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Cloning and Characterization of the Catalytic Subunit of Human Histone Acetyltransferase, Hat1

Hyo-Young Chung, Na-Young Suh and Jong-Bok Yoon*

Department of Biochemistry, College of Science, Yonsei University, 134, Shinchon-dong, Seodaemoon-ku, Seoul 120-749, Korea

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Acetylation of lysine residues within the amino-terminal domains of the core histones plays a critical role in chromatin assembly as well as in regulation of gene expression. To study the biochemical function of histone acetylation, we have cloned a cDNA encoding the catalytic subunit of human histone acetyltransferase, Hat1. Analysis of the predicted amino acid sequence of human Hat1 revealed an open reading frame of 419 amino acids with a calculated molecular mass of 49.5 kDa and an isoelectric point of 5.5. The amino acid sequence of human Hat1 is homologous to those of known and putative Hat1 proteins from various species throughout the entire open reading frame. The recombinant human Hat1 protein expressed in bacteria possesses histone H4 acetyltransferase activity *in vitro*. Both RbAp46 and RbAp48, which participate in various processes of histone metabolism, enhance the histone acetyltransferase activity of the recombinant human Hat1, indicating that they are both able to functionally interact with the human Hat1 *in vitro*.

Keywords: Chromatin assembly, Cloning, Hat1, Histone acetyltransferase, RbAp46/48.

Introduction

Eukaryotic genome is packaged into a nucleoprotein complex known as chromatin. Chromatin is organized in arrays of a regularly repeating unit called the nucleosome, which consists of approximately two turns of DNA wrapped around a core histone octamer. This structural organization has fundamental effects on many chromosomal processes, such as gene expression, DNA replication, and recombination (van Holde, 1989).

Structural studies on the nucleosome revealed that each core histone contains a structured domain, called histone fold, and two unstructured tails (Luger *et al.*, 1997). The histone fold domains interact with each other to form the heterodimers H2A-H2B and H3-H4, which in turn oligomerize to form the histone octamer (Arents *et al.*, 1991). In addition to the formation of the histone octamer, the histone fold domains are responsible for directly contacting nucleosomal DNA. The unstructured amino-terminal tails extend beyond the nucleosomal DNA surface and are involved in nucleosome–nucleosome interactions which would lead to higher-order chromatin structures (Luger *et al.*, 1997).

The amino-terminal domains of histones are subject to several post-translational modifications, including acetylation, phosphorylation, ubiquitination, and polyADP-ribosylation (van Holde, 1989). Acetylation of specific lysine residues within the core histones is shown to play a critical role in transcriptional regulation (Brownell and Allis, 1996). Although the exact relationship between histone acetylation and transcriptional activation is not yet known, various experiments have indicated that transcription of certain genes is affected by acetylation of core histones (Roth and Allis, 1996; Sternglanz, 1996; Wade *et al.*, 1997). The correlation between histone acetylation and transcriptional activation was further supported by the recent finding that several transcriptional coactivators, such as GCN5, CBP/p300, P/CAF, SRC-1, and $TAF_{II}250$, possess intrinsic histone acetyltransferase activities (Bannister and Kouzarides, 1996; Brownell *et al.*, 1996; Mizzen *et al.*, 1996; Ogryzko *et al.*, 1996; Yang *et al.*, 1996; Candau *et al.* 1997; Chen *et al.*, 1997; Wang *et al.*, 1997). Furthermore, some transcriptional repressors, including Mad and unliganded nuclear receptors, were found to recruit histone deacetylases as a component of corepressor complexes (Hassig *et al.* 1997; Heinzel *et al.*,

* To whom correspondence should be addressed.
Tel: 82-2-361-2704; Fax: 82-2-362-9897
E-mail: yoonj@bubble.yonsei.ac.kr

1997; Laherty *et al.*, 1997; Nagy *et al.*, 1997). Taken together, these studies indicate that targeted histone (de)acetylation by the recruitment of histone acetyltransferases and deacetylases is an important means to modulate transcription.

Another function of histone acetylation concerns assembly of newly synthesized histones into nucleosomes. Immediately after the synthesis, histone amino-terminal tails are acetylated. Biochemical studies on replication-coupled chromatin assembly have shown that the chromatin assembly factor, CAF-1, mediates deposition of H3-H4 dimers onto actively replicating DNA (Stillman, 1986; Smith and Stillman, 1989). More importantly, CAF-1 interacts specifically with newly synthesized and acetylated histones H3-H4, but not with histones isolated from chromatin, and provides a direct link between histone acetylation at specific sites and the chromatin assembly machinery (Kaufman *et al.*, 1995; Verreault *et al.*, 1996).

Recently, a yeast histone acetyltransferase has been reported which acetylates free histone H4 (Kleff *et al.*, 1995; Parthun *et al.*, 1996). The yeast HAT1 enzyme contains two subunits, Hat1p and Hat2p. Hat1p is the catalytic subunit of the enzyme and specifically acetylates lysines at positions 5 and 12 of histone H4. Hat2p enhances Hat1p activity and belongs to the RbAp46/48 family which participate in various processes of histone metabolism, such as histone acetylation, deacetylation, chromatin assembly, and nucleosome remodeling (Parthun *et al.*, 1996; Tyler *et al.*, 1996; Verreault *et al.*, 1996; Hassig *et al.*, 1997; Martinez-Balbas *et al.*, 1998). In an effort to study the role of histone acetylation in transcriptional regulation and chromatin assembly, we have undertaken molecular cloning of human genes for histone acetyltransferases. Here, we report the cloning and characterization of the catalytic subunit of the human HAT1 enzyme.

Materials and Methods

***E. coli* strains and media** XL1-Blue was the transformation recipient for all plasmid construction. BL21(DE3)pLysS (Studier *et al.*, 1990) was used as the host for the expression of all recombinant proteins in *E. coli*. For propagation of plasmids, XL1-Blue was grown in LB medium with 100 μg/ml ampicillin. BL21(DE3)pLysS was grown in 2×TY medium containing 16 g/l Bacto Tryptone, 10 g/l yeast extract, 5 g/l NaCl, 100 μg/ml ampicillin, and 25 μg/ml choloramphenicol.

Cloning of a cDNA encoding the human Hat1 To clone the 5′-end of the Hat1 cDNA, antisense oligonucleotide primers (GSH1, 5′-GATGCATATTCAACACGGAACA-3′; GSH2, 5′-TGTTGACAGGCTACCAGCAA-3′; GSH3, 5′-CATCATCCCCAAAGAGTTGA-3′) annealing to the 5′-end of EST cDNA clone T78280 were designed. Using these primers, 5′ RACE (rapid amplification of cDNA ends) was performed with HeLa-cell mRNA according to the manufacturer's instructions (Gibco BRL, Gaithersberg, USA). The PCR product was cloned into pGEM-T easy vector (Promega, Madison, USA) and sequenced. For the expression of human Hat1 as a GST fusion protein in *E. coli*, the PCR amplification was performed with primers, GSH3 and GSH4 (containing the *Nde*I site, 5′-TAGCGCGCATATGGCGGGATTTGGTGCTAT-3′), using the 5′ RACE product as a template. The PCR product was digested with *Nde*I and *Bgl*II. The resulting fragment was ligated with *a Bgl*II–*Eco*RI fragment derived from EST cDNA clone T78280 and inserted into the *Nde*I and *Eco*RI sites of the pGEX-2TL vector to generate pGEX-2TL-hHat1.

Expression and purification of recombinant Hat1 BL21(DE3)pLysS harboring pGEX-2TL-hHat1 was grown at 37°C to an OD_{600} of 0.4–0.6. IPTG was added to a final concentration of 0.4 mM, and the culture was allowed to grow for an additional 3 h. Cells were harvested by centrifugation at 5000 × *g* for 10 min and lysed by sonication in PBS (140 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 1.8 mM KH_2PO_4) containing 1 mM PMSF and 10% glycerol. The lysate was cleared by centrifugation, and the supernatant was loaded onto a 1.0 ml glutathione-Sepharose column equilibrated with the same buffer. The column was washed extensively with the same buffer and eluted with elution buffer containing 10 mM reduced glutathione and 50 mM Tris-HCl (pH 8.0). The eluted GST-Hat1 was directly used in enzyme activity assays.

Expression and purification of recombinant histones H3 and H4 Cells carrying expression plasmids for *Xenopus* histone proteins (Luger *et al.*, 1997) were grown at 37°C to an OD_{600} of 0.8 and histones induced by addition of IPTG to a final concentration of 0.4 mM. Following further incubation for 2.5 h for histone H3 and for 1.5 h for histone H4, cells were harvested and the pellets resuspended in 20 ml of washing buffer containing 50 mM Tris-HCl (pH7.5), 100 mM NaCl, 1 mM benzamidine, and 1 mM 2-mercaptoethanol. Cells were lysed by sonication and the lysate spun at 23,000 × *g* for 10 min at 4°C. The pellet was washed by resuspension and centrifugation three times with 20 ml of wash buffer containing 1% Triton X-100 and twice with wash buffer containing no detergent. The pellet was resuspended and incubated in 5 ml of a 7 M urea solution containing 20 mM Tris-HCl (pH 7.5) for 1 h at room temperature. After centrifugation at 23,000 × *g* for 10 min, the washed pellet containing inclusion bodies was dissolved in 5 ml of a 7 M guanidinium hydrochloride solution containing 20 mM Tris-HCl (pH 7.5) and 1 mM DTT by gentle mixing for 1 h at room temperature. The equimolar mixture of unfolded histones H3 and H4 was dialyzed at 4°C against three changes of a 100-fold excess of refolding buffer A containing 10 mM Tris-HCl (pH 7.5), 2 M NaCl, 1 mM Na-EDTA, and 1 mM 2-mercaptoethanol. Precipitated material was removed by centrifugation and the soluble protein dialyzed against a 100-fold excess of refolding buffer B containing 10 mM Tris-HCl (pH 7.5), 100 mM NaCl, 1 mM Na-EDTA and 1 mM 2-mercaptoethanol. Precipitated material was removed by centrifugation and the soluble protein containing $(H3\text{-}H4)_2$ tetramers was concentrated to 5–10 mg/ml.

Histone acetyltransferase assays Histone acetyltransferase assays were performed for 45 min at 37°C in a 50 μl reaction

mixture with 0.2 μM [^{3}H]acetyl-coenzyme A (3.40 Ci/mmol, Amersham) and 100 μg/ml recombinant $(H3\text{-}H4)_2$ tetramers. The standard reaction mixtures contained 25 mM Tris-HCl (pH 8.0), 150 mM NaCl, 10% glycerol, 0.5 mM Na-EDTA. Incorporation of [^{3}H]acetate into histones was analyzed by electrophoresis on SDS-15% polyacrylamide gels. The gels were subjected to fluorography with Amplify (Amersham) and exposed to film at −70°C.

Expression and purification of recombinant human GCN5 To construct an expression plasmid for hGCN5, PCR amplification was performed with two primers, G5-1 (containing the *Nde*I site, 5′-AATTAACATATGCTG GAGGAGGAGATCTAT-3′) and G5-2 (containing the *Sac*II site, 5′-AACTCGATGATGCCGCGG-3′), using hGCN5 cDNA (a generous gift from Shelly L. Berger) as a template. *Nde*I–*Sac*II fragments from the PCR product and *Sac*I–*Eco*RI fragments from hGCN5 cDNA were inserted into the *Nde*I and *Eco*RI sites of pFLAG(AS)-7 (Chiang and Roeder, 1993). The *Nco*I–*Xho*I fragment from the resulting pFLAG(AS)-hGCN5 was inserted into the *Nco*I and *Xho*I sites of pET15b vector to generate pET15b-FLAG-hGCN5. This plasmid was introduced into BL21(DE3)pLysS and transformants grown to an OD_{600} of 0.6 at 37°C. Expression of hGCN5 was induced for 3 h with 0.4 mM IPTG. Cells were harvested, and pellets were resuspended in TBS buffer (50 mM Tris-HCl, pH 7.4, 150 mM NaCl, 5 mM Na-EDTA, and 0.5 mM PMSF) and lysed by sonication. The lysate was clarified with centrifugation and loaded to an anti-FLAG M2 affinity gel charged with an anti-FLAG M2 monoclonal antibody (Eastman Kodak company). The gel was washed extensively with TBS buffer and eluted with steps of 25, 50, 100, and 200 μg/ml FLAG peptide dissolved in TBS buffer.

Expression and purification of recombinant RbAp46 and RbAp48 To construct an expression plasmid for RbAp46, PCR amplification was performed with two primers, p46-1 (containing the *Nde*I site, 5′-AGGGAATTCCATATGGCGAGTAAAGAGATGTT-3′) and p46-2 (containing the *Xho*I site, 5′-TTGGGAATATGT ACTCGAGC-3′), using RbAp46 cDNA (EST clone N24521) as a template. The *Nde*I–*Xho*I fragment of the PCR product and the *Xho*I–*Bam*HI fragment of RbAp46 cDNA were inserted into the *Nde*I and *Bam*HI sites of the pET15b vector. This plasmid was introduced into BL21(DE3)pLysS and cells were grown to OD_{600} of 0.6 at 37°C. After induction for 3 h with 0.4 mM IPTG, cells were harvested, and pellets were resuspended in EBC buffer (50 mM Tris-HCl, pH 8.0, 120 mM NaCl, 0.5% Nonidet P-40, 1 mM 2-mercaptoethanol, 1 mM EDTA, 2 μg/ml aprotinin, 1 mM PMSF) and lysed by sonication. Inclusion bodies were collected by centrifugation and extensively washed with EBC. The pellet was dissolved in 8 M urea and dialyzed sequentially against 6, 4, 2, 1 M urea and finally in renaturation buffer (0.2 M Tris-HCl, pH 8.0 and 0.5 M NaCl). The refolded protein was further purified by ion exchange column chromatography using Q-sepharose.

For the expression of recombinant RbAp48, the *Nco*I–*Xho*I fragment of RbAp48 cDNA (EST clone AA227133) was inserted into the *Nco*I and *Xho*I sites of the pGET11 vector to generate pGET11-RbAp48. Expression and purification of GST-RbAp48 from cells harboring pGET11-RbAp48 was done by following essentially the same procedure for purification of GST-Hat1.

Results and Discussion

By searching the GenBank database of ESTs for sequences with homology to *S. cerevisiae* Hat1, we identified a human cDNA clone (accession number T78280) encoding a partial open reading frame (410 amino acids) with significant sequence similarity to yeast Hat1. To obtain a full-length human Hat1 cDNA, we performed 5′ RACE PCR using HeLa cell mRNA as described in Materials and Methods. The 200-bp PCR product was subsequently cloned and sequenced. The sequence at the 3′ region of the PCR product was identical to that of the 5′-end of the EST clone, indicating that the product was derived from human Hat1 mRNA and was not due to some PCR artifact. Figure 1 illustrates the complete DNA and deduced amino acid sequence of a human Hat1 cDNA assembled from the PCR and EST clones. Analysis of the sequence revealed an open reading frame of 419 amino acids with a calculated molecular mass of 49.5 kDa and an isoelectric point of 5.5. The first in-frame ATG codon closely matches the Kozak consensus sequence (Kozak, 1984). The human Hat1 protein produced *in vitro* using a transcription–translation coupled system migrates as a 45 kDa protein (data not shown), which is in a good agreement with the size reported for the enzyme partially purified from HeLa cells (Chang *et al.*, 1997).

Database searches for homologous proteins to human Hat1 revealed putative *Z. mays* and *C. elegans* Hat1 proteins as well as *S. cerevisiae* Hat1p. Figure 2 illustrates their sequence homology alignment that shows the sequence similarity throughout the entire protein. It was recently reported that GCN5-related histone acetyltransferases, including yeast Hat1p, belong to a protein superfamily of various N-acetyltransferases (Neuwald and Landsman, 1997). In the subfamily of GCN5-related histone acetyltransferases, regions of sequence similarity, named motifs A, B, and D, were identified. Sequence alignment of the Hat1 proteins from different species, however, shows that while motifs A and B are conserved in the Hat1 proteins, gaps of different sizes have to be introduced in motif D to maximize the sequence alignment. This suggests that the region corresponding to motif D may not be critical for the Hat1 function. Motifs A and B are well conserved in most N-acetyltransferases and may function as a binding domain for acetyl-coenzyme A.

To determine whether human Hat1 possesses histone H4 acetyltransferase activity, the enzyme was expressed as a glutathione-S-transferase fusion protein in bacteria and assayed for its ability to acetylate histones. As a substrate, we used recombinant $(H3\text{-}H4)_2$ tetramers assembled *in vitro* with histones H3 and H4 produced in *E. coli*

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CGTCCTTCCTCAGCCGCGGGTGATCGTAGCTCGGAAATGGCGGGATTTGGTGCTATGGAGAAATTTTTGGTAGAA      75
                                  M  A  G  F  G  A  M  E  K  F  L  V  E           (13)

TATAAGAGTGCAGTGGAGAAGAAACTGGCAGAGTACAAATGTAACACCAACACAGCAATTGAACTAAAATTAGTT      150
 Y  K  S  A  V  E  K  K  L  A  E  Y  K  C  N  T  N  T  A  I  E  L  K  L  V      (38)

CGTTTTCCTGAAGATCTTGAAAATGACATTAGAACTTTCTTTCCTGAGTATACCCATCAACTCTTTGGGGATGAT      225
 R  F  P  E  D  L  E  N  D  I  R  T  F  F  P  E  Y  T  H  Q  L  F  G  D  D      (63)

GAAACTGCTTTTGGTTACAAGGGTCTAAAGATCCTGTTATACTATATTGCTGGTAGCCTGTCAACAATGTTCCGT      300
 E  T  A  F  G  Y  K  G  L  K  I  L  L  Y  Y  I  A  G  S  L  S  T  M  F  R      (88)

GTTGAATATGCATCTAAAGTTGATGAGAACTTTGACTGTGTAGAGGCAGATGATGTTGAGGGCAAAATTAGACAA      375
 V  E  Y  A  S  K  V  D  E  N  F  D  C  V  E  A  D  D  V  E  G  K  I  R  Q      (113)

ATCATTCCACCTGGATTTTGCACAAACACGAATGATTTCCTTTCTTTACTGGAAAAGGAAGTTGATTTCAAGCCA      450
 I  I  P  P  G  F  C  T  N  T  N  D  F  L  S  L  L  E  K  E  V  D  F  K  P      (138)

TTCGGAACCTTACTTCATACCTACTCAGTTCTCAGTCCAACAGGAGGAGAAAACTTTACCTTTCAGATATATAAG      525
 F  G  T  L  L  H  T  Y  S  V  L  S  P  T  G  G  E  N  F  T  F  Q  I  Y  K      (163)

GCTGACATGACATGTAGAGGCTTTCGAGAATATCATGAAAGGCTTCAGACCTTTTTGATGTGGTTTATTGAAACT      600
 A  D  M  T  C  R  G  F  R  E  Y  H  E  R  L  Q  T  F  L  M  W  F  I  E  T      (188)

GCTAGCTTTATTGACGTGGATGATGAAAGATGGCACTACTTTCTAGTATTTGAGAAGTATAATAAGGATGGAGCT      675
 A  S  F  I  D  V  D  D  E  R  W  H  Y  F  L  V  F  E  K  Y  N  K  D  G  A      (213)

ACGCTCTTTGCGACCGTAGGCTACATGACAGTCTATAATTACTATGTGTACCCAGACAAAACCCGGCCACGTGTA      750
 T  L  F  A  T  V  G  Y  M  T  V  Y  N  Y  Y  V  Y  P  D  K  T  R  P  R  V      (238)

AGTCAGATGCTGATTTTGACTCCATTTCAAGGTCAAGGCCATGGTGCTCAACTTCTTGAAACAGTTCATAGATAC      825
 S  Q  M  L  I  L  T  P  F  Q  G  Q  G  H  G  A  Q  L  L  E  T  V  H  R  Y      (263)

TACACTGAATTTCCTACAGTTCTTGATATTACAGCGGAAGATCCATCCAAAAGCTATGTGAAATTACGAGACTTT      900
 Y  T  E  F  P  T  V  L  D  I  T  A  E  D  P  S  K  S  Y  V  K  L  R  D  F      (288)

GTGCTTGTGAAGCTTTGTCAAGATTTGCCCTGTTTTTCCCGGGAAAAATTAATGCAAGGATTCAATGAAGATATG      975
 V  L  V  K  L  C  Q  D  L  P  C  F  S  R  E  K  L  M  Q  G  F  N  E  D  M      (313)

GCGATAGAGGCACAACAGAAGTTCAAAATAAATAAGCAACACGCTAGAAGGGTTTATGAAATTCTTCGACTACTG     1050
 A  I  E  A  Q  Q  K  F  K  I  N  K  Q  H  A  R  R  V  Y  E  I  L  R  L  L      (338)

GTAACTGACATGAGTGATGCCGAACAATACAGAAGCTACAGACTGGATATTAAAAGAAGACTAATTAGCCCATAT     1125
 V  T  D  M  S  D  A  E  Q  Y  R  S  Y  R  L  D  I  K  R  R  L  I  S  P  Y      (363)

AAGAAAAAGCAGAGAGATCTTGCTAAGATGAGAAAATGTCTCAGACCAGAAGAACTGACAAACCAGATGAACCAA     1200
 K  K  K  Q  R  D  L  A  K  M  R  K  C  L  R  P  E  E  L  T  N  Q  M  N  Q      (388)

ATAGAAATAAGCATGCAACATGAACAGCTGGAAGAGAGTTTTCAGGAACTAGTGGAAGATTACCGGCGTGTTATT     1275
 I  E  I  S  M  Q  H  E  Q  L  E  E  S  F  Q  E  L  V  E  D  Y  R  R  V  I      (413)

GAACGACTTGCTCAAGAGTAAAGATTATACTGCTCTGTACAGGAAGCTTGCAAATTTTCTGTACAATGTGCTGTG     1350
 E  R  L  A  Q  E  *                                                            (419)

AAAAATCTGATGACTTTAATTTTAAAATCTTGTGACATTTTGCTTATACTAAAAGTTATCTATCTTTAGTTGAAT     1425
ATTTTCTTTTGGAGAGATTGTATATTTTAAAATACTGTTTAGAGTTTATGAGCATATATTGCATTTAAAGAAAGA     1500
TAAAGCTTCTGAAATACTACTGCAATTGCTTCCCTTCTTAAACAGTATAATAAATGCTTAGTTGTGATAAAAAAA     1575
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Fig. 1. Nucleotide and deduced amino acid sequences of the human histone acetyltransferase, Hat1. The numbers at the right indicate the nucleotide and amino acid positions. The sequence obtained from the RACE product is underlined.

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ceHat1    1 MKIPGDKRKI SEKSIKIHPE REKQHGSIRR SLATCNSHGV FRKKRDERVC GRERRATLRQ  60
ceHat1   61 SERTPSLMRG CESQRVSPRR DTFVSIYEVR KPAGNFFKFY SFIFVGIPSF FLIFHAKSVQ 120

hsHat1    1 MAGFGAMEKF LVEYKSAVEK KLAEYKCNTN TAIELKLVRF PEDLENDIRT FFPEYTHQLF  60
zmHat1    1 ---------- ---------- ---------- -MKVFLVWNP GEVGSVDCTA IQPFDLNHFF  29
ceHat1  121 WRFQLFNSFS MVMEDVTNVN RNRRYISDGL AVVKMTFLKN MQEISTAPR. YEPEMVYQHF 179
scHat1    1 ---------- ---------M SANDFKPETW TSSANEALRV SIVGENAVQ. FSPLFTYPIY  40

hsHat1   61 GDDETAFGYK GLKILLYYIA GSLSTMFRVE YASKVDENFD CVEADDVEGK IRQII.PPGF 119
zmHat1   30 GEDGKIYGYK NLKINVWISA KSFHGYADVS FDETSDGGKG ITDLKPVLQN IFGEN.LVEK  88
ceHat1  180 GDEETIFGYE DLEVTIHHTA QTLYSYINVS YSSKAKNENG LEADDVIDKL VHPDVRPNVL 239
scHat1   41 GDSEKIYGYK DLIIHLAFDS VTFKPYVNVK YSAKLGDD.. ..NIVDVEKK LLSFL.PKDD  95

hsHat1  120 CTNTNDFLSL LEKEVDFKPF GTLLHTYSVL SPTGGENFTF QIYKADMTCR GFREYHERLQ 179
zmHat1   89 EEFLHTFSKE CEYIRTAVTN GSAIKHDGSY ESDPAVEIVR VELQGAAAFL .....YSRLV 143
ceHat1  240 VSGKEEFQQK LIKQKDFKPF GEMVHKFELK GK......SY EVYKVAEQTE EFNLFLERIQ 293
scHat1   96 VI.VRDEAKW VDCFAEERKT HNLSDVFEKV SEYSLNGEEF VVYKSSLVDD FARRMHRRVQ 154

                                        ┌──────────── D ────────────┐
hsHat1  180 TFLMWFIETA SFIDVDDERW HYFLVFEKYN K...DGATLF ATVGYMTVYN YYVYPD.... 232
zmHat1  144 PLVLLLVEGS TPIDIGEHGW EMLLVVKKAT Q...EAGSKF ELLGFAAVHN FYHYPE.... 196
ceHat1  294 TLGMFFIECC SLTDNTEDNW LHYFIYERCD TGEGDGSTVA NVAGYATLFK FYNYID.... 349
scHat1  155 IFSLLFIEAA NYIDETDPSW QIYWLLNKKT K......... ELIGFVTTYK YWHYLGAKSF 205

                  ┌──────────────────────── A ────────────────────────┐      ┌─
hsHat1  233 ......KTRP RVSQMLILTP FQGQGHGAQL LETVHRYYTE FPTVLDITAE DPSKSYVKLR 286
zmHat1  197 ......SIRL RISQILVLPP YQGEGHGLGL LEAINYIAQ. SENIYDVTIE SPSDYLQYVR 249
ceHat1  350 ......RIRP RIAQMLLLPQ YRKSGIGASF MESFLRDLRA SPEVFDVTVE SPGDQFVSLR 403
scHat1  206 DEDIDKKFRA KISQFLIFPP YQNKGHGSCL YEAIIQSWLE DKSITEITVE DPNEAFDDLR 265

          ┌──── B ────┐
hsHat1  287 DFVLVKLCQD LPCFSREKLM QGFN..... ......... ..EDMAIEAQ QKFKINKQHA 328
zmHat1  250 SSIDCLRLLM FDPIKPALGA IVSSLKETNL SKRAQSLRMU PPADLMETVR QKLKINKKQF 309
ceHat1  404 DYVDCVNCMT LREFAPENLK RGYS..... ......... ..DKMRQAAL EKLKISRQQA 445
scHat1  266 DRNDIQRLRK LGYDAVFQKH SDLS..... ......... ..DEFLESSR KSLKLEERQF 307

hsHat1  329 RRVYEILRLL VTDMSDAEQY RSYRLDIKR. .RLISPYKKK QRDLAKMRKC LRPEELTNQM 386
zmHat1  310 LRCWEILIFL SLDSQDHKSM DNFRACIYD. .RMKGEILGS ASGTNRKRLL QMPTSFNKEA 367
ceHat1  446 RRVYEILRYR ATNKKDKEEL KAQRIDVKR. .RLYAPMKKS DQDWKRLNLA LTPDELRQAA 503
scHat1  308 NRLVEML.LL LNNSPSFELK VKNRLYIKNY DALDQTDPEK AREALQNSFI LVKDDYRRII 366

hsHat1  387 N.QIEISMQH EQLEESFQEL VEDYRRVIER LAQE------ ---------- ---------- 419
zmHat1  368 SFAVYWTQEI EDEDEQTVEQ QPEDLKTQEQ QLNELVDIQI EEIAGVAKNV TSRRKDKMAE 427
ceHat1  504 CGQMDEETKF STLSQNYDRL MEAYQKTIDR IEQHPSIF-- ---------- ---------- 541
scHat1  367 E.SINKSQG- ---------- ---------- ---------- ---------- ---------- 374

zmHat1  428 LVVQ
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Fig. 2. Sequence alignment of known and putative Hat1 proteins from various species. Sequences shown are *Homo sapiens* (hsHat1), *Z. mays* (zmHat1), *C. elegans* (ceHat1), and *S. cerevisiae* (scHat1) proteins. Amino acids that are identical for at least three proteins are shown in boldface. Motifs A, B, and D that are conserved in many N-acetyltransferases are indicated.

(Fig. 3A). The recombinant histone proteins are known to be free of post-translational modifications such as acetylation (Luger *et al.*, 1997). Histone acetyltransferase activity of human Hat1 was compared to that of a well-characterized enzyme, hGCN5 (Candau *et al.*, 1997). As shown in Fig. 3B, the recombinant human Hat1 specifically acetylated histone H4 (lane 2), while human GCN5 acetylated histone H3 (lane 1). In conjunction with the sequence homology of the human Hat1 to the yeast enzyme, these data strongly suggest that the cloned histone acetyltransferase is the human orthologue of the yeast Hat1.

Although the recombinant human Hat1 had histone H4 acetyltransferase activity by itself, its specific activity was at least 5 times lower than that of the recombinant hGCN5. Since the yeast HAT1 enzyme consists of two subunits, and since the noncatalytic subunit Hat2p increases the activity of the catalytic subunit Hat1p (Parthun *et al.*, 1996), we tested whether the histone acetyltransferase activity of the recombinant human Hat1 could be enhanced by either RbAp46 or RbAp48, which is a probable human counterpart of yeast Hat2p. As shown in Fig. 4, incubation of the human Hat1 with increasing amounts of recombinant RbAp46 greatly stimulated histone H4 acetyltransfrerase activity (lanes 3–5). This stimulation of the enzyme activity was not due to a nonspecific enzyme-stabilizing effect of the protein, since addition of bovine serum albumin to the reaction did not significantly increased the activity of human Hat1 (lane 2). Recombinant RbAp48 expressed as a GST fusion protein, but not GST itself, also activated the enzyme activity in a dose-dependent manner (lanes 6–9). Both RbAp46 and RbAp48 did not affect the substrate specificity of the enzyme. Since the experiments shown in Fig. 4 did not contain any cellular proteins but recombinant ones purified from bacteria, these results strongly indicated that both RbAp46 and RbAp48 were capable of functionally interacting with human Hat1 under the experimental conditions used.

While this report was in preparation, Stillman and colleagues also reported cloning of the gene encoding human Hat1 (Verreault *et al.*, 1998). They found that the recombinant protein possessed the anticipated properties of human Hat1 *in vitro* and that the purified human HAT1 holoenzyme contained two subunits, Hat1 and RbAp46. Since our data indicated a functional interaction of the human Hat1 with both RbAp46 and RbAp48 (Fig. 4), the apparently specific association of the Hat1 only with RbAp46 in the native Hat1 holoenzyme is intriguing. So far, the difference of RbAp46 and RbAp48 in their function has not been rigorously examined *in vitro* and the determinant of their specific *in vivo* association with interacting proteins is not known. The discrepancy between *in vivo* complex formation and *in vitro* interaction data may be explained if endogenous RbAp46 and RbAp48 are

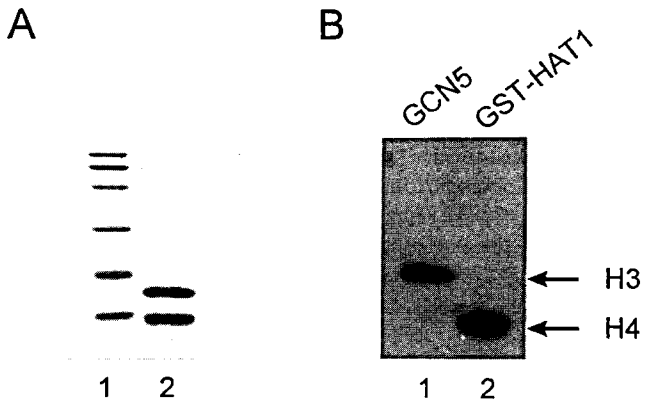

Fig. 3. Recombinant human Hat1 has histone H4 acetyltransferase activity. A. SDS-PAGE anaysis of recombinant $(H3\text{-}H4)_2$ tetramers. *In vitro* assembled histone $(H3\text{-}H4)_2$ tetramers were resolved by electrophoresis on an SDS-15% polyacrylamide gel (lane 2). The polypeptides were visualized by Coomassie blue staining. Lane 1 shows protein molecular size markers containing 97, 66, 45, 31, 22, and 14 kDa polypetides. B. Comparison of substrate specificity for human histone acetyltransferases, hHat1 and hGCN5. Recombinant human GCN5 (lane 1) and human GST-Hat1 (lane 2) were assayed for histone acetyltransferase activity using [^{3}H]acetyl-coenzyme A and $(H3\text{-}H4)_2$ tetramers as substrates. The acetylated histones were detected by fluorography following SDS-PAGE. The labeled histones H3 and H4 are indicated by the arrows.

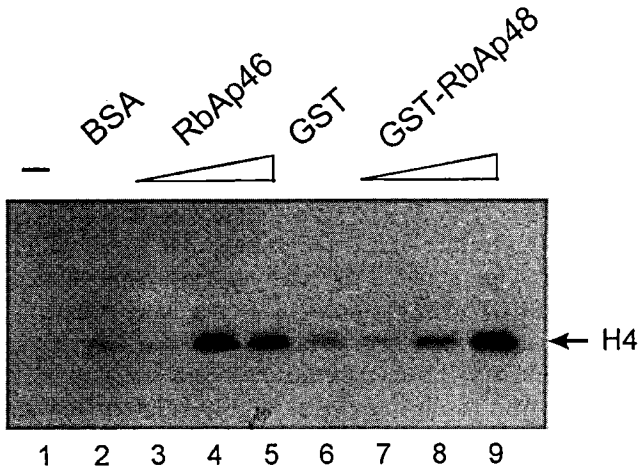

Fig. 4. Both RbAp46 and RbAp48 stimulate the acetyltransferase activity of human Hat1. Histone acetyltransferase activity of the recombinant human Hat1 enzyme (50 ng) was assayed with increasing amounts of recombinant RbAp46 (50 ng in lane 3; 100 ng in lane 4; 150 ng in lane 5) or GST-RbAp48 (50 ng in lane 7; 100 ng in lane 8; 150 ng in lane 9) proteins. Reactions in lanes 1, 2, and 6 were supplemented with no protein, 150 ng of BSA, and 150 ng of GST, respectively. The acetylated histones were analyzed by fluorography following SDS-PAGE. The histone H4 is indicated by the arrow.

differentially modified in human cells so that RbAp46, but not RbAp48, interacts with the Hat1. Alternatively, specific protein–protein interactions involving another protein may affect the association of Hat1 with RbAp46 or RbAp48. In this regard, it is noted that two histone acetyltransferase complexes containing Hat1p have been found in yeast cells (Ruiz-Garcia *et al.*, 1998). These complexes were different in their molecular sizes and subcellular localization. However, it has not been rigorously tested whether human cells also have more than one Hat1-containing complex.

Members of the RbAp46 family in various organisms are involved in histone metabolism and function as a subunit of the chromatin assembly-related histone acetyltransferase HAT1, the chromatin assembly factor CAF-1, the corepressor complex containing histone deacetylase HDAC1, or the nucleosome remodeling factor NURF. Yeast cells contain two members, Hat2p and Msi1p. Biochemical and genetic evidence indicated that Hat2p and Msi1p are a subunit of yeast HAT1 and CAF-1 complexes, respectively (Parthun *et al.*, 1996; Verreault *et al.*, 1996). In contrast, *Drosophila* cells appear to have only one homologue, p55, which is a constituent of dCAF-1, NURF, and a histone deacetylase complex. In human cells, two family members, RbAp46 and RbAp48, have been identified. Their striking sequence identity (89%) is far greater than that (20%) between yeast Hat1p and Msi1p. Remarkably, however, it has been reported that purified human CAF-1 contained only RbAp48 while native human HAT1 included exclusively RbAp46 (Verreault *et al.*, 1996; 1998). Biochemical dissection of complexes containing RbAp46 or RbAp48 should help to understand the mode of the specific association of RbAp46 and RbAp48 with various interacting proteins.

In summary, we have cloned and characterized the catalytic subunit of the human histone acetyltransferase, Hat1. The molecular cloning of the Hat1 subunit should facilitate future studies on the function of histone acetylation in chromatin assembly and gene regulation.

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References

Arents, G., Burlingame, R. W., Wang, B. C., Love, W. E. and Moudrianakis, E. N. (1991) The nucleosomal core histone octamer at 3.1 Å resolution: a tripartite protein assembly and a left-handed superhelix. *Proc. Natl. Acad. Sci. USA* **88,** 10148–10152.

Bannister, A. J. and Kouzarides, T. (1996) The CBP co-activator in a histone acetyltransferase. *Nature.* **384,** 641–643.

Brownell, J. E. and Allis, C. D. (1996) Special HATs for special occasions: linking histone acetylation to chromatin assembly and gene activation. *Curr. Opin. Genet. Dev.* **6,** 176–184.

Brownell, J. E., Zhou, J., Ranalli, T., Kobayashi, R., Edmondson, D. G., Roth, S. Y. and Allis, C. D. (1996) *Tetrahymena* histone acetyltransferase A: a homolog to yeast Gcn5p linking histone acetylation to gene activation. *Cell* **84,** 843–851.

Candau, R., Zhou, J. X., Allis, C. D. and Berger, S. L. (1997) Histone acetyltransferase activity and interaction with ADA2 are critical for GCN5 function *in vivo. EMBO J.* **16,** 555–565.

Chang, L., Loranger, S. S., Mizzen, C., Ernst, S. G., Allis, C. D. and Annunziato, A. T. (1997) Histones in transit: cytosolic histone complexes and diacetylation of H4 during nucleosome assembly in human cells. *Biochemistry* **36,** 469–480.

Chiang, C. M. and Roeder, R. G. (1993) Expression and purification of general transcription factors by FLAG epitope-tagging and peptide elution. *Peptide Res.* **6,** 62–64.

Chen, H., Lin, R. J., Schiltz, R. L., Chakravarti, D., Nash, A., Nagy, L., Privalsky, M. L., Nakatani, Y. and Evans, R. M. (1997) Nuclear receptor coactivator ACTR is a novel histone acetyltransferase and forms a multimeric activation complex with p/CAF and CBP/p300. *Cell* **90,** 569–580.

Hassig, C. A., Fleischer, T. C., Billin, A. N., Schreiber, S. L. and Ayer, D. E. (1997) Histone deacetylase activity is required for full transcriptional repression by mSin3A. *Cell* **89,** 341–347.

Heinzel, T., Lavinsky, R. M., Mullen, T. M., Soderstrom, M., Laherty, C. D., Torchia, J., Yang, W. M., Brard, G., Ngo, S. D., Davie, J. R., Seto, E., Eisenman, R. N., Rose, D. W., Glass, C. K. and Rosenfeld, M. G. (1997) A complex containing N-CoR, mSin3 and histone deacetylase mediates transcriptional repression. *Nature* **387,** 43–48.

Kaufman, P. D., Kobayashi, R., Kessler, N. and Stillman, B. (1995) The p150 and p60 subunits of chromatin assembly factor I: a molecular link between newly synthesized histones and DNA replication. *Cell* **81,** 1105–1114.

Kleff, S., Andrulis, E. D., Anderson, C. W. and Sternglanz, R. (1995) Identification of a gene encoding a yeast histone H4 acetyltransferase. *J. Biol. Chem.* **270,** 24674–24677.

Kozak, M. (1984) Compilation and analysis of sequences upstream from the translational start site in eukaryotic mRNAs. *Nucleic Acids Res.* **12,** 857–872.

Laherty, C. D., Yang, W. M., Sun, J. M., Davie, J. R., Seto, E. and Eisenman, R. N. (1997) Histone deacetylase associated with the mSin3 corepressor mediate mad transcriptional repression. *Cell* **89,** 349–356.

Luger, K., Rechsteiner, T. J., Flaus, A. J., Waye, M. M. Y. and Richmond, T. J. (1997) Characterization of nucleosome core particles containing histone proteins made in bacteria. *J. Mol. Biol.* **272,** 301–311.

Martinez-Balbas, M. A., Tsukiyama, T., Gdula, D. and Wu, C. (1988) *Drosophila* NURF–55, a WD repeat protein involved in histone metabolism. *Proc. Natl. Acad. Sci. USA.* **95,** 132–137.

Mizzen, C. A., Yang, X. J., Kokubo, T., Brownell, J. E., Bannister, A. J., Owen-Hughes, T., Workman, J., Wang, L., Berger, S. L., Kouzarides, T., Nakatani, Y. and Allis, C.D. (1996) The TAF(II) 250 subunit of TFII D has histone acetyltransferase activity. *Cell* **87,** 1261–1270.

Nagy, L., Kao, H. Y., Chakravarti, D., Lin, R. J., Hassig, C. A, Ayer, D. E., Schreiber, S. L. and Evans, R. M. (1997) Nuclear receptor repression mediated by a complex containing SMRT, mSin3A, and histone deacetylase. *Cell* **89,** 373–380.

Neuwald, A. F. and Landsman, D. (1997) GCN5-related histone N–acetyltransferases belong to a diverse superfamily that includes the yeast SPT10 protein. *Trends Biochem. Sci.* **22,** 154–155.

Ogryzko, V. V., Schiltz, R. L., Russanova, V., Howard, B. H. and Nakatani, Y. (1996) The transcription coactivators p300 and CBP are histone acetyltransferases. *Cell* **87,** 953–959.

Parthun, M. R., Widom, J. and Gottschling, D. E. (1996) The major cytoplasmic histone acetyltransferase in yeast: links to chromatin replication and histone metabolism. *Cell* **87,** 85–94.

Roth, S. Y. and Allis, C. D. (1996) Histone acetylation and chromatin assembly: A single escort, multiple dances? *Cell* **87,** 5–8.

Ruiz-Garcia, A. B., Sendra, R., Galiana, M., Pamblanco, M., Perez-Ortin, J. E. and Tordera, V. (1998) HAT1 and HAT2 proteins are components of a yeast nuclear histone acetyltransferase enzyme specific for free histone H4. *J. Biol. Chem.* **273,** 12599–12605.

Smith, S. and Stillman, B. (1989) Purification and characterization of CAF-1, a human cell factor required for chromatin assembly during DNA replication *in vitro*. *Cell* **58,** 15–25.

Sternglanz, R. (1996) Histone acetylation: a gateway to transcriptional activation. *Trends Biochem. Sci.* **21,** 357–358.

Stillman, B. (1986) Chromatin assembly during SV40 DNA replication *in vitro*. *Cell* **45,** 555–565.

Studier, F. W., Rosenberg, A. H., Dunn, J. J. and Dubendorff, J. W. (1990) Use of T7 RNA polymerase to direct expression of cloned genes. *Methods Enzymol.* **185,** 60–89.

Tyler, J. K., Bulger, M., Kamakaka, R. T., Kobayashi, R. and Kadonaga, J. T. (1996) The p55 subunit of *Drosophila* chromatin assembly factor1 is homologous to a histone deacetylase–associated protein. *Mol. Cell. Biol.* **16,** 6149–6159.

van Holde, K. E. (1989) *Chromatin*, Springer-Verlag, New York.

Verreault, A., Kaufman, P. D., Kobayashi, R. and Stillman, B. (1996) Nucleosome assembly by a complex of CAF-1 and acetylated histones H3/H4. *Cell* **87,** 95–104.

Verreault, A., Kaufman, P. D., Kobayashi, R. and Stillman, B. (1998) Nucleosomal DNA regulates the core–histone–binding subunit of the human Hat1 acetyltransferase. *Curr. Biol.* **8,** 96–108.

Wade, P. A., Pruss, D. and Wolffe, A. P. (1997) Histone acetylation: chromatin in action. *Trends Biochem. Sci.* **22,** 128–132.

Wang, L., Mizzen, C. A., Ying, C., Candau, R., Barlev, N., Brownell, J., Allis, C. D. and Berger, S. L. (1997) Histone acetyltransferase activity is conserved between yeast and human GCN5 and is required for complementation of growth and transcriptional activation. *Mol. Cell. Biol.* **17,** 519–527.

Yang, X. J., Ogryzko, V. V., Nishikawa, J., Howard, B. H. and Nakatani, Y. (1996) A p300/CBP-associated factor that competes with the adenoviral oncoprotein E1A. *Nature* **382,** 319–324.