mediated acetylation to specific genes.

It has recently been proposed that the acetylation of Lys-16 in the histone H4 tail disrupts higher order chromatin compaction, promoting the transition from a transcriptionally silenced heterochromatin state to a transcriptionally activated euchromatic state (61). MOZ has been shown to acetylate lysine 16 on histone H4, and our recent demonstration of the capacity of MOZ to bind DNA and preferentially acetylate nucleosomes over free histones, suggests a cellular context in which MOZ may bind nucleosomes in a transcriptionally silent condensed state, acetylate the histone H4 tail at position lysine 16, and thereby instigate the decondensation of the chromatin fiber and activation of transcription.

AML is characterized by the expression of fusion genes involving coactivators and also including the HAT domain of MOZ. Using a bone marrow transplant model in mouse, it was previously shown that pathogenesis of the MOZ-TIF2 fusion protein is dependent on the integrity of the CREB-binding protein domain of TIF2 and the C2HC zinc finger of the MOZ HAT domain (33). It had previously been proposed for the Drosophila MOF protein that this conserved C2HC zinc finger acts as a nucleosome binding motif (62), although in our hands Drosophila MOF did not show appreciable affinity for DNA (Fig. 2b). Here we propose that the C2HC zinc finger in MOZ coupled with the helix-turn-helix region represents a newly discovered DNA-targeting module for subsequent acetylation. In the mouse model, a single point mutation in the C2HC zinc finger abrogates MOF-TIF2-mediated transformation, whereas a deleterious mutation for HAT activity still resulted in the onset of AML, albeit at a lower incidence rate (33), consistent with our findings that MOZ binds DNA at sites mutually exclusive and distinct from the catalytic site. These results underscore the importance of the DNA-binding function of MOZ in vivo and suggest that the DNA-binding activity of MOZ may be at the heart of the oncogenic effect in AML-associated chromosomal translocations.

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