Cloning and Characterization of a cDNA Encoding the Histone Acetyltransferase Monocytic Leukemia Zinc Finger Protein (MOZ) in the Rat

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Many DNA-binding transcription factors require coactivators for their function. Some of these coactivators have histone acetyltransferase (HAT) activity, which is important for transcription from chromatin template. We cloned a cDNA encoding the rat homolog of monocytic leukemia zinc finger protein (MOZ), a member of the MYST (MOZ, Ybf2/Sas3, Sas2, and Tip60) acetyltransferase family. Rat MOZ (rnMOZ) encoded 1998 amino acids and was composed of 16 exons. Comparison of the rnMOZ and human MOZ amino acid sequences revealed 89% identity over the whole sequence and 100% identity in the MYST region, which is essential for HAT activity. Further, we identified physical interaction between rnMOZ and basic leucine zipper (bZIP)-type DNA-binding proteins, including c-Jun and CCAAT/enhancer binding proteins. This finding suggests that MOZ may function in multiple cellular processes through various bZIP-type transcription factors.

Key words —— histone acetyltransferase, monocytic leukemia zinc finger protein, transcription, c-Jun, CCAAT/enhancer binding protein, basic region leucine zipper family

INTRODUCTION

Transcription of eukaryotic genes is controlled by various regulatory elements, termed promoters, enhancers, and silencers. These elements are recognized by sequence-specific DNA-binding proteins.[1)] A virtual explosion of information in the field of eukaryotic gene regulation has revealed that many DNA-binding transcription factors require coactivators that have histone acetyltransferase (HAT) activity.[2)] Active chromatin has been associated with hyperacetylation of histones, binding of transcriptional regulators, and active transcription.[3)]

Some HATs in multiple-protein complexes are recruited by DNA-binding transcription factors to chromatin, whereas others are physically associated with DNA-binding proteins. Monocytic leukemia zinc finger protein (MOZ) is a member of the MYST (MOZ, Ybf2/SAS3, SAS2, and TIP60) family of HATs, which play key roles in various nuclear functions[2)] and frequently are rearranged in leukemia.[2,4)] Only human MOZ (hsMOZ) cDNA had been cloned previously.[5)] Although the MOZ complex has been identified,[4)] physical interaction between MOZ and DNA-binding transcription factors has not yet been reported. Candidates for associating factors include the basic region leucine zipper (bZIP) proteins, which are a large class of transcription factors including c-Jun, c-Fos, and CCAAT/enhancer binding proteins (C/EBPs) that are crucial for cell proliferation, cell differentiation, and cancer development.[6)]

We cloned rat MOZ (rnMOZ) cDNA and showed that rnMOZ interacted with various bZIP-type transcription factors.

MATERIALS AND METHODS

Cloning of rnMOZ cDNA and Plasmid Construction —— All animal care and handling procedures were approved by the animal care and use committee of Osaka University. Total RNA was prepared from Wister rat liver by using TriZol reagent

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Table 1. PCR Condition

Amplifed Position	Primer Sequence	Denature	Annealing	Extension	Cycles
1-993	5′-ATAGAATTCATGGTAAAACTCGCTAACC-3′				
	5′-AAGCGGCCGCGTTAATAGCGCCGTTTTATC-3′	94 °C, 1 min	54°C, 1 min	72°C, 1 min	36
804-2336	5′-TCGCGATCAAGGCAAAAACG-3′				
	5′-ACTATGACTGGAGTCCAGCG-3′	94 °C, 1 min	54°C, 1 min	72°C, 2 min	43
1969-3524	5′-GGCAGGTTTCTCATCGATTTCA-3′				
	5′-TTAAATCCTGGTTTCCGTCCAGG-3′	94 °C, 1 min	54°C, 1 min	72°C, 2 min	40
3271-4722	5′-AAGACATCCTTAGGTGTCAGGCTT-3′				
	5′-GTTATTCCCACAAATACTGCTG-3′	94 °C, 1 min	56°C, 1 min	72°C, 1.5 min	40
4576-5386	5′-AAATGGATGTGCCTTCCGTATC-3′				
	5′-AACGGCTAAGGGATGAGATGGA-3′	94 °C, 1 min	56°C, 1 min	72°C, 2 min	40
4576-5997	5′-AAATGGATGTGCCTTCCGTATC-3′				
	5′-TTTGCGGCCGCATCATCTTCTCATGTAAGG-3′	94 °C, 1 min	54°C, 1 min	72°C, 2 min	40
5100-5997	5′-ATTGAATTCATGAACAACAGCTTCACTGC-3′				
	5′-TTTGCGGCCGCATCATCTTCTCATGTAAGG-3′	94 °C, 1 min	56°C, 1 min	72°C, 1 min	36

(Invitrogen, Carlsbad, CA, U.S.A.) according to the manufacturer's recommendations. The open reading frame (ORF) of rnMOZ was amplified by reverse transcriptase-polymerase chain reaction (RT-PCR); PCR conditions are found in Table 1. The seven fragments we obtained were subcloned into pBluescript KS (Stratagene, La Jolla, CA, U.S.A.) and sequenced. To subclone the full-length rnMOZ ORF into the *Eco*RI-*Not*I site of the mammalian expression vector pCI (Promega, Madison, WI, U.S.A.), cDNA fragments were assembled using suitable internal restriction sites. Glutathione *S*-transferase (GST)-fused rat c-Jun constructs were generated by PCR from pRJ101[7] (kindly provided Dr. Masayoshi Imagawa of Nagoya City University). GST-c-Jun [contains full-length (FL) Jun], GST-c-Jun [contains the Jun transactivation domain (AD)], and GST-c-Jun [includes the DNA-binding domain (DBD) of Jun] were constructed by inserting fragments corresponding to amino acid residues 1-334, 1-146, and 257-334, respectively, into the *Bam*HI-*Xho*I site of pGEX4T-1 (Amersham Biosciences, Piscataway, NJ, U.S.A.). All fragments amplified by PCR were verified by DNA sequencing. GST-fused C/EBPα and C/EBPβ expression plasmids[8] were kindly provided by Dr. Robert Hache of Ottawa University.

GST Pull-Down Assay —— GST fusion proteins were expressed in *Escherichia coli* as described by the manufacturer (Amersham Biosciences) and cross-linked to glutathione-Sepharose 4B with dimethylpimelimidate. [^{35}S]-labeled MOZ protein was produced from pCI-MOZ by *in vitro* transcription-translation with the TNT T7-coupled reticulocyte lysate system (Promega). A 5 μl aliquot of the reticulocyte lysate reaction containing [^{35}S]-labeled MOZ protein was incubated for 3 hr at 4°C with GST fusion proteins in buffer containing 50 mM Tris–HCl (pH 8.0), 150 mM KCl, 10% glycerol, 0.1% Tween 20, 1 mM phenylmethylsulfonyl fluoride, and 1 mM dithiothreitol. After extensive washes, bound proteins were separated by SDS-polyacrylamide gel electrophoresis and detected by autoradiography.

RESULTS AND DISCUSSION

Molecular Cloning of cDNA Encoding rnMOZ

To generate PCR primers for identification of rnMOZ cDNA by RT-PCR, we compared the sequence of hsMOZ (GenBank accession no. U47742) with mouse expressed sequence tags (accession nos. AK028058, BC024786, and AK054354) and designed our primers on the basis of sequences identical between human and mouse. Seven fragments amplified from rat total RNA were sequenced and covered the entire ORF. The predicted primer-generated sequences, except for primers including the translation start and stop codons, were confirmed by sequencing of the amplified regions. The rnMOZ ORF (accession no. AB195309) included 5994 nucleotides and encoded 1998 amino acids (Fig. 1A).

Comparison of rnMOZ cDNA with Rat Genome Sequence

The genome sequence of the Brown Norway rat,

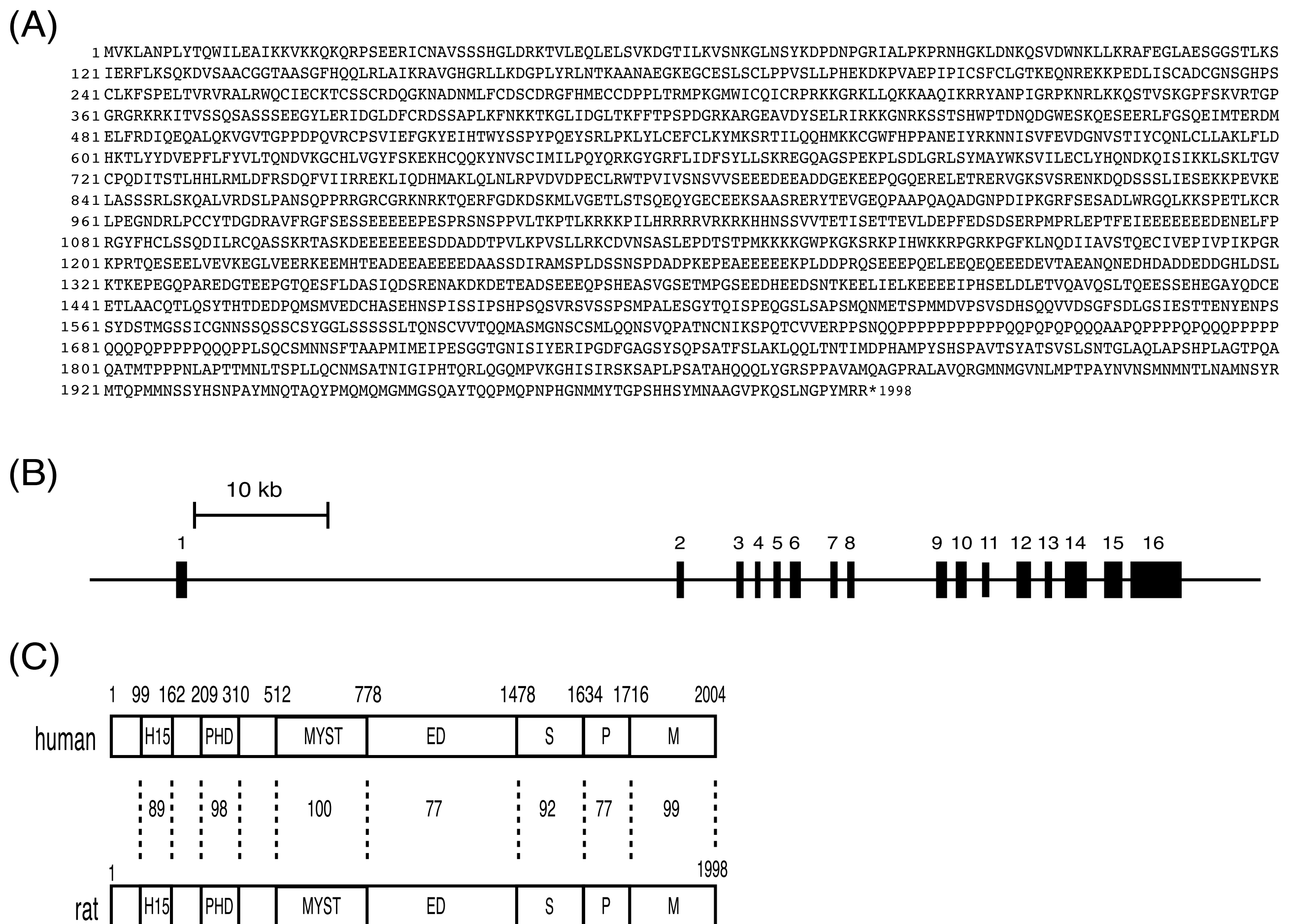

Fig. 1. Molecular Cloning of CDNA Encoding rnMOZ

(A) Deduced amino-acids sequence of rnMOZ. The numbers on the left refer to the corresponding to amino acid positions. (B) Schematic representation of the rnMOZ gene structure. The boxes indicate the relative sizes and positions of exons. (C) Comparison of the functional domains of orthologous MOZs. H15, histone H1- and H5-1ike motif; PHD, plant homeodomain zinc fingers; MYST, MYST acetyltransferase domain; ED, S, P, and M are Glu/Asp-, Ser-, Pro-, and Met-rich regions, respectively.

recently determined by the Rat Genome Sequencing Project Consortium, is available in the international sequence databases.[9] Comparison of rnMOZ with the genomic sequence revealed that the *rnMOZ* gene was located at 16q12.5 and comprised 16 exons spanning > 75 kb (Fig. 1B); all of the exon-intron boundaries were consistent with the GT/AG rule (data not shown). The sequence of rnMOZ cDNA was the same as the genomic sequence, except that the C, T, and C at nucleotides 3699, 4329, and 5058 of the cDNA were T, G, and T, respectively, in the genome. These differences may be due to differences in the stem lines. The predicted sequences of primers including the translation start and stop codons were also identical to the genome sequences, except that A at position 9 of cDNA was G in the genome sequence; however the predicted amino acids of rnMOZ were completely identical with those from the genomic sequence.

Comparison of rnMOZ with hsMOZ

Functional domains of hsMOZ have been identified.[10] Comparison of rnMOZ with hsMOZ revealed 89% identity over the whole sequence, and the percentage of identity at each domain is shown in Fig. 1C. All domains were highly conserved. In particular, the sequences of the MYST, PHD, and Met-rich domains, which are important for HAT activity and transcriptional regulation, were nearly identical between the orthologs.

Association of rnMOZ with bZIP-type Transcription Factors

Recruitment of HAT activity by DNA-binding transcription factors is a trigger for gene expression from chromatin. We observed the *in vitro* interac-

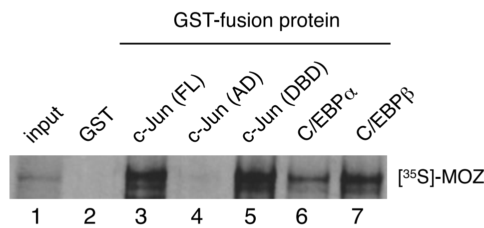

Fig. 2. Association of rnMOZ with bZIP-Type Transcription Factors

[35S]-MOZ was incubated with GST (lane 2) or GST-fused c-Jun (FL), c-Jun (AD), c-Jun (DBD), C/EBPα. and CIEBPβ (1anes 3-7). MOZ protein retained on the GST-conjugated beads after extensive washes was analyzed by SDS-polyacrylamide gel electrophoresis and autoradiography The amount of input (lane 1) was equivalent to 10% of the reaction in the assay.

tion between rnMOZ and various DNA-binding proteins by using the GST pull-down assay. c-Jun is a bZIP-type DNA-binding transcription factor,[6] and the GST pull-down assay revealed that [35S]-labeled MOZ was retained on beads cross-linked with GST-c-Jun (FL) but not with GST alone. This finding suggests that rnMOZ is physically associated with c-Jun. The AD and the DBD of c-Jun have been determined.[11] We tried to identify the region in c-Jun that is required for interaction with rnMOZ and found that DBD, but not AD, of c-Jun was necessary for interaction with rnMOZ (Fig. 2). Further, we showed that both C/EBPα and C/EBPβ — other bZIP family members — also physically associate with rnMOZ (Fig. 2). These findings are the first evidence that MOZ interacts with DNA-binding transcription factors, and they suggest that MOZ may influence multiple cellular functions through bZIP-type transcription factors.

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