Revealing the Protein Propionylation Activity of the Histone Acetyltransferase Males absent on the first (MOF)

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Running Title: MOF and lysine propionylation

Key words: Acetyltransferase, Crystal structure, Post-translational modification (PTM), Protein acylation, Proteomics, Males absent on the first (MOF), Lysine propionylation

ABSTRACT

Short-chain acylation of lysine residues recently emerges as a group of reversible posttranslational modifications in mammalian cells. The diversity of acylation further broadens the landscape and complexity of the proteome. Identification of regulatory enzymes and effector proteins for lysine acylation is critical to understand functions of these novel modifications at the molecular level. Here, we report that the MYST family of lysine acetyltransferases (KATs) possesses strong propionyltransferase activity both in vitro and in cellulo. Particularly, the propionyltransferase activity of MOF, MOZ, and HBO1 is as strong as their acetyltransferase activity. Overexpression of MOF in human embryonic kidney 293T cells induced significantly increased propionylation in multiple histone and non-histone proteins, which manifests that the function of MOF goes far beyond its canonical histone H4 lysine-16 acetylation. We also resolved the X-ray co-crystal structure of MOF bound with propionyl coenzyme A, which provides a direct structural basis for the propionyltransferase activity of the MYST KATs. Our data together defines a novel function for the MYST KATs as lysine propionyltransferases and suggests much broader physiological impacts for this family of enzymes.

INTRODUCTION

Short-chain acylations of lysine residues in cellular proteins such as acetylation, propionylation, and crotonylation are reversible...
posttranslational modifications (PTMs) that modulate functions and properties of protein targets (1). The variety of lysine acylation has been proposed to correlate with divergent biological outputs (2,3). Etiologically, lysine acylations rely on acetyl-coenzyme A (acyl-CoA) molecules to serve as the acyl donors, which are key metabolic intermediates in the Krebs cycle, fatty acid oxidation, and amino acid degradation (4). The level of acyl-CoA intermediates fluctuates with nutritional status and altered activities of metabolic enzymes. Toxic accumulation of acyl-CoA molecules is associated with a broad variety of complications (5,6). A typical example is the propionic acidemia, an autosomal recessive metabolic disorder caused by the deficiency of propionyl-CoA carboxylase (PCC) (7). Increase of propionyl-CoA and protein lysine propionylation level were reported in PCC deficient patient fibroblast cells, but the disease relevant targets and the corresponding regulatory enzymes are not defined (5). It is also increasingly recognized that the gut microbiota-related short-chain fatty acids interacts with and modulates the mammalian epigenetic machinery, especially histone acylation and methylation (8,9). Nevertheless, it remains poorly understood how the alterations of acyl-CoA abundance and protein acylation levels are causatively linked with the related diseased phenotypes. Understanding the regulatory mechanisms of short-chain lysine acylation such as lysine propionylation will have profound significance to elucidate the pathophysiological mechanisms of metabolic diseases.

To understand the mechanistic links of acylation diversity with varied physiological outputs, a significant challenge is to resolve how different acylations are deposited, interpreted, and erased in the cell. In eukaryotes, protein acetylation marks are introduced by lysine acetyltransferases (KATs), which are classified into three major families based on primary amino acid sequence and domain organization including the MYST, the PCAF/GCN5, and the p300/CBP family (10). These KATs, together with lysine deacetylases (KDACs), orchestrate the dynamics of lysine acetylation in the cell. Numerous studies have shown that aberrant expression and dysfunction of KATs are associated with various kinds of disease phenotypes such as inflammation, neurodegenerative disorders, and cancers (11-13). In 2007, Chen et al. reported that p300 and CBP can propionylate human proteins histone H4 and p53 with high resolution mass spectrometry. (14). Later on, GCN5 and PCAF, members of the GNAT superfamily, were also found to possess propionyltransferase activity (15-17). These findings largely increased mechanistic complexity and diversity of the KAT related physiological activities. The MYST KATs represent a distinct family whose sequence and function are dramatically different from the members of p300/CBP and PCAF/GCN5 families. It remains an open question whether or not the members of the MYST family possess lysine propionyltransferase (KPT) activity (Figure 1) (18). In this work, we conducted a combined suite of biochemical, cellular, and structural studies and demonstrated that the MYST KATs have genuine KPT activity both in vitro and in cellulo.

RESULTS

Propionyl-CoA is an abundant metabolite in human cells.

Several novel acylations other than acetylation on protein lysine residues have been identified, such as propionylation, butyrylation, crotonylation, succinylation, and malonylation (14,19,20). Thus far, it remains poorly understood as regards to the cellular abundance of the acyl-CoA molecules for corresponding
acetylations. To understand the regulation of protein propionylation, we first quantified and compared the cellular abundance of endogenous propionyl-CoA and acetyl-CoA in the human embryonic kidney 293T (HEK293T) cells. Following cell culture, acetyl-CoA molecules were extracted in 50% methanol and subjected to the LC-MS/MS detection. Deuterated acetyl-CoA and propionyl-CoA were used as internal standards for calibration. Triplicate experiments were performed and showed that the cellular propionyl-CoA concentration is 92 ng/mg cellular proteins and acetyl-CoA is 754 ng/mg cellular proteins. Thus, the abundance of propionyl-CoA is about 12% that of acetyl-CoA (Figure 2). Considering that acetyl-CoA is a rich and principal metabolite for cell growth, propionyl-CoA, even 8 times less abundant than acetyl-CoA, has the potential to serve as an ample source for lysine propionylation.

**The recombinant MYST KATs have strong lysine propionyltransferase activity.**

A few studies have demonstrated that GCN5/PCAF and p300/CPB members were able to carry out lysine propionylation, butyrylation, crotonylation, etc. in addition to their intrinsic acetylation activity (3,14,17). In contrast, although the MYST enzymes represent the largest KAT family in human cells, their cofactor promiscuity is much less studied. Very recently, MOF was shown to carry out butyrylation and crotonylation on histone substrates (21,22). These evidence hints for the cofactor promiscuity of the MYST enzymes. Herein, we thoroughly investigated the novel acyltransferase activity of MYST KATs comparatively and from different perspectives. First, we screened and compared the KPT activity of the three families of KATs on their histone H3 or H4 peptide substrates (H3-20 and H4-20, the N-terminal 20-aa sequence of histone H3 and H4). A fluorogenic assay was used to determine the kinetic constants of KATs including $K_m$ and $k_{cat}$ with respect to acetyl-CoA or propionyl-CoA under the initial velocity condition (23). The $k_{cat}/K_m$ ratio was calculated to quantitatively compare the KPT and KAT activities of individual KATs. In consistence with the previous studies showing that PCAF/GCN5 and p300/CPB members possess KPT activity (15,17,24), our kinetic measurement showed that the KPT activity of GCN5 and HAT1 is almost equally strong compared to their KAT activity while the KPT activity of PCAF and p300 is about 40% and 30% of their acetyltransferase activity (Table S1). Interestingly, the MYST KAT members, including MOF, HBO1, and MOZ, show strong KPT activity with ratios of their KPT/KAT activity to be 0.84, 0.99, and 1.04 respectively. The MYST KATs Tip60 and MORF also showed appreciable KPT activities, which are 12% and 17% of their KAT activity. After we demonstrated the propionyltransferase activity of the KAT enzymes on histone peptides, we then tested the KPT activity of the MYST KATs on the proteome of the 293T cell using an anti-propionyllysine antibody (Figure 3). Compared with the untreated cell lysate, sole propionyl-CoA co-incubation did not induce increase of lysine propionylation on cellular proteins while treatment of the cell lysate with propionyl-CoA and individual MYST KATs induced extensive propionylation of multiple histone and non-histone proteins. In particular, MOF showed the strongest KPT activity on cellular proteins. Together, the steady-state kinetic data and the western blot analysis of whole protein propionylation strongly demonstrate that the MYST KATs are *bona fide* KPTs. Next, we focus on MOF for further biological, structural, and biochemical investigation of the novel KPT activity of the MYST enzymes.
MOF acetylates and propionylates largely shared substrates with subtle variation.

After proving the KPT activity of the MYST enzymes on cellular histones and non-histones, we investigated the similarity of MOF-mediated sub-acylome and sub-propionylome. Peptide proteomic analyses were applied to identify MOF acetylation and propionylation sites on the H4-20 peptide. Mono-acetylated H4-20 is the major product from MOF catalyzed acetylation reaction and the di-acetylated H4-20 also present with lower yield than the mono-acetylated product (Supplementary Figure S1A). Notably, the acylation sites were slightly different: K8, K12, and K16 were found acetylated while K8 and K16 were propionylated. In further detail, mono-acetylation of H4-20 occurs on K8 and K16 while di-acetylated peptides have acetylated K8 and K12, K16 and K12, and K8 and K16, respectively. For H4-20 propionylation, K8- and K16- mono-propionylated peptides were detected, and di-propionylated peptides have propionylated K8 and K16 simultaneously (Supplementary Figure S1B and C, Supplementary Table S2). Thus, both K8 and K16 are prone to undergo both acetylation and propionylation by MOF while K12 may be a weak acetylation site of MOF. Next, we tested MOF substrate specificity on the histone proteins and nucleosomes with western blot analysis. Using free histones as substrates, MOF was able to modify all the four core histones for both acetylation and propionylation (Figure 4A) while MOF cannot acylate histone H3 on the nucleosomes (Figure 4B). Both MOF catalytic domain and full-length MOF showed the same substrate specificity, suggesting that the chromo domain did not affect the interaction between MOF and nucleosomes. Accumulated studies indicated that the histone proteins are not the only substrate of MOF (2,25-27); therefore we next explored MOF substrate profile on cellular proteome. Since the extracted cellular proteome has gone through acylations by endogenous KAT enzymes, it is hard to accurately discriminate MOF sub-acylome from the bulk acylome with immunoblot assays. Thus, a radiometric gel assay was performed to compare the substrates acetylation and propionylation driven by MOF (Figure 5). In accord with the western blot results shown in Figure 3 and Figure 4, little non-enzymatic labeling on lysine residues driven by the chemical reactivity of acetyl-CoA and propionyl-CoA was observed. Treatment of the cell lysate with acyl-CoA and MOF together induced strong acylation of multiple proteins from the cellular proteome. A highly identical modification pattern was observed between lysine acetylation and propionylation, which is suggestive that MOF acetylome and propionylome may largely overlap. MOF auto-acylation was previously reported by Yang et al (28). We observed that MOF underwent auto-propionylation with similar activity compared to auto-acylation. The fact that multiple non-histone proteins were being acylated by MOF is in agreement with the previous findings that MOF targets both histone and non-histone proteins (2,26,29-31). Overall, MOF may have highly identical acetylome and propionylome while the modification levels at individual lysine residues could differ to varying degrees.

MOF is a genuine lysine propionyltransferase in cellulo with a distinct substrate profile.

The above biochemical experiments demonstrated that the recombinant MYST KATs catalyze propionylation of histone and non-histone proteins in vitro. We next focused to test MOF propionyltransferase activity in the cell and determine the MOF propionylome. A model of MOF overexpression was created in HEK293T cells by lentivirus transient transfection (Figure 6A). Following cell culture
and cell lysate extraction, the acylation levels of cell proteome were examined with western blot analyses. Both lysine acetylation and propionylation in extracted histones were moderately upregulated in the presence of MOF overexpression (Figure 4B). Particularly, MOF overexpression induced increased levels of acetylation on both histone H4 and H2A/2B while histone H3 acylation did not change, which are consistent with the in vitro data shown in Figure 4B. Therefore, H3 is likely not a significant in cellulo substrate of MOF. Of note, H4K16 propionylation was upregulated with MOF overexpression (Figure 6C), in accord with the previous knowledge that H4K16 is a primary substrate of MOF (32). We also observed that multiple non-histone proteins were acetylated and propionylated at elevated levels in response to MOF overexpression, and the western blot profiles of substrate propionylation and acetylation are highly identical (Figure 6D). This result is in consistence with the radiometric gel assay (Figure 5) and strengthens our hypothesis that MOF have shared substrates for KAT and KPT activities. In addition, we detected the change of lysine propionylation level on cellular proteome in response to varying propionyl-CoA concentrations (Supplementary Figure S2). The 293T cells were treated with sodium propionate, which can be converted to propionyl-CoA in the cells through the acyl-CoA synthase pathway (33), and cellular proteins were extracted for western blot detection. Treatment of both the control and MOF-overexpressed cells with sodium propionate induced increase of lysine propionylation on both histone and non-histone proteins. Interestingly, the western blot patterns show appreciable difference between the control cells and MOF-overexpressed cells upon propionate treatment, suggesting that some proteins are only propionylated in the MOF-overexpressed cells. These results indicate that MOF may have a distinct substrate propionylome and the unique MOF function cannot be compensated by other KPT enzymes. Moreover, we knocked down MOF expression in the 293T cells and found that the lysine propionylation level was slightly decreased upon downregulation of MOF expression (Figure S3). Together, MOF displayed in cellulo KPT activity on both histone and non-histone proteins. The propionylation substrate profile of MOF is highly similar to that of acetylation, but is partially distinct from other KPTs.

**Proteomic profiling of MOF-mediated propionylome.**

Tandem MS was carried out to further investigate the propionylation substrates of MOF in the 293T cellular proteome. The MOF-overexpressed and control cells were treated with deuterated sodium propionate (Sodium propionate_D5), followed by cell lysate extraction, affinity enrichment with anti-propionyllysine antibody, and LC-MS/MS analyses. As shown in Figure 7A, the number of d5-propionylated lysine sites (Kpr_D5) in cellular proteins greatly increased, from 36 to 60, upon MOF overexpression. Excitingly, 43 unique propionylation sites were identified in MOF-overexpressed cells which were not present in the control cells. These emerging sites most likely represent bona fide cellular substrates of MOF (Figure 7B and Supplementary Table S3). These proteins include known MOF substrates such as p53 and MOF (2,28). H4K16 was found in the propionylation list, which is consistent with the in vitro and in cellulo studies (Supplementary Table S2 and Figure 6) and also matches the previous literature showing that MOF can acetylate H4K16 in mammalian cells (31). Additionally, we identified several new histone propionylation sites at H4K12, K16, and
H2AK5, K9 in the MOF-overexpressed cells, which is in agreement with the western blot data that the propionylation levels of histone H2A/H2B and H4 were upregulated upon MOF overexpression (Figure 6B). It is worth mentioning that 17 out of the total 60 Kpr_D5 sites in the MOF-overexpressed cells were found in the control cells as well. These shared modification marks could be the common substrates of MOF and other KPTs. Also, 19 Kpr_D5 sites were only identified in the control cells but not in the MOF-overexpressed cells. These lysine sites might be the substrates of other KPTs such as GCN5 or p300 and their propionylation level are not high enough to be detected in the MOF-overexpressed cells due to the strong competition from the overexpressed MOF. Overall, these proteomic data demonstrate that MOF propionylates a wide range of cellular proteins. The finding that MOF propionylates multiple protein substrates indicates broader involvement of MOF in biological regulation and its versatile functions.

The crystal structure of MOF in complex with propionyl-CoA further supports the KPT activity of the MYST enzymes.

To gain structural insight into the KPT activity of MOF, we solved the crystal structure of MOF catalytic domain in complex with propionyl-CoA at 1.78 Å resolution (Table S4 and Figure 8A). Binding of propionyl-CoA to the MOF catalytic domain did not cause any major structural change in MOF structure, as the MOF-propionyl-CoA binary structure is almost identical to that of the previously solved MOF-acetyl-CoA complex (PDB: 2GIV). The Ca of these two structures can be superimposed with root-mean-square deviation (RMSD) of just 0.17 Å. Similar to the MOF-acetyl-CoA structure, the overall fold of MOF-propionyl-CoA adopts an elongated shape. The structure is comprised of a central core region, flanked on the opposite sides by N- and C-terminal domains (Figure 8A). The catalytic site of the MYST family of KATs involves a catalytic cysteine (Cys316 in MOF), and a conserved glutamate (Glu350 in MOF) function as general base. An auto-acetylated lysine at the active site (Lys274 in MOF) is also critical for the KAT activity (34,35). Our structure shows that the active site of MOF can well accommodate propionyl-CoA and Fo-Fc omit map shows clear density for the terminal methyl group in propionyl-CoA (Figure 8B). Like acetyl-CoA, propionyl-CoA binds to MOF between the core domain and C-terminal domain in a bent conformation, interacting mostly with the β10-loop-α4 region of the core domain and loop-α5 region of the C-terminal domain (Figure 8A). When the two complex structures are overlaid together, the propionyl-CoA molecule in the MOF-propionyl-CoA structure can superimpose with the corresponding moiety of the acetyl-CoA molecule in the MOF-acetyl-CoA complex structure very well (Figure 8C). Most of the interactions between MOF and acetyl-CoA in the MOF-acetyl-CoA structure are still conserved in the MOF-propionyl-CoA structure. However, propionyl–CoA makes two extra hydrophobic interactions through its propionyl group with Val314 and Pro349 in MOF (Figure 8C). Sequence alignment reveals that both Pro349 and Val 314 in MOF are conserved amino acid residues through all the five eukaryotic MYST KATs (Figure 9). These two interactions could contribute to the proper orientation of the propionyl group. The crystallographic data provide direct structural evidence that the MYST KATs behave as a dual activity enzyme for catalyzing both propionylation and acetylation. To test the necessity of the two residues Val314 and Pro349 of MOF for its KPT activity, we conducted site-directed mutagenesis to replace MOF-Val314 or Pro349 with Ala and then tested the KAT and KPT activity of wt-MOF.
MOF-V314A, and MOF-P349A using the fluorescent CPM assay to obtain the kinetic constants $k_{cat}$ and $K_m$ with respect to acetyl-CoA and propionyl-CoA (Supplementary Table S5). Both MOF-V314A and P349A showed decreased intrinsic enzymatic activity on acetylation, retaining 30% and 20% activity compared with the wt-MOF. The KPT activity of MOF-V314A abolished 55% of wt-MOF while mutation of Pro349 to Ala abolished more than 95% the KPT activity of MOF. These data suggest that the interaction between the propionyl group and MOF involves Pro349 more than Val314; therefore, the conserved Pro residue contributes more to the KPT activity of MYST KATs.

**DISCUSSION**

The discovery of chemically diverse acylations in proteins represents an exciting area of research in biology. It embodies the potential and important regulatory roles of cellular acyl-CoA metabolites in the modulation of epigenetics and signal transduction. It would be vitally important to determine the cofactor promiscuity of different KATs in order to elucidate the biochemical etiology of cellular protein acylations. In this study, we found that the MYST family of KATs showed strong *bona fide* KPT activity. Especially, MOF, MOZ, and HBO1 exhibited as strong KPT activity as their classic KAT activity on histone substrates. The analysis of cellular proteins with Western blot showed that all members of the MYST KATs promote extensive lysine propionylation, not only on histones but also on non-histone proteins. These findings that the MYST enzymes exhibited a widespread propionylome suggest their much broader physiological roles in the regulation of biological processes. We found that MOF has highly identical acetylome and propionylome profiles, but the modification levels at the individual lysine residues were slightly distinct. That the propionylome of MOF being highly identical to its acetylome strongly advocates for the cofactor promiscuity of the MYST enzymes and predicts that the dynamics of distinct acylation marks in proteins is greatly influenced by the metabolic fluctuation of cellular acyl-CoA variants. We, for the first time, solved the crystal structure of a MYST enzyme bound with propionyl-CoA, which provides direct evidence supporting the KPT activity of the MYST KATs. Moreover, our data suggest that the residue Pro349 in MOF, conserved through the MYST family of KATs, is required for its KPT activity. Future efforts will be needed to clarify how the sub-propionylomes of individual KATs differ from each other and to address how lysine propionylation impacts on the properties of the substrate proteins in various biological pathways.

**EXPERIMENTAL PROCEDURES**

**Quantification of cellular acetyl- and propionyl-CoA.**

Human embryonic kidney 293T (HEK293T) cells were cultured to 90% confluence with DMEM medium supplemented with 10% fetal bovine serum (FBS) and 1% streptomycin-penicillin. Cells were washed with ice-cold PBS buffer followed by fixing in methanol at −80 °C for 15 minutes. 75 ng deuterated acetyl-CoA and propionyl-CoA were added as internal standards. Cells were collected in 50% of methanol with gentle scrape. Cell suspension was centrifuged at 16000g and supernatant was collected for LC-MS/MS analysis. An Atlantis® T3 (4.6×150 mm, 3 μm) column coupled with a Phenomenex SecurityGuard C-18 guard column (4.0 mm×2.0 mm) was applied for separation. An Agilent 1100 binary pump HPLC system (Santa Clara, CA) coupled to a Waters Micromass Quattro Micro triple quadrapole mass spectrometer with an ESI
source (Milford, MA) was applied for LC–MS/MS analysis. The column temperature was controlled at 32 °C. The mobile phase A was 10 mM ammonium acetate, and mobile phase B was acetonitrile. Analytes were separated using a gradient method, with a 0.4 mL/min flow rate, (time/minute, % mobile phase B): (0, 6), (15, 30), (15.01, 100), (22.50, 100), (22.51, 6). The injection volume was 30 μL, and the autosampler injection needle was rinsed with methanol after each injection. Samples were analyzed by the mass spectrometer in positive ion ESI mode. Nitrogen was used as the desolvation gas at a flow rate of 500 L/h. The desolvation temperature was 500 °C and the source temperature was 120 °C. Argon was used as the collision gas, and the collision cell pressure was $3.5 \times 10^{-3}$ mbar. The capillary voltage was 3.2 kV, the cone voltage was 42 V and the collision energy was 22 eV. Multiple reaction monitoring (MRM) functions were applied for the detection of each acyl-CoA and internal standards. The ion transitions monitored were 810→303 for acetyl-CoA, 813→306 for d3-acetyl-CoA, 824→317 for propionyl CoA and 829→322 for d5-propionyl-CoA.

**Kinetic characterization of acetyl-CoA and propionyl-CoA in KAT mediated histone modification.**

Synthetic histone peptides H3-20 or H4-20 (20 amino acids from the N-terminal of histone H3 and H4, the sequence of H3-20 is Ac-ARTKQTARKSTGGKAPRKQL, the sequence of H4-20 is Ac-SGRGKGGKGLGKGGAKRHRK) were used as acyl acceptor substrates. Acyl-CoA at varied concentrations was incubated with individual KAT enzymes and peptide at fixed concentration. KAT enzymes deposit acyl groups on the ε-amine of peptide lysines and release CoASH simultaneously. The fluorogenic probe 7-diethylamino-3-(4’-maleimidylphenyl)-4-methylcoumarin (CPM) was added to react with by-product CoASH to form the fluorescent CoAS-CPM complex (23). The fluorescence intensities were measured with a microplate reader (FlexStation® 3). The catalytic rate was determined from the fluorescence intensity. Kinetic constants including binding affinity ($K_m$) and catalytic efficiency ($k_{cat}$) were determined by fitting the acyl-CoA concentration-catalytic rate to the Michaelis-Menten equation. All the samples for the kinetic assays were duplicated and the final results were presented in Value±SD.

**Detection of acetylation and propionylation level on proteins.**

For *in vitro* studies, recombinant or extracted cellular proteome were incubated with acyl-CoA and KAT enzymes, followed by western blot detection with anti-acetyllysine (PTM Biolabs, Product#: PTM-101) and anti-propionyllysine antibodies (PTM Biolabs, Product#: PTM-210). The radiometric gel analysis was used to compare the acetylome and propionylome of MOF. For the *in cellulo* studies, cellular proteome or core histone proteins were extracted from the control and MOF-overexpressed cells and detected with different antibodies.

**Profiling of MYST KATs in vitro propionylome.**

The whole lysate of HEK293T cells was extracted using a gentle cell lysis buffer M-PER Mammalian Protein Extraction Reagent (Thermo Scientific, Product#: 78501) together with sonication at 30% amplitude using a sonicator (Fisher Scientific, Model 120 Sonic Dismembrator). Protein propionylation level was tested with western blot using pan anti-propionyllysine antibody (PTM Biolabs, product number PTM-201).
Identification of MOF acylation sites on H4-20 peptide using LC-MS/MS analysis.

100 μM H4-20 peptide, 200 μM acyl-CoA were incubated with 1 μM MOF enzyme for 1 hour at 30 °C followed by desalting with C18 Zip-tip. The desalted samples were analyzed by ACQUITY UPLC system (Waters, Milford, MA) coupled to a Waters SYNAPT G2 mass spectrometer (Milford, MA). Peptides were separated on the HALO C18 peptide column (2.7 μm, 4.6 x 100mm, Advanced Materials Technology, Wilmington, DE). Mobile phase A is water containing 0.01% formic acid and B is acetonitrile (ACN). The injection volume was 10 μL. Peptides were separated with a 0.3 mL/min, isocratic flow of 5 % B. MS tune parameters were as follows: capillary 2.00 kV, sample cone 35 V, extraction cone 4.0 V, source temperature 120 °C, desolvation temperature 500 °C, desolvation gas 500 L/h. Data were first collected in the full scan mode in the mass range of 300-1900. To identify acetylation/propionylation sites, data were acquired by the data-dependent acquisition (DDA) mode. For DDA parameters, 1 s MS survey scan in the m/z range of 300–1900 were followed by MS/MS scans of up to 3 ions, when intensity rose above 1500 counts per second. MS/MS was acquired in the m/z range of 100-1900, with a 2 s scan rate. MS/MS scan was switched to MS survey scan after 3 scans. Trap collision energy was set using charge state recognition, applying the default files for 1-4 charge states. Files containing MS/MS spectra were processed with Proteinlynx Global Server 2.4 software (Waters, Milford, MA) to identify PTM sites.

Comparative analysis of MOF substrate specificity on both acetylation and propionylation

20 μg cell lysate was incubated with 2 μM MOF and 50 μM carbon-14 labeled acetyl-CoA or propionyl-CoA for 3 hours at 30 °C. The reaction was quenched with the addition of 5x SDS-PAGE loading dye and the proteins were resolved on a 4-20% gradient SDS-PAGE gel (BioRad). The gel was dried in vacuum and exposed to a phosphorscreen for 72 hours. The autoradiograph was scanned with the GE storm 865 imager (GE Healthcare) and the in gel proteins were imaged with coomassie brilliant blue stain for protein loading control.

Study of in vivo MOF acyltransferase activity

Full-length MOF encoding sequence was inserted into Xbal and EcoRI sites of lentivirus vector FuCRW to generate the MOF-overexpression plasmid (36). Plasmid transfections were performed using Lipofectamine 3000 (ThermoFisher). Twenty-four hours before transfection, the HEK 293T cells were seeded at a density of 1x10^6 cells/well for six-well plate. The cells were transfected with 8 μg MOF-vector or vector plasmid as described in the manufacturer’s protocol and the transfected cells were maintained for 72 h before harvest. 20 mM sodium propionate was added into the medium 24 hours before the cellular proteins were extracted. Whole cell lysate and histones were extracted from both MOF-overexpressed and normal cells for the study of cellular protein acetylation and propionylation changing in response to the overexpression of MOF. The extracted proteins were subjected to western blot analyses.

For the MOF knockdown study, control siRNA and siRNAs targeting KAT8 (ON TARGET plus™ siRNA) were purchased from Dharmacon (Dharmacon, Lafayette, CO). siRNA transfections were performed using Lipofectamine 3000 (Invitrogen, Carlsbad, CA). HEK293T cells with ~80% confluence in 6-cm dish were transfected with 300 pmol siRNA (15 μl of 20 μM stock) and 30 μl Lipofectamine 3000
and maintained for 72 hr. Then the cellular proteins were extracted for western blot analyses.

**Proteomic profiling of MOF sub-propionylome**

The whole cell lysates and core histone proteins were extracted from the HEK293T cells treated with 20 mM deuterated sodium propionate with or without MOF overexpression. The extracted whole cell lysates or histones were precipitated using trichloroacetic acid. The resulting protein precipitate was washed twice with ice-cold acetone and digested with trypsin using a procedure described previously (37). To enrich the propionylated peptides, the tryptic digest in NETN buffer (100 mM NaCl, 1mM EDTA, 50 mM Tris-HCl, 0.5% NP40, pH 8.0) was incubated with pan anti-Kpr antibody (PTM-201, PTM Bio, Chicago, IL) that was immobilized to protein A agarose beads at 4 °C for 6 hours with gentle rotation. After incubation, the beads were washed 3 times with NETN buffer and twice with ddH$_2$O. The enriched peptides were eluted with 0.1% trifluoroacetic acid (TFA). The eluates were vacuum concentrated and the peptides were suspended in 0.1% trifluoroacetic acid (TFA). The eluted peptides were loaded directly onto a home-made capillary column (10 cm length, 75 μm internal diameter) packed with Reprosil C18 resin (3 μm particle size, 100 Å pore size, Reprosil) on an EASY-nLC™ 1000 System (Thermo Fisher Scientific, Waltham, MA). The binding peptides were eluted with a gradient of 5% to 90% HPLC buffer B (0.1% formic acid in 90% acetonitrile, v/v) in buffer A (0.1% formic acid in water, v/v) over 60 min at a flow rate of 200 nL/min. The eluted peptides were ionized and introduced into a Q-Exactive mass spectrometer (Thermo Fisher Scientific, Waltham, MA) using a nanospray source. Full MS scans were acquired over the range of m/z 300–1400 with a resolution of 70000, which was followed by data-dependent MS/MS fragmentation of the 15 most intense peaks with a resolution of 17500 at 27% normalized collision energy. For all the experiments, the dynamic exclusion time was set to 25 s. Peptides identification was performed with MaxQuant (v 1.3.0.5) against the UniProt Human protein database. Oxidation on methionine, protein N-terminal acetylation, lysine acetylation, lysine propionylation, lysine D5-propionylation, lysine mono-/di-/tri-methylation, and arginine mono-/di-methylation were set as variable modifications. Carbamidomethylation on cysteine was set as fixed modification. False discovery rate (FDR) thresholds for protein, peptide and modification site were specified at 0.01. Peptides with MaxQuant score below 40 or site localization probability below 0.75 were removed. In addition, all the identified peptides were manually verified.

**Cloning, expression and purification of recombinant MOF.**

The DNA fragment encoding the histone acetyltransferase domain of human MOF (residues 174-449) was sub-cloned into pET28a-LIC vector (GenBank ID: EF442785). Recombinant MOF was over-expressed in *E. coli* BL21 (DE3) codon plus RIL strain (Stratagene) as an N-terminal Hexa-His fusion protein at 15 °C in Terrific Broth (Sigma). The harvested cells were resuspended in 50 mM HEPES buffer, pH 7.4, supplemented with 500 mM NaCl, 5 mM imidazol, 2 mM β-mercaptoethanol, 5% glycerol, 0.1% CHAPS. The cells were lysed by passing through Microfluidizer (Microfluidics Corp.) at 20,000 psi. The lysate was loaded onto 10 ml Chelating Sepharose column (GE Healthcare) charged with Ni$^{2+}$. After washing the column with 20 mM HEPES buffer, pH 7.4, containing 500 mM NaCl, 50 mM imidazole, 5% glycerol, the protein was eluted with 20 mM HEPES, pH 7.4, 500 mM NaCl, 250 mM imidazole, 5%
glycerol. The eluted protein was loaded onto a Superdex 200 column (26x60) (GE Healthcare), equilibrated with 20 mM HEPES buffer, pH 7.4, containing 500 mM NaCl. The fractions containing recombinant MOF were combined and the protein was further purified to homogeneity by ion-exchange chromatography.

**Crystallization of MOF with propionyl-CoA and X-ray crystal structure determination**

Purified recombinant MOF protein (5 mg/mL) was mixed with propionyl CoA (Sigma) at 1:5 molar ratio of protein:compound and crystallized using hanging drop vapor diffusion method at 20 °C by mixing 1µl of the protein solution with 1 µl of the reservoir solution. MOF-propionyl CoA was crystallized in buffer containing 20% PEG 3350, 0.2 M sodium malonate, pH 5.0. Crystals were soaked in the corresponding mother liquor supplemented with 20% glycerol as cryoprotectant before freezing in liquid nitrogen.

X-ray diffraction data for MOF + propionyl CoA was collected at 100K on a Rigaku FR-E superbright X-ray generator. Data set was processed using the HKL-3000 suite (38). The structures of MOF—propionyl CoA complex is isomorphous with PDB entry 2GIV. REFMAC (39) was used for structure refinement. Graphics program COOT (40) was used for model building and visualization. MOLPROBITY (41) was used for structure validation.

**ACKNOWLEDGEMENTS**

This work is supported by the NSF grant 1507741 to YGZ. Y.Z. was supported by the NIH (GM105933, DK107868, and GM115961) and the Nancy and Leonard Florsheim Family Foundation. Z.H. is partly supported by a scholarship from Chinese Scholarship Council. The SGC is a registered charity (number 1097737) that receives funds from AbbVie, Bayer Pharma AG, Boehringer Ingelheim, Canada Foundation for Innovation, Eshelman Institute for Innovation, Genome Canada through Ontario Genomics Institute, Innovative Medicines Initiative (EU/EFPIA) [ULTRA-DD grant no. 115766], Janssen, Merck & Co., Novartis Pharma AG, Ontario Ministry of Economic Development and Innovation, Pfizer, São Paulo Research Foundation-FAPESP, Takeda, and the Wellcome Trust.

**CONFLICT OF INTERESTS**

The authors declare that they have no conflicts of interest with the contents of this article.

**AUTHOR CONTRIBUTIONS**

Z.H. and Y.G.Z. designed and performed the enzyme kinetic, western blot, and radiometric gel imaging experiments and gathered and analyzed all the data. X.Y. and M.G.B designed and performed the acyl-CoA quantification and peptide proteomic experiments. H.W., A.D., H.Z., P.J.B., and C.H.A. designed, performed, and analyzed the MOF-Propionyl-CoA co-crystallization experiment. Q.L. and H.C. created the MOF overexpressed and MOF knockdown 293T cells. S.K., H.H., and Y.Z. designed and performed the MOF propionylation proteomic profiling experiments. Z.H. and Y.G.Z. wrote the manuscript with the help of all the other authors.

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Figure 1. Dual enzymatic activity of eukaryotic KAT enzymes. The three major eukaryotic KAT families catalyze acetylation of lysine residues using acetyl-CoA as the acetyl donor. p300/CBP and GCN5/PCAF KATs have been reported to possess lysine propionyltransferase activity. In this study, we found that all the MYST KATs possess strong KPT activity, providing a holistic view of KATs as lysine propionyltransferase.
Figure 2. Quantification of acetyl- and propionyl-CoA abundance in 293T cells using LC-MS/MS.

A. LC-MS chromatograms of acetyl- and propionyl-CoA. The cellular acyl-CoA concentration was calculated based on the deuterated acyl-CoA molecules which serve as internal standards. Acetyl-CoA and propionyl-CoA were sufficiently separated with the retention time at 9.43 and 11.43 minute respectively; B. Summary of triplicate experiments. The cellular abundance of propionyl-CoA is about 12% of the abundance of acetyl-CoA.
Figure 3. Test of lysine propionyltransferase activity of the MYST KATs on cellular proteome with western blot analysis. HEK 293T cell lysate was incubated with individual KATs in the presence or absence of propionyl-CoA. Treatment of cell lysate with propionyl-CoA and MYST KATs, especially MOF and HBO1, induced strong lysine propionylation on multiple histone and non-histone proteins.
Figure 4. Study of MOF acetyl- and propionyl-transferase activity on recombinant histones and nucleosomes. Both recombinant free histones and reconstituted nucleosomes were used as substrates for MOF acetyl- and propionyl-transferase activity study. Histone proteins with acyl-CoA were incubated with or without MOF; the reaction mixtures were then subjected to western blot analysis with pan anti-acetyllysine and pan anti-propionyllysine antibodies. A. MOF acylates four free core histones; B. MOF acylates histone H2A/H2B and H4 on nucleosome.
Figure 5. Imaging of MOF in vitro acetylome and propionylome using radioactive gel assay. Carbon-14 labeled acetyl-CoA and propionyl-CoA were used for cell lysate acylation by MOF. Proteins were resolved on SDS-PAGE and the MOF acylome was imaged using a phosphor imager.
Figure 6. Detection of the cellular acetyltransferase and propionyltransferase activities of MOF. 
A. MOF was overexpressed in transfected 293T cells compared to the control cells where the vector plasmid was used for transfection; B. Histone lysine acylation level was tested with pan anti-acyllysine antibodies. MOF acylated histone H2A/H2B and H4; C. MOF overexpression induced increase of H4K16 propionylation; D. Acylation of the cellular proteome was tested with western blot. Increase of lysine acetylation and propionylation of multiple proteins were induced by MOF overexpression.
Figure 7. A comparison of lysine propionylation sites between the control and MOF overexpressed cells. **A.** The numbers of Kpr_D5 modified sites in histones and non-histone proteins were compared between the control and MOF overexpressed cells; **B.** The numbers of overlapped and non-overlapped Kpr_D5 sites between both the control and MOF-overexpressed cells.
Figure 8. X-ray crystal structure of MOF-propionyl CoA complex. A. Overall structure of MOF-propionyl-CoA. MOF structure is shown in cartoon model, with the N-terminal, central, and C-terminal domains colored in green, magenta and blue, respectively. The bound compounds are shown in sticks, with propionyl CoA colored in grey and Ac-CoA (from 2GIV) in yellow; B. Active site of MOF. Catalytic residues C316, E350 and auto-acetylated K274 are shown in stick model. Fo-Fc omit map of propionyl-CoA contoured at 2.5 sigma shows the density for the extra methyl group in propionyl CoA; C. Structural comparison of the binding sites for propionyl CoA (PDB: 5WCI) and Ac-CoA (PDB: 2GIV) in MOF. The two structures are superimposed, with MOF-propionyl CoA in pink and MOF-acetyl CoA in green. The MOF residues interacting with the compounds are shown in stick model. Dashed lines represent hydrogen bonds. The two extra interactions in MOF-propionyl CoA are circled.
Figure 9. Sequence alignment of MYST KATs. A. MOF Pro349 is a conservative amino acid residue through all the five eukaryotic MYST KATs; B. MOF Val314 is a conservative amino acid residue through all the five eukaryotic MYST KATs.
