The Histone Acetyltransferase NCOAT Contains a Zinc Finger-like Motif Involved in Substrate Recognition*

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Nuclear cytoplasmic O-GlcNAcase and acetyltransferase (NCOAT) is a bifunctional enzyme with both glycoside hydrolase and alkyltransferase activity. Its O-GlcNAcase active site lies in the N terminus of the enzyme and its histone acetyltransferase (HAT) domain lies in the C terminus. Whereas the HAT domain of the enzyme is catalytically and structurally similar to other acetyltransferases across subfamilies, NCOAT has a motif resembling a zinc finger-like domain unique to the MYST family of HATs. Among the MYST family, this zinc finger, or zinc-finger-like domain, is responsible for making contacts with the histone tails within nucleosomes for the HAT to catalyze its respective reaction. Here, we show that NCOAT has the ability to directly associate with both an acetylated and unacetylated histone H4 tail in vitro, and a potential zinc finger-like motif found in NCOAT is implicated in this nucleosomal contact, and is necessary for fully efficient enzymatic activity. Subsequent to the catalysis of acetyltransfer to lysine 8 of histone H4 for the enzyme, however, the substrate is released and NCOAT can no longer bind H4 in our assays. Furthermore, this finger domain by itself is sufficient to bind histone H4.

Acetyltransferases (ATs) are a superfamily of enzymes capable of catalyzing the transfer of acetate groups from acetyl coenzyme A (CoA) to specific target lysines on proteins. ATs are catalogued into several subfamilies based on sequence, substrate specificity, and the context of other conserved protein domains (1). Members of the Gcn5, GNAT, and Hat1 families share a conserved mechanism of catalysis (1). This mechanism involves an active site carboxylate-containing residue that abstracts a proton from the ε-amino group of the target lysine, causing its direct attack on acetyl-CoA. The acetyl group is transferred from the thioester of acetyl-CoA to the lysine side chain and an active site acid abstracts a proton from the sulfur atom of coenzyme A to bring the reaction to completion (2, 3). Such a mechanism requires the formation of an enzyme-substrate-acetyl-CoA ternary complex before any events occur, thereby requiring the enzyme to have distinct substrate and acetyl-CoA binding sites.

Members of the MYST family of ATs, which are believed to undergo a variation of this mechanism involving a self-acetylated intermediate (4), also require distinct acetyl-CoA and substrate binding sites. MYST family members, with the exception of Esa1, share a Cx7Cx13HxC锌 finger motif in the N-terminal portion of the active site that is responsible for making contacts with the histone tail substrates (5–7). Esa1 lacks a complete zinc-coordinating tetrahedral motif and therefore does not bind zinc, yet the crystal structure reveals that a zinc finger-like structure is present within this site (4). The histone acetyltransferases p300 and CBP also possess a zinc finger motif, a PHD-type finger, in a comparable region in the N termini of their HAT domains, which has been shown to be important in the binding of nucleosomes (8), as well as for enzymatic activity (9, 10).

Nuclear-cytoplasmic O-GlcNAcase and acetyltransferase (NCOAT) is a protein with both N-acetylglucosaminidase activity and a recently described histone acetyltransferase activity (11). The HAT active site resides in the C terminus of the enzyme, and is structurally related, with four sequence motifs A–D in the catalytic core, to other ATs across many subfamilies (1, 11). NCOAT carries out its acetyltransfer by the same mechanism as that described for the GNAT, Gcn5, and Hat1 families and here we demonstrate that NCOAT displays biochemical properties consistent with its possession of a zinc finger-like domain as seen in the MYST family of HATs. Like MYST family member Esa1, NCOAT lacks a proper complete set of tetrahedral zinc-coordinating residues and does not bind zinc. NCOAT can directly bind histone H4 in vitro, both free and in the context of oligonucleosome substrates, and this binding ability is abrogated upon H4 acetylation on lysine 8. Select point mutations to this finger domain disrupt both its histone binding ability as well as its HAT activity. Furthermore, a construct containing this motif as well as 40 amino acids surrounding it on each side is sufficient to confer histone H4 binding ability to an otherwise non-histone-binding protein. This study characterizes the histone interacting domain of NCOAT, and these features of the HAT domain can collectively help better define its mode of action and its family relationships.

MATERIALS AND METHODS

Recombinant NCOAT and Mutagenesis—A pUC118-pTM hybrid expression vector was designed as described previously for expression of GST alone or as an N-terminal fusion protein with mouse NCOAT or the NCOAT HAT domain (11). For point mutations, the pUC-pTM-NCOAT was modified by site-directed mutagenesis using a four-primer cassette strategy to introduce the substitution (mutated residues underlined) and a selection site (bold). PCR amplifications were performed with the following oligonucleotides (5’–3’): D774A, GAAGATGAA CCGGCAATTGTGTTATGCC; C777A, GAAGATGAGATGGCC ATTGCCGCGTATGCC; F789A, GATGACGTCACCCCCGCGCAT TAAAAAGTGT; C793A, GTAGACGTCACCCCCCTTCTATTAAA AGGCCAAAAAT; S796A, TGTAAAAATGCGTGGATACCC. Flanking oligonucleotides were: upstream, GGGTGTCGATGCTACTCTT CAGCAGTGTCC and downstream, GGCTTTGATACCTTC TCTTGGCATGAGG. Products were separated by electrophoresis, extracted, and purified by the QiAquick extraction kit (Qiagen). Products were digested with Bsu36I and MfeI and ligated into pUC-pTM-NCOAT digested with the same. Ligations were transformed into DH5α cells (Invitrogen), screened for the selection sites, and sequenced.
Histone Binding Assays—Binding assays performed with a synthetic biotinylated H4 tail substrate (Upstate) were achieved by incubating 2 µg of H4 tail with 50 µl of streptavidin beads (Pierce) at 4 °C in avidin binding buffer. Resin was washed three times with RIPA buffer, then purified and eluted GST-tagged NCOAT protein was added for 1 h at 4 °C in HAT buffer. Washes were repeated, and resin-bound material was run on 8% SDS-PAGE and analyzed by Western blot with anti-GST according to standard protocols. Where indicated, 1, 2, 3, or 4 µg of free H4 tail peptide (Upstate) were added into the reaction mixture to test competition. Oligonucleosomes were reconstituted as described elsewhere (11). For nucleosome retention assays, 20 µg of reconstituted nucleosomes were incubated with the indicated purified, immobilized protein in 500 µl of HAT buffer for 1 h at room temperature, with 500 µg of bovine serum albumin to prevent nonspecific binding. Free peptide competition assays were performed by adding 10, 20, 30, or 40 µg of free H4 tail to the reconstituted nucleosome/NCOAT mixture. In some assays, 10 µg of acetyl-CoA was added to the mixture to determine binding on acetylated substrates, and where indicated, 1 m hydroxyamine, pH 7.4, was added to 10 µg of acetyl-CoA for 30 min at 37 °C to remove the acetyl-CoA acetate groups. Washed samples were then resolved on 15% SDS-PAGE and Western blotted with anti-histone H4, or unacetylated H4, acetylated H4, or H4 only acetylated on lysine 8, (Upstate). Where indicated, immobilized wild type or Y891F mutant enzymes were incubated with 500 µl of BSC40 whole cell lysate for 1 h at 4 °C, then washed. In some assays, 1 m hydroxyamine, pH 7.4, was added to the whole cell lysate for 30 min or 1 h prior to incubation with GST-NCOAT. In other binding assays, the indicated DTT concentration was incubated, as described above, with the GST-NCOAT HAT domain or GST finger construct. Tail-less nucleosomes were prepared as described by Ragvin et al. (8) in HAT buffer with final integrity of the histones analyzed by 15% SDS-PAGE followed by Coomassie stain, and the integrity of the nucleosomes analyzed by the agarose gel shift assay as described previously (11).

RESULTS

NCOAT Binds Histone H4 in Vitro—We have previously characterized the HAT activity and active site of NCOAT. Given its high degree of similarity to other HATs, which have the ability to directly bind histones in addition to their HAT activity, we wished to determine whether NCOAT could stably interact with histones as well. To examine direct NCOAT-histone interaction, we utilized a biotin-tagged histone H4 tail substrate, containing the N-terminal 24 amino acids of histone H4. Whereas NCOAT can acetylate all four core histones in vitro, we chose H4 for our binding experiments because much of our previous characterization of NCOAT HAT activity was done using this histone. The biotin tag allows for the fixation of this substrate to streptavidin-conjugated agarose. The resin-bound H4 tail was then incubated with purified, eluted GST-NCOAT in HAT assay buffer. After stringent washing, the resin mixture was run on 8% SDS-PAGE and NCOAT-histone binding was examined by Western blot with anti-GST. As shown in Fig. 1A, the N-terminal 24 amino acids of histone H4 can be used successfully to affinity purify mammalian-expressed NCOAT in this in vitro system (first lane), and this binding could be abated by the addition of free H4 tail peptide in a dose-dependent manner (second to fifth lanes). The H4 tail was unable to co-precipitate GST-NCOAT from whole cell lysates for reasons that will be described below. Incubations with GST alone did not result in the co-purification of GST by the H4 tail, indicating that the GST tag was not responsible for the observed histone binding by NCOAT. Lastly, the observed binding was not the result of NCOAT being tethered to avidin beads, as reactions carried...
out in the absence of biotin-H4 peptide could not be used to effectively purify NCOAT (Fig. 1A, sixth lane).

With NCOAT appearing to have an affinity for binding a synthetic histone tail substrate, we wished to examine if NCOAT would bind histone H4 under more biologically relevant conditions. Oligonucleosomes were reconstituted using histone octamers and α-satellite DNA purified from HeLa cells by PCR. The oligonucleosome arrays were incubated with purified resin-bound GST-NCOAT in the HAT activity buffer. Following incubation and washing, the material still bound to GST-NCOAT was run on 15% SDS-PAGE and the presence of unacetylated H4 was probed by Western blot. As shown in Fig. 1B, the NCOAT protein could effectively co-precipitate histone H4 in these oligonucleosome retention assays, and competition for this binding could be achieved with free H4 tail peptide. Again, the glutathione resin could not be used to purify H4 (sixth lane) and, likewise, resin-bound GST alone was unable to do so (seventh lane).

NCOAT has been demonstrated to acetylate lysine 8 of histone H4 (11). We placed GST-NCOAT in the same oligonucleosome retention assays in the presence of acetyl-CoA and repeated the procedure with a Western blot for H4 acetylated on Lys8. Whereas analysis of the supernatant shows that NCOAT effectively acetylates H4 on Lys8 (Fig. 1C, first lane), the enzyme no longer has the ability to co-precipitate the H4 that had been acetylated on Lys8 (Fig. 1C, second lane). However, again, the minor fraction of histone H4 that was left unmodified by NCOAT even when acetyl-CoA was added to the reaction mixture (Fig. 1D, second lane), could still be purified by GST-NCOAT (Fig. 1D, third lane). GST-NCOAT could not be co-purified by the H4 tail in the presence of acetyl-CoA either (Fig. 1E), suggesting that acetylation upon
other histones within nucleosomes are not wholly responsible for the lack of NCOAT binding to H4 when acetylated on Lys8. When GST-NCOAT was used in attempts to co-purify unacetylated H4 from cell lysates, unacetylated H4 could not be detected (Fig. 2A). Because acetyl-CoA is present in whole cell lysate preparations, and this enzyme is catalytically active, unacetylated histone that binds to these proteins would be subject to the activity of the enzyme. After the catalysis of acetyltransfer, just as seen in Fig. 1, C and E, the enzyme releases the substrate. Therefore, the inability of these enzymes to co-purify unacetylated histone H4 from a whole cell lysate is unsurprising. An enzyme that is catalytically inactive, however, an NCOAT with a Tyr891 → Phe mutation (11), was able to co-purify histone-unacetylated H4 from a whole cell lysate (Fig. 2A). This protein contains the necessary histone binding domain, and can therefore bind H4, but cannot catalyze the reaction that results in substrate release. Another way to show binding in this acetyl-CoA inclusive system would be to add the thioester-cleaving reagent, hydroxylamine, to the whole cell lysate reaction mixture to deplete the acetyl-CoA pool. To test this principle, 1 mM hydroxylamine was able to significantly prevent H4 binding to GST-bound NCOAT when added with acetyl-CoA 30 min prior to the nucleosome retention assay (Fig. 2B, left). Hydroxylamine cleavage of acetyl-CoA to CoA is verifiable by measuring the absorbance of the resultant hydroxamic acid at 520 nm (data not shown). When added to a whole cell lysate, 1 mM hydroxylamine effectively depletes the acetyl-CoA pool in a time-dependent manner (1 h, rather than 30 min), and this then allows the wild type NCOAT to bind unacetylated H4 in a whole cell lysate (Fig. 2B, right). These data support the idea that NCOAT does have an ability to bind histone H4 in a natural cellular environment.

We next addressed whether NCOAT could bind H4 that may be acetylated at sites other than lysine 8. To test this possibility, the Y891F mutant reaction was repeated, and the co-precipitate was probed for acetylated histone H4. As shown in Fig. 2C, the histone sample co-purified by Y891F NCOAT still contained a generous fraction of acetylated histone H4. Just as in Fig. 1C, we have not investigated which of the four alternative lysines on the H4 tail are acetylated within this sample, but the result is sufficient to suggest that NCOAT can bind both unacetylated and acetylated H4, although it appears that acetylation on at least lysine 8 eradicates the binding ability of the enzyme. Not surprisingly, therefore, NCOAT can bind unacetylated H4, acetylation at these other sites are not required for recognition and binding by NCOAT. The addition of an acetyl-lysine analog, Nω-acetylhistamine, prior to the reaction could not compete for NCOAT binding in any of our assays, nor could it impair HAT activity (data not shown), revealing that prior acetylations on H4 are not a requirement for our observed interaction.

Finally, H4 detection by Western blot in these nucleosome retention assays is not a result of H4 being indirectly co-purified by NCOAT-DNA interaction. When reconstituted nucleosomes were treated briefly with trypsin to remove the histone tails, H4 could no longer be detected in these assays (Fig. 2D), demonstrating the necessity of the histone tails, but not the DNA, for NCOAT-histone interaction. Trypsin treatment did not result in the digestion of the entire histone proteins, which are still visible by Coomassie stain (Fig. 2E) and the protein-DNA nucleosomal arrays remain intact as well, as seen by DNA gel shift assay (Fig. 2F). These data indicate that NCOAT has the ability to bind histone H4 in the context of nucleosomes, but after acetylation, at least on Lys8, this ability is eliminated. Presumably, the need of the enzyme to bind the substrate is no longer necessary after the enzyme has catalyzed its reaction to completion. Whereas H4 may be co-purified from oligonucleosomes via an indirect interaction with another histone, the direct H4 tail

**FIGURE 2.** NCOAT directly binds the histone H4 tail and does not require acetyl-lysine contacts. A, a catalytically inactive mutant (Y891F), but not the wild type (WT), can purify unacetylated histone H4 from whole cell lysates (WCL). B, the H4 fraction that is co-purified by Y891F contains acetylated histone. C, addition of hydroxylamine to nucleosome retention assays in the presence of acetyl-CoA (left) or to a whole cell lysate (right) allows NCOAT to co-precipitate unacetylated H4. D, removal of the histone tails by trypsin abolishes the ability of NCOAT to retain unacetylated H4 in retention assays. E, the trypsin protocol above does not result in the digestion of the whole histone proteins, as seen by Coomassie Blue staining. F, trypsinization also leaves the oligonucleosomes intact for the binding assays, as seen by the gel shift assay.
binding and elimination of binding after acetylation on lysine 8 argue for the direct binding of NCOAT to H4.

NCOAT Contains a Motif Similar to the Histone Binding Domain in the MYST Family of HATs—Members of the MYST family of histone acetyltransferases have been elucidated to contain a CX2CX3HXXC zinc finger domain in the N terminus of their active sites, overlapping the first α helix (helix C) in the conserved active site globular core. Biochemical characterization of these motifs implicates them in a role in the mediation of contact with the histone tails during nucleosome recognition and thus they are also essential for HAT activity (5, 7). MYST family member Esa1 is an exception, and does not possess a zinc finger, although the region does form a classic zinc finger-like fold as determined by crystal structure (12). Whereas aspartates can occasionally be utilized as zinc coordinating residues (13), Esa1 contains a DX5TX12YX3C motif at this site and lacks at least two zinc-coordinating residues, and does not bind zinc. All mammalian NCOATs we examined possess a DX5CX11FX2X4C motif in this exact region as seen in the primary sequence and a large number of secondary structure prediction programs (11, 14) (Fig. 3A). This motif contains an additional cysteine residue, like those in standard zinc fingers, and has a conservative replacement of a tyrosine to a phenylalanine when compared with the sequence of Esa1 at the comparable position. This led us to postulate that NCOAT may possess a zinc finger-like motif similar to that of Esa1. This region of mammalian NCOAT also has resemblance to Ca2+/binding epidermal growth factor-like domains as identified by SMART analysis (15), giving further substantiation that this may be a domain similar to those that bind divalent cations. Interestingly, other HATs such as CBP and p300 also possess a PHD-type zinc finger in a comparable region of their AT domain and these have also been implicated in histone substrate interactions (8).

Because the DX5CX11FX2X4C domain of NCOAT lacks an internal residue and at least one amino acid required to co-ordinate a zinc ion, its ability to bind such a divalent cation within this motif is unlikely. However, to confirm this likelihood, increasing concentrations of the metal chelator 1,10-phenanthroline up to 15 mM was added to purified NCOAT preparations to observe its effect on subsequent HAT assays. Under such conditions, HATs, which contain zinc fingers, such as p300/ CBP and MOF, have their HAT activities severely hindered, whereas HATs, such as Hat1 or PCAF, which do not bind zinc remain unaffected (5, 10). With increasing concentrations of 1,10-phenanthroline up to 15 mM, the ability of NCOAT to catalyze acetyltransfer remained largely unchanged, whereas the HAT activity of p300 was abolished (Fig. 3B). NCOAT incubated in the ethanol solvent alone, then washed, produced negligible changes in activity (data not shown). This result does not resolve the issue of zinc binding, rather only displays that NCOAT does not require zinc ions for HAT activity. When purified NCOAT protein was independently denatured, the characteristic ionizable peaks for zinc were not detectable by MS-MS, nor were changes in absorbance detectable when denatured protein was mixed with a commercially available zinc-specific fluorescent dye Newport Green, which shifts spectrophotometric absorbance from 510 to 325 nm upon binding zinc (data not shown). Taken together, these results reveal that NCOAT neither needs zinc ions for activity, nor does the enzyme bind zinc for a non-catalytic purpose.

Although NCOAT may not bind zinc, like Esa1, it may still form a zinc finger-like domain. To address this possibility, we attempted to characterize the secondary structure of this region. Wild type NCOAT was treated with a biotinylated sulfhydryl-specific reagent, HPDP, to tag free cysteine residues in the native enzyme. This labeled enzyme was subjected to tryptic digest, followed by affinity chromatography on...
HAT NCOAT Contains a Zinc Finger-like Motif

FIGURE 4. The putative finger domain is involved in histone H4 binding and HAT activity. A, mutation to residues within the finger (D774A, C777A, F789A, and C793A), but not those outside the finger domain, impair the ability of the NCOAT to retain H4 from reconstituted nucleosomes. Negative control (NC) refers to binding or HAT assays in the absence of NCOAT. B, whereas NCOAT can acetylate lysine 8 of histone H4 (supernatant, first lane in each panel of B), it can no longer bind lysine 8-acetylated H4 when assays are carried out in the presence of acetyl-CoA. Catalytically dead mutants (D833N, D884N, and Y891F) retain the ability to bind H4. C, HAT activity upon the H4 tail is lessened after point mutation to these residues, whereas those outside the finger (S796A) are unaffected. D and E, HAT activity upon all histones is also reduced when point mutations are introduced within the finger domain, as seen by the autoradiograph following the HAT assay with oligonucleosomes. F, the GST finger construct (amino acids 695–814) has the ability to co-purify H4 from reconstituted oligonucleosomes. G, the synthetic histone H4 tail can be used to co-precipitate the GST-tagged finger from whole cell lysates. The addition of 20 mM DTT, as seen in Fig. 3, to the lysate reduces the effectiveness of binding (lane 3).

streptavidin-agarose. This strategy purified those peptides that had previously contained a free cysteine thiol. The HPDP-cysteine bond, a disulfide linkage, is readily reversible with DTT. The resulting purified peptide mixture was analyzed by MALDI-MS and revealed the presence of two peaks, corresponding to peptides between residues 157–167 and 875–886, as confirmed by LC-MS sequencing, in the amino acid sequence of NCOAT, both containing a single cysteine (Fig. 3C). Mutation of the cysteine residue at position 793, shown in Fig. 2A, to an alanine resulted in the appearance of a third peptide peak observable by MALDI-MS, indicating the presence of a new free cysteine (Fig. 3D). Because of the size of the target peptide that would be generated by tryptic digest, we utilized the protease Glu-C for this second experiment. Two of the primary peaks after Glu-C digestion were M, 1790 and 2800, with sequences overlapping the two peptides shown in Fig. 4A to have free cysteines after tryptic digest. A third major peak had a M, of 1139 and corresponds to amino acids 775–785 in the mouse NCOAT. The cysteine at position 777 is the only cysteine within this stretch, indicating that Cys777 and Cys793 are linked by a disulfide bridge in the native state(s) of the wild type enzyme. We feel it is possible that the disulfide linkage between Cys777 and Cys793 acts much like the structural zinc-coordinating property in zinc fingers (Fig. 3E), and maintains the integrity of the tertiary structure of the finger. In the absence of a crystal structure, a finger motif cannot be proven, however, a model of our suggested secondary structure is shown in Fig. 3F.

Consequently, if a disulfide bond is needed to maintain the hypothesized integrity of the finger, incubating NCOAT with an increasing concentration of the reducing agent DTT should deteriorate this structure and accordingly affect histone binding and HAT activity. We took the HAT domain of mouse NCOAT (amino acids 614–917), which retains full HAT activity, and incubated it with up to 50 mM DTT prior to the binding or activity assays. Using the HAT domain eliminates the possibility that DTT indirectly affects binding or activity through disruption of a distant protein domain. As shown in Fig. 3G, first lane, the HAT domain alone has the ability to co-precipitate H4 in our nucleosome retention assays. Any concentration of DTT over 10 mM added prior to the retention assay effectively reduced the ability of NCOAT to bind histone H4 ~2–3-fold. A similar result was witnessed when the synthetic H4 tail was incubated with NCOAT after DTT treatment (Fig. 3H).

Accordingly, the HAT activity was also reduced ~3-fold after incubation with concentrations of DTT above 10 mM (Fig. 3I), compared with the activity of the p300 HAT domain, whose cysteine-rich finger domain has been documented to be unaffected by concentrations of DTT up to 125 mM (9). In conclusion, given the striking sequence similarity, the characterization of the free cysteines, and the effects of the cysteine mutations and DTT administration, the histone H4 binding capability of NCOAT is consistent with its possession of a zinc finger-like domain like those in MYST ATs.

A Potential Finger Motif in NCOAT Is Responsible for Histone Binding—To determine whether the region of NCOAT, which shows similarity to the zinc finger-like domains in the MYST family, is similarly important in substrate binding, we constructed proteins with point mutations to residues within this motif. Whereas NCOAT does not bind zinc, we chose to mutate the four amino acids that align to those used to coordinate zinc in analogous enzymes: Asp774, Phe789, and the Cys777 and Cys793 mentioned above. Expression and purity was analyzed by Coomassie stain and yielded results identical to the constructs previously reported (11). Each mutant protein exhibited a decrease in the ability to bind unacetylated H4 compared with the wild type in nucleosome retention assays (Fig. 4A). As expected, each mutant enzyme, like the wild type NCOAT, could not be used to purify H4 acetylated at lysine 8 when acetyl-CoA was added to the reaction (Fig. 4B).

Three residues essential for catalysis, Asp753, Asp804, and Tyr891 (11), showed no decrease in histone binding ability (Fig. 4A), demonstrating that the loss of histone binding is not a generalized effect because of any mutation within the active site, but rather only observable when specific muta-
HAT NCOAT Contains a Zinc Finger-like Motif

DISCUSSION

The inherent histone acetyltransferase activity of mammalian-expressed NCOAT raised the probability of the ability of the enzyme to directly bind its histone substrates. This likelihood was corroborated by two independent assays. First, histone H4 tails can successfully co-precipitate NCOAT in an in vitro assay system (Fig. 1A). Second, NCOAT possesses the ability to bind unacetylated and acetylated H4 in nucleosome retention assays (Figs. 1B and 2B). Co-purification in these assays is the result of direct NCOAT-histone tail binding, not the result of NCOAT-DNA contacts (Fig. 2). Sufficient substrate contacts appear to be made within the active site because the HAT domain alone can bind H4 (Fig. 3, G and H). NCOAT does not have a detectable bromodomain, a domain implicated in acetyl-lysine recognition. As a result, NCOAT does not require prior acetylations on histone H4 for substrate recognition and an acetyl-lysine analog is ineffective in competition assays. However, NCOAT can still bind acetylated histone H4 (Fig. 2). Acetylation on lysine 8 of H4, however, abolishes the ability of NCOAT to associate with this histone. The inability of NCOAT to co-precipitate histone H4 acetylated on lysine 8 appears to be because of the need to no longer bind substrate after catalysis is complete, because catalytically inept enzymes do not release histone even in the presence of acetyl-CoA (Figs. 2 and 4). The binding of Gcn5 or P/CAF to histone H3 has likewise been shown to be short-lived after acetylation occurs (16, 17).

Given the bisubstrate character of the acetyltransferase mechanism, regardless of subfamily, binding sites are required for both the target lysine acceptor as well as for acetyl coenzyme A. Acetyl-CoA binding is typically made by contacts in the A and D motifs within the active site (4, 18). This region tends to be more highly conserved among families because, regardless of their targets, they must all utilize acetyl-CoA during catalysis. Specific lysine acceptors, however, are different between members of AT subfamilies. Their lysine substrate binding sites are therefore more divergent in character than those for acetyl-CoA. In the MYST family of ATs, a principle region of the active site responsible for lysine recognition is a \( CX_3CX_2X_1HX_2C \) zinc finger motif, or in the case of Esa1 a zinc finger-like \( DXX_TX_12YX_5C \) motif. CBP/p300 also possess zinc fingers that are implicated in substrate contacts. Substrate contacts in the Gcn5/PCAF, GNAT, and Hat1 families have been mapped to a comparable region in the C and B motif in the catalytic core, and although they form a cleft that allows substrate entry, the presence of a finger-like motif to facilitate selectivity has not been demonstrated (18–20). Here, we show that NCOAT possesses a \( DX_3CX_11FX_2C \) finger motif similar to that in the MYST family. This domain, with the surrounding 40 amino acids on each side, is alone sufficient to bind unacetylated histone H4 (Fig. 4, F and G), although other histone tail contacts are likely made. Like Esa1, NCOAT does not bind zinc within this structure (Fig. 3) but a disulfide bond within this stretch could likely serve to maintain the structure of a finger. Select point mutations within this site, but not others around it, hinder the histone binding capability of NCOAT and consequently encumber HAT activity (Fig. 4). Acetyl-CoA binding has been shown to alter HAT active sites and facilitate variations of contact with substrate (17, 20), and this may occur in NCOAT. Whereas these other contacts are not required for H4 binding in our nucleosome retention assays, NCOAT can acetylate other histones (11) and other contacts in the active site may encourage specificity to other lysine targets. In some cases such as in HpaII, hetero- or homomultimerization can affect substrate binding (21). Whereas NCOAT purifies and functions as a monomer in hexosaminidase assays (22), dimerization may play a role.

NCOAT has been referred to as MGEA5, after its detection within meningioma-expressing libraries, and was perceived to have hyaluron-
idase activity (23). The enzyme was also discovered, as described above, to have the ability to remove O-GlcNAc protein modifications, thus resulting in its common referral as O-GlcNAcase (22). The recent discovery of HAT activity within this enzyme prompted an acknowledgement of this bifunctionality, and hence the name NCOAT (11). Several glucosaminidases, from bacteria to higher organisms, have strong similarity to the N-terminal half of NCOAT, where the O-GlcNAcase domain resides. As more genetically complex organisms developed, a need for more stringent post-translational control of their gene expression was required. For example, O-GlcNAc modifications act concertedly with histone deacetylation to repress genes (24). Consequently, the mammalian O-GlcNAcase enzyme may have acquired, then utilized this HAT activity to more efficiently contribute to the reversal of this gene repression. According to published cDNA sequences, many non-mammalian NCOATs lack or possess a truncated HAT domain (chicken, honeybee, and zebrafish) or possess an intact HAT domain with substitutions around the finger domain (bonyfish, Drosophila, mosquito, and worm). In less complex organisms, the acquisition of a HAT domain in NCOAT may not have been of primary importance because HAT activity is encoded by other gene products. Therefore, the maintenance of this activity and domain was expendable, whereas in the more complex organisms this activity became beneficial, then necessary for evolutionary success.

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