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Short sequence-paper

A new human member of the MYST family of histone acetyl transferases with high sequence similarity to *Drosophila* MOF

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Abstract

We have identified a novel human gene product, hMOF, which exhibits significant similarity to the *Drosophila* dosage compensation regulator, MOF. A recombinant C-terminal portion of hMOF has histone acetyltransferase activity directed toward histones H3, H2A and H4, a specificity characteristic of other MYST family histone acetyltransferases. Based on hMOF's chromodomain, we discuss possible interactions with other proteins.

Keywords: Histone acetyltransferase; Chomodomain; MYST family; MSL3 family

Studies in many organisms have shown a correlation between acetylation of the N-terminal tails of core histones and a higher level of transcriptional activity [for examples see [1–3]]. The histone acetyltransferase enzymes responsible for this modification are termed type A and are localized in the nucleus. A subgroup of these enzymes constitutes the MYST family, so called for its founding members MOZ, YBF2/SAS3, SAS2 and Tip60 [4–6]. This family also includes the *Saccharomyces cerevisiae* essential protein ESA1 [7,8], the human protein HBO1 that has been shown to interact with member of the DNA replication initiator complex [9], and the *Drosophila* dosage compensation protein MOF [10]. MOF is a member of the multiprotein complex responsible for the acetylation of histone H4 at lysine-16, a histone isoform found predominantly on the hypertranscribed X chromosome of *Drosophila* males [11]. All MYST family members share a region of similarity of approximately 240 amino acids in length containing the canonical acetyl CoA binding site found in most acetyl transferases [12]. With the exception of ESA1, this region, known as the MYST domain, also contains a C2HC-type zinc finger motif. In addition to the MYST domain, a chromodomain is also present in Tip60, ESA1, MOF and in other, albeit uncharacterized, members of the MYST family that have been found in *Schizosaccharomyces pombe* and *Caenorhabditis elegans* through their respective genome sequencing projects (GenBank accession numbers CAA22591, AAC78211), and in the carrot, *Daucus carota* (GenBank accession number BAA32822). Chromodomains are involved in protein–protein interactions and may target transcriptional regulators to chromatin [13]. Here we report the characterization of a human protein with a similarity to *Drosophila* MOF that extends well beyond

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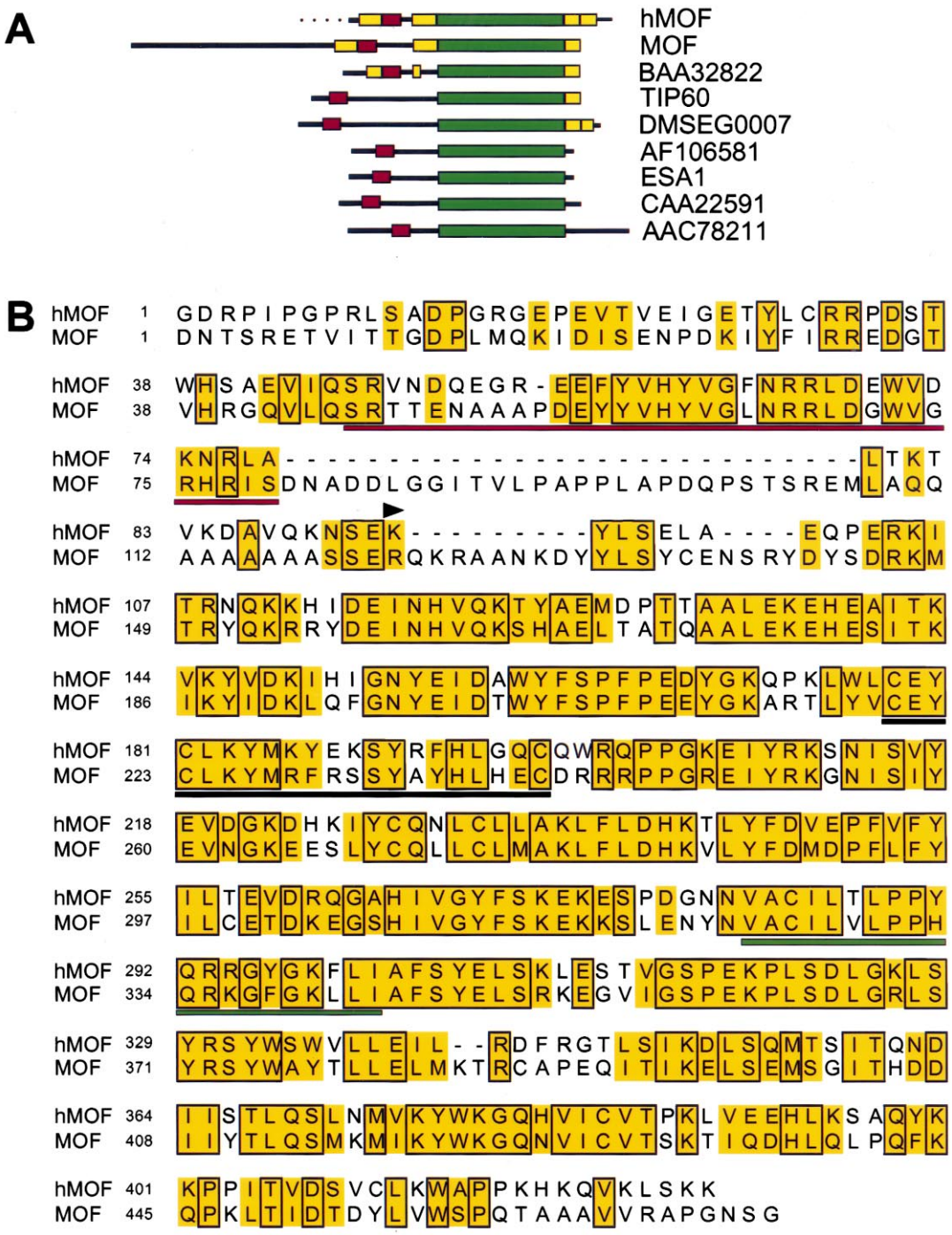

Fig. 1. (A) Schematic representation of the MYST family members with the conserved MYST domain indicated by a green box, the chromodomain regions indicated by a red box. The yellow-boxed regions of homology are derived from pairwise comparisons with hMOF. AF106581 is from *C. elegans*. (B) Sequence comparison of the predicted partial sequence of hMOF compared with the homologous region of *Drosophila* MOF. The arrowhead indicated the initial amino acid of hMOFC, the region used for immunizations and HAT assays. The chromodomain region is underlined in red. The zinc finger is underlined is black. The acetyl CoA binding site is underlined in green. Identities are boxed and homologies are shown in yellow.

the MYST region and the chromodomain. We also discuss the possibility that human MOF may interact with members of another chromodomain containing protein family.

The human EST database was screened for homology to *Drosophila* MOF. Several cDNAs encoding the same predicted protein were identified, the longest of which was 1416 bp long. A comparison between the conceptual human protein and MOF revealed levels of 52% identity and 69% similarity (Fig. 1). Analysis of the human genome databases shows that the gene responsible for the human protein maps to the short arm of chromosome 16 in region 11.2. This mapping is based on the presence of hMOF 3′ cDNA sequences within the 3′ flanking region of the PRSS8 gene that encodes a serine protease [14] and the fact that an STS (SHGC-15904) is contained within the hMOF cDNA.

A 470-bp *Ava*I fragment of the cDNA was used for Northern analysis of poly(A)$^+$ RNA from Raji cells (a human B-cell line). A single transcript of 1.8 kb was identified (Fig. 2A). Identical results were obtained using a 5′ *Eco*RI fragment (data not shown). Northern analysis of RNA from other human tissues also shows a 1.8-kb transcript in all tissues analyzed (data not shown).

Rabbit antisera were generated against a C-terminal region of the protein (hMOF C) and used for Western analysis of nuclear extracts from Raji cells (Fig. 2B). A single band of 52 kDa, absent when the

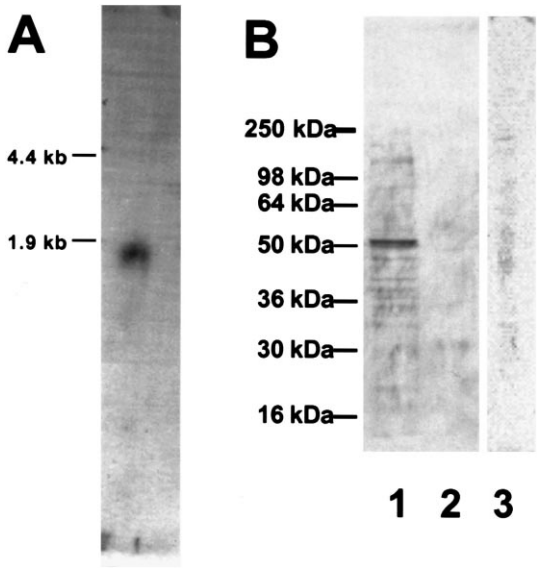

Fig. 2. (A) Northern analysis of hMOF shows a transcript of approximately 1.8 kb. The 470-bp *Ava*I fragment was random-primed and used as a probe. (B) Western analysis of hMOF shows a single protein product of approximately 50 kDa (Lane 1). The antibodies were raised in rabbit against recombinant C-terminal portion of the protein expressed in bacteria, hMOFC (see Fig. 1). The preimmune serum does not recognize the 52-kDa band (Lane 3). Lane 1: 30 μg Raji cell nuclear extract. Lane 2: 30 μg Raji cell cytoplasmic extract. Lane 3: 30 μg Raji cell nuclear extract.

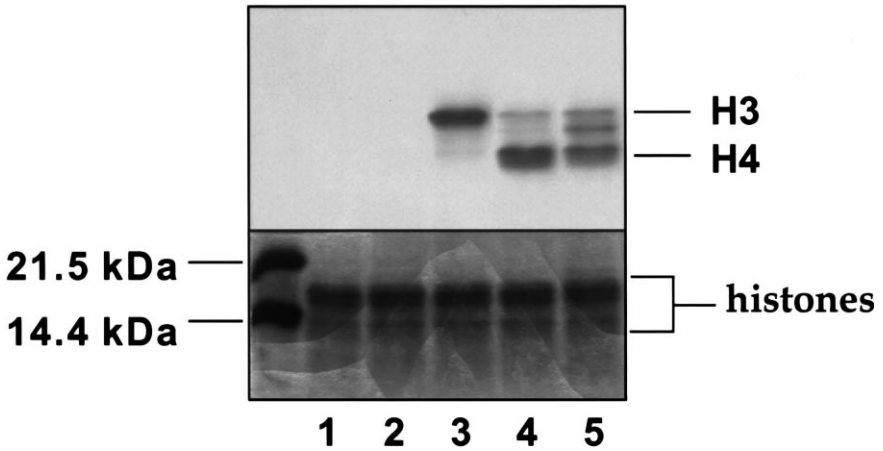

Fig. 3. Histone acetyltransferase activity of hMOFC has activity against histones H4, H3 and H2A. Upper box: Fluorogram indicates histones that have been labeled with the tritiated acetyl group. For comparison, the specificity of known histone acetyltransferases GCN5 [17] and ESA1 [7] was assayed. Lower box: Coomassie-stained gel shows the position of the histones. Lane 1: no protein extract. Lane 2: pET19b vector only. Lane 3: GCN5. Lane 4: ESA1. Lane 5: hMOFC.

extracts were probed with the preimmune serum, was identified. Immune serum recognized no protein when Raji cell cytoplasmic extracts were probed. Identical results were obtained with HeLa cell nuclear extracts (data not shown). These results localize the hMOF protein to the nucleus as is expected for a type A HAT.

A C-terminal region of human MOF (hMOFC), with an N-terminal 10-histidine tag, was expressed in bacteria using the pET19b vector (Novagen). The recombinant protein was purified using Qiagen nickel agarose and was used in a histone acetyltransferase activity liquid assay [15]. The recombinant protein acetylates purified histones H3, H2A and H4, with obvious preference for the latter (Fig. 3). This substrate specificity is similar to that exhibited by the other MYST family proteins that have been successfully assayed to date: Tip60 [16], ESA1 [7], HBO1 [9] and MOF (E. Smith, A. Pannuti, C.D. Allis and J.C. Lucchesi, in preparation).

MOF is a key factor in the regulatory mechanism of dosage compensation in *Drosophila*. Dosage compensation operates under completely different principles in flies, where it is the result of enhanced X-chromosome transcription in males, and in mammals, where one X chromosome is inactivated in females. Therefore, it is improbable that human MOF may be involved in this particular regulatory mechanism. Regardless of its cellular function, it is very likely, though, that hMOF will function while complexed with other proteins. This contention is based on work that shows that the two best characterized MYST family members to date, ESA1 and MOF, are members of multi-subunit aggregates, the NuA4 and MSL complexes, respectively [10,17]. The NuA4 complex includes a protein with significant similarity to the *Drosophila* MSL3 subunit of the MSL complex (A. Eisen, J.C. Lucchesi and J. Cote, in preparation) and we have found that there are at least two human proteins with significant similarity to MSL3 encoded in the human genome (GenBank accession numbers AF100615, AF117065). Based on this information, we propose that individual MYST family histone acetyl transferases may associate with specific MSL3 family proteins.

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