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LETTER TO THE EDITOR

Regulation of the histone acetyltransferase activity of hMOF via autoacetylation of Lys274

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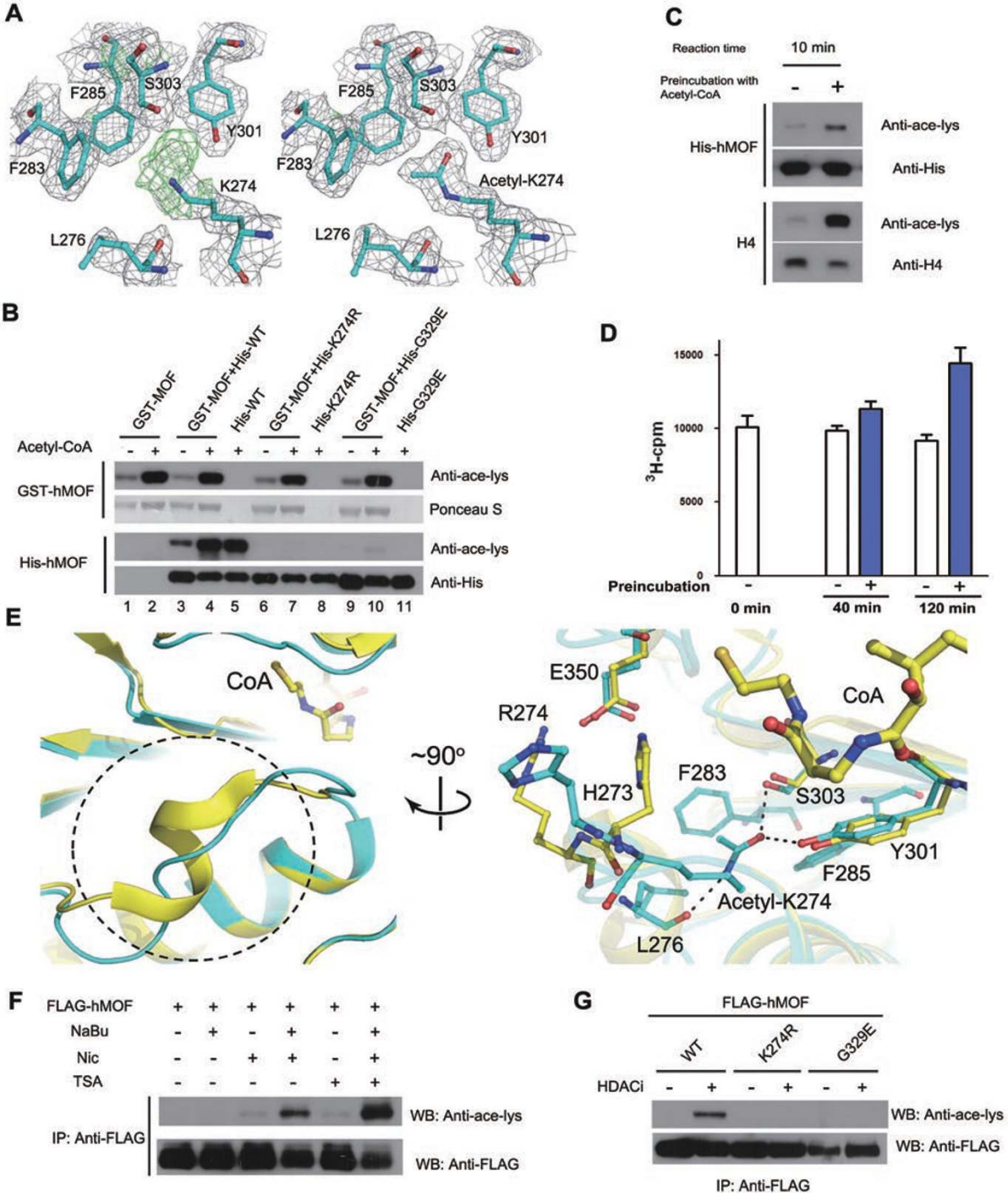
Dear Editor,

Males-absent-on-the-first (MOF, also called MYST1 or KAT8) is a histone acetyltransferase (HAT) belonging to the MOZ, Ybf2/Sas3, Sas2 and Tip60 (MYST) family. MOF has been shown to possess a specific HAT activity towards Lys16 of histone H4 (H4K16) [1]. Homozygous knockout of MOF in mice results in loss of H4K16 acetylation and embryonic lethality, indicating that MOF and H4K16 acetylation are essential for embryogenesis and genome stability in mammals [2]. Downregulation of human MOF (hMOF) leads to dramatic nuclear morphological deformation and inhibition of cell cycle progression [3], and has recently been correlated with primary breast carcinoma and medulloblastoma [4].

Here we report the crystal structure of the catalytic domain (residues 174-449) of hMOF at 2.1 Å resolution (Figure 1 and Supplementary information, Data S1, Figure S1A and Table S1). Intriguingly, in the initial difference Fourier maps there was strong residual electron density at the tip of the side chain of Lys274 at the catalytic active site (Figure 1A, left panel). Modeling of the density as an acetyl group and further refinement of the acetylated Lys274 led to a good fit of the map (Figure 1A, right panel). Structural comparison indicates that the conformation of the catalytic domain of the apo hMOF is similar to that of hMOF in complex with acetyl-coenzyme A (acetyl-CoA) alone (PDB code 2GIV) and in complex with acetyl-CoA and an male-specific lethal 1 (MSL1) fragment [5]. In particular, the acetylation of Lys274 is found in all these structures. The acetylation of Lys274 in the purified hMOF protein (hereafter the hMOF protein will refer to the catalytic domain of hMOF unless otherwise specified) was verified with liquid chromatography-tandem mass spectrometry (LC-MS/MS) (Supplementary information, Figure S1B and S1C). Although other acetylation sites were also identified, the intensity of the peptide containing the acetylated Lys274 constituted 83% of the total intensity of the acetylated peptides, indicating that Lys274 is the major acetylation site (**S**upplementary information, Table S2). Furthermore, hMOF is also acetylated at Lys274 *in vivo* (see discussion later). Sequence alignment of hMOF with other members of the MYST family HATs reveals that among all the identified acetylation sites, Lys274 is the only strictly conserved residue, suggesting that acetylation of this site might be common and probably functionally important for the MYST family HATs. Lys274 and two other strictly conserved residues (Cys316 and Glu350) which are in the vicinity of the acetyl group of the cofactor acetyl-CoA were mutated to study their functional roles (Supplementary information, Data S1 and Figure S2). Strikingly, mutation of Lys274 to Arg (K274R) almost completely abolished the hMOF activity (0.1%), while the K274Q mutant retained about 2.9% activity (Supplementary information, Figure S2A), suggesting that the loss of the HAT activity by the K274R mutation could be due to the inability of Arg to be acetylated.

To investigate whether Lys274 is autoacetylated, the wild-type and mutant hMOF proteins were incubated with acetyl-CoA and subsequently subjected to western blot with an antibody specific to acetylated lysine. Intriguingly, for all the hMOF proteins except the K274Q mutant, the acetylation levels prior to the acetyl-CoA incubation and the extents of the increase after the incubation correlated well with their respective HAT activities (Supplementary information, Figure S2), indicating that the acetylation of hMOF is catalyzed by hMOF itself. Although the K274Q mutant had an HAT activity comparable to that of the G327E mutant, its acetylation was undetected, which is in agreement with our MS data that Lys274 is the main acetylation site of the hMOF catalytic domain.

Next we investigated whether the autoacetylation of Lys274 of hMOF occurs inter- or intra-molecularly. The GST-tagged hMOF was incubated with either His-tagged wild-type or mutant hMOF in the presence or absence of acetyl-CoA (Figure 1B). Both GST-tagged and His-tagged wild-type hMOF could autoacetylate themselves; however, the His-tagged K274R and G329E mutants exhibited no or very weak activity. The presence of any of the His-tagged hMOF proteins had no appreciable effect



on the acetylation level of the GST-tagged hMOF and vice versa. These results indicate that Lys274 of hMOF is autoacetylated in an intra-molecular manner.

To investigate whether acetylation of this residue is functionally important, the HAT activity of hMOF with or without pre-incubation with low concentration of acetyl-CoA (200 μM) was examined with the HAT activity assays in the presence of high concentration of acetyl-CoA (1 mM). As shown in Figure 1C, hMOF pre-incubated with acetyl-CoA can acetylate H4 more efficiently. To exclude the possibility that pre-incubation of hMOF with acetyl-CoA allows binding of acetyl-CoA in a higher percentage of the enzyme hence leading to higher activity, we carried out more quantitative HAT activity

Figure 1 Regulation of the histone acetyltransferase activity of hMOF via autoacetylation of Lys274. **(A)** A close-up view of $2F_o$-F_c and F_o-F_c maps at Lys274 and surrounding residues. The $2F_o$-F_c (gray, 1σ) and F_o-F_c (green, 2.5 σ) maps are shown after refinement of the side chain of Lys274 as unmodified (left) or acetylated (right). **(B)** Intra-molecular autoacetylation of hMOF. Approximately 10 μg of GST-tagged and 5 μg of His-tagged hMOF proteins were incubated with 200 μM acetyl-CoA (+) or the mock solution (–) for 2.5 h at 30 °C. The acetylation level of the proteins was examined. **(C)** Stimulation of the HAT activity of hMOF by Lys274 autoacetylation. Approximately 0.8 μg of hMOF proteins with (+) or without (–) pre-incubation with 200 μM acetyl-CoA were subjected to HAT activity assays. **(D)** Quantitative HAT activity assays of autoacetylated hMOF. Approximately 1 μM hMOF protein was pre-incubated with 25 μM non-labeled acetyl-CoA (+) or the mock solution (–) for 40 or 120 min at 30 °C. HAT activity assays were carried out in the presence of 25 μM ^{3}H-labeled acetyl-CoA with 50 μM recombinant H4 as substrate for 10 min. The activities were detected with liquid scintillation. **(E)** Comparison of the active site of the wild-type hMOF (cyan) with that of the K274R mutant (PDB code 2PQ8, yellow). Left: the major structural difference of the α3-β7 segment (residues 272-278) (marked with a circle). Right: A detailed view of the differed region and nearby residues. The hydrogen-bonding interactions between the side chain of acetylated Lys274 and the surrounding residues are indicated with dashed lines. **(F)** Enhanced acetylation of the full-length hMOF with the treatment of HDAC inhibitors. 293T cells transfected with FLAG-hMOF were treated with 10 mM sodium butyrate (NaBu, an inhibitor of Class I and Class II HDACs except for HDAC6 and HDAC10), 1 mM nicotinamide (Nic, an inhibitor of the Sirtin family HDACs), and/or 5 μM trichostatin A (TSA, an inhibitor of Class I and Class II HDACs) for 6 h. **(G)** Autoacetylation of hMOF in 293T cells. 293T cells transfected with the plasmids encoding the full-length wild-type, K274R or G329E mutant hMOF were treated with (+) or without (–) a combination of the three HDAC inhibitors (HDACi). For **(F** and **G)**, the FLAG-hMOF proteins were immunoprecipitated with anti-FLAG M2 beads and then assayed with western blot using anti-ace-lys and anti-FLAG antibodies.

assays with addition of non-labeled acetyl-CoA at the pre-incubation step and supplementation of ^{3}H-labeled acetyl-CoA at the activity assay step. The results show that pre-incubation with acetyl-CoA increases the hMOF activity in a time-dependent manner (Figure 1D), indicating that autoacetylation of hMOF potentiates its HAT activity. It has been reported that moderate modulation of the activities of HATs could have a significant impact on the biological processes in which they are involved. For example, an approximate 25% increase in the enzymatic activity of TIP60, which is most closely related to hMOF in the MYST family, by the binding of PDCD5 led to acceleration of DNA damage-induced apoptosis [6]. Therefore, the increase in the enzymatic activity of hMOF we observed in Figure 1C and 1D is not trivial and suggests an important role for acetylation of Lys274 in the HAT activity of hMOF.

The structures of the wild-type hMOF (PDB code 2GIV and in this study) were compared with the structure of the K274R mutant (PDB code 2PQ8) which might mimic the structure of hMOF with an unacetylated Lys274. Although the overall structures are similar, the region encompassing residues 272-278 (the α3-β7 segment) exhibits significant conformational difference which forms a loop in the wild-type hMOF but a 3_{10}-helix in the K274R mutant (Figure 1E). Within this segment, the side chains of acetylated Lys274 and Arg274 point to opposite sides although their main chains occupy similar positions.

In the K274R mutant structure, the side chain of Arg274 flips outwards from the active site and forms a salt bridge with the carboxyl group of Glu350 (2.7 Å) (Supplementary information, Table S3). Consequently, the side chain of His273 occupies the space between Glu350 and cofactor coenzyme A (CoA) (Figure 1E, right panel). Superposition of this structure onto that of *Tetrahymena* Gcn5 bound with CoA and a histone H3 peptide [7] shows that the side chain of hMOF His273 occupies a site corresponding to that between the highly conserved Glu and the cofactor in the Gcn5 structure which is the binding site for the substrate lysine of the H3 peptide (Supplementary information, Figure S3). Therefore, the complete loss of the HAT activity of hMOF by the K274R mutation might be due to impaired binding and/or improper positioning of the side chain of the substrate lysine, and hence the active site of the K274R mutant hMOF represents an inactive conformation.

In contrast, in the wild-type hMOF, the side chain of acetylated Lys274 points inwards forming part of the active site (Figure 1E). Correspondingly, His273 occupies a position different from that in the mutant structure and the space between Glu350 and cofactor CoA is vacant, allowing for proper binding and positioning of the substrate lysine (Figure 1E, right panel). A detailed analysis shows that Lys274 makes extensive interactions with the surrounding residues. The acetyl carbonyl group forms two hydrogen bonds with the side chains of two conserved residues Tyr301 and Ser303. The acetyl methyl group makes hydrophobic interactions with two aromatic residues Phe283 and Phe285. In addition, the ε-nitrogen of Lys274 forms a hydrogen bond with the main-chain carbonyl group of Leu276 (Figure 1E, right panel). Besides these interactions via the side chain of acetylated

Lys274, there are only three additional hydrogen bonds between the α3-β7 segment and the surrounding residues (Supplementary information, Table S4). Therefore, the interactions made by the acetylated side chain of Lys274 constitute a major part of the interaction network which helps maintain the loop conformation of the α3-β7 segment and the corresponding structure of the active site.

The loop conformation of the α3-β7 segment might also exist in solution, as in the wild-type hMOF structures no residue in this region was involved in crystal packing and this conformation was captured independently under different crystallization conditions [5]. Thus, the active site of the wild-type hMOF structures is likely to represent a biologically active conformation. On the other hand, as lysine shares a similar electrostatic property as arginine, hMOF with an unacetylated Lys274 might assume an inactive conformation of the catalytic site similar to that of the K274R mutant which, intriguingly, might block the binding of MSL1 due to the steric hindrance between the main-chain carbonyl group of hMOF-Tyr277 and the side chain of MSL1-Glu516 (Supplementary information, Figure S4). Therefore, acetylation of Lys274 may play a critical role for the enzymatic activity through stabilization of the loop conformation of the α3-β7 segment and hence the active conformation of the catalytic active site.

The effect of Lys274 acetylation of hMOF on its HAT activity was further investigated *in vivo*. Acetylation was detected for the full-length hMOF overexpressed in 293T cells although the acetylation level was low (Supplementary information, Figure S5A). Treatment of the cells with 10 mM sodium butyrate, 1 mM nicotinamide or 5 μM TSA alone had no or minor effect on the acetylation level of the full-length hMOF; however, treatment with a combination of sodium butyrate and nicotinamide or the three inhibitors resulted in significant increase of the hMOF acetylation level (Figure 1F), indicating that multiple classes of histone deacetylases (HDACs) are likely responsible for the deacetylation of hMOF. In contrast, no acetylation was detected for the K274R and G329E mutants even when the cells were treated with the HDAC inhibitors (Figure 1G). Consistently, LC-MS/MS analysis of the full-length hMOF immunoprecipitated from 293T cells treated with the three HDAC inhibitors confirmed that Lys274 is acetylated *in vivo* (Supplementary information, Figure S5B). The potential biological function of Lys274 acetylation of hMOF was further investigated by examination of the H4K16 acetylation level in COS7 cells. Overexpression of the wild-type hMOF resulted in higher level of H4K16 acetylation compared with the control, while the K274R mutant functions as a dominant negative form (Supplementary information, Data S1 and Figure S5C), supporting the notion that Lys274 and probably its acetylation are critical for the hMOF function and the regulation of H4K16 acetylation level *in vivo*.

Recently the Akhtar and Liu groups also report that hMOF can be autoacetylated at Lys274 [5, 8]. Compared with their studies, our work reveals that hMOF is autoacetylated in an intra-molecular manner, indicates that the autoacetylation of hMOF at Lys274 has a significant impact on the catalytic activity of the enzyme, and provides the structural basis for the modulation of the enzymatic activity by Lys274 acetylation. Thus, our study investigates independently the function of this modification from different aspects. It was shown that the acetyltransferase p300/CBP can be autoacetylated at multiple lysine residues on a pseudo substrate inhibitory loop leading to substantial increase of the HAT activity [9]. Another HAT, Rtt109 was found to be autoacetylated at Lys290 of the activation domain, which is essential for its HAT activity towards H3K56 [10]. Lys274 of hMOF and Lys290 of Rtt109 are not at equivalent positions (Supplementary information, Figure S6), and the regulatory scheme of hMOF by Lys274 acetylation proposed in this study is quite different from those of p300 and Rtt109. Therefore, hMOF employs a novel regulatory mechanism of acetyltransferase activities, providing another example of great divergence in the regulation of acetyltransferases as well as other enzymes by acetylation.

The structure of the hMOF catalytic domain has been deposited with the RCSB Protein Data Bank under accession code 3QAH.

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(**Supplementary information** is linked to the online version of the paper on the *Cell Research* website.)