SUPPLEMENTAL EXPERIMENTAL PROCEDURES FOR

Subunit Composition and Substrate Specificity of a MOF-containing Histone Acetyltransferase Distinct From the Male-Specific Lethal (MSL) Complex

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Materials—Anti-FLAG (M2) agarose (cat. no. A2220), anti-FLAG (M2) monoclonal antibody (cat. no. F1804), FLAG peptide, anti-HA agarose, HA peptide, and anti-human H4K16 (cat. no. H9164) antibody were purchased from Sigma. Anti-human H4K5 (cat. no. 07-327), anti-human H4K8 (cat. no. 07-328) and anti-human H4K12 (cat. no. 07-595) antibodies were from Millipore/Upstate. Anti-human MOF (MYST1) (cat. no. A300-992A) and anti-human H4 (cat. no. A300-646A) polyclonal antibodies were purchased from Bethyl Laboratories, Inc. Anti-human MSL1 and anti-human MSL3L1 antisera (1) were a kind gift from Edwin R. Smith (Stowers Institute for Medical Research).

Generation and Growth of Mammalian Cell Lines—Full-length cDNAs encoding the human FLJ20436 (accession number BC009746), KIAA1267 (accession number NM_015443), FLJ10081 (accession number BC063792), PHF20 (accession number NM_016436), WDR5 (accession number NM_017588), OGT1 (accession number NM_181672), and human MSL3L1 (accession number BC031210) proteins were obtained from the American Type Culture Collection (ATCC). Full length cDNA encoding human MSL1/LOC339287 (accession number XM_496217) was cloned by RT-PCR from total RNA from HEK293 cells. cDNAs were subcloned with N-terminal FLAG epitope tags into pcDNA5/FRT, and introduced into HEK293/FRT cells using the Invitrogen Flp-in system. cDNA encoding human MOF (MYST1, accession number BC037773, was stably expressed in HeLa S3 cells with an N-terminal FLAG tag. Parental and stably transformed HEK293/FRT and HeLa S3 cells were maintained in Dulbecco's modified Eagle's medium with 5% glucose and 10% fetal bovine serum. For large scale cultures, HEK293/FRT cells were grown in roller bottles with 5% glucose and 10% fetal bovine serum, and HeLa cells were grown in spinner culture in Joklik medium with 5% bovine calf serum.

Immunoaffinity purification of protein complexes from mammalian cells—Nuclear extracts were prepared from HEK293/FRT or HeLa S3 cells according to the method of Dignam *et al.* (2). FLAG-tagged proteins and their associated proteins were purified on anti-FLAG (M2) agarose beads as described (3).

Expression of recombinant proteins in Sf21 insect cells —cDNA encoding human MOF (accession number BC037773) was subcloned with an HA tag into pBacPAK8, and recombinant baculoviruses were generated. Sf21 cells were cultured at 27° C in Sf-900 II SFM (Invitrogen with 5% fetal calf serum. Sixty hours after infection, Sf21 cells were collected and resuspended in ice-cold buffer containing 40 mM Hepes-NaOH (pH 7.9), 300 mM NaCl, 0.5% Triton X100, 5 mM $MgCl_2$, 10% glycerol, and protease inhibitor cocktail (Sigma) and disrupted using a French press (Thermo Spectronic). The lysate was centrifuged at 400,000 *x g* for 1 h at 4°C, and the resulting supernatant was subjected to anti-HA agarose chromatography as described (3).

Mass Spectrometry— Identification of proteins was accomplished using a modification of the MudPIT procedure (4,5). The SEQUEST algorithm (6) was used to match tandem mass spectrometry

spectra to human peptides extracted from the NCBI NR database (61,430 human protein sequences, consisting of 37,742 human proteins as of March 4, 2008). Spectra/peptide matches were retained if they had a normalized difference in cross-correlation scores of at least 0.08 and minimum cross-correlation score of 1.8 for +1, 2.5 for +2, and 3.5 for +3 spectra and if the fully tryptic peptides were at least 7 amino acids long. To estimate relative protein levels, Normalized Spectral Abundance Factors (NSAFs) were calculated for each protein as described (7,8).

Preparation of histone octamers and polynucleosomes—To generate recombinant human histone octamers, human histone H2A, H2B, H3, and H4 were expressed in *E. coli*, purified, and reconstituted into octamers as described (3). To obtain polynucleosomes, a ~1500-bp DNA fragment was generated by PCR using biotinylated oligonucleotides as primers (forward: 5'-TAT CGA ATT CCT GCA GCC CGG GGG A -3'; reverse: 5'-AGG GCC GAG CGC AGA AGT GGT CCT-3') and the plasmid pBluescriptR as template. Purified recombinant histone octamers (3 µg) and DNA (150 ng) were used to reconstitute polynucleosomes as described (9). The final assembly reaction was mixed with 200 µl of avidin-coupled Dynabeads (Dynal Biotech) and incubated at room temperature for 3 hrs. The beads were washed with 0.6 M NaCl in 20 mM Hepes-NaOH, pH 7.5, 1 mM EDTA, 10 % glycerol, 0.5 mM DTT, and 0.5 mM PMSF and finally resuspended in 200 µl of the same buffer. Bead-bound polynucleosomes were stored at 4°C for a maximum of ~2-3 weeks.

Histone acetyltransferase (HAT) assay—HAT assays were performed essentially as described (10). 40 µl reaction mixtures contained 50 mM Tris-HCl (pH 8.0), 50 mM KCl, 0.1 mM EDTA, 1 mM dithiothreitol, 5% (v/v) glycerol, 1 mM phenylmethylsulfonyl fluoride, 10 mM sodium butyrate, either 0.5 µCi of [^{3}H]acetyl coenzyme A (3.4 Ci/mmol; Amersham Biosciences) or 12.5 µM cold acetyl coenzyme A (Sigma), and varying amounts of anti-FLAG agarose eluates prepared from the indicated cell lines. Substrates for HAT reactions were either ~3 µg of HeLa oligonucleosomes, ~0.5 µg *E.coli* expressed and purified core histones, or ~1.5 µg reconstituted recombinant polynucleosomes. After a 60 min incubation at 30°C, aliquots of reaction mixtures were subjected to 18% SDS-PAGE (29:1 acrylamide:bis) or 4-20% gradient gels (BioRad). Proteins were visualized by Coomassie R-250 Blue staining. Radioactively labeled proteins were visualized by autoradiography using EN^{3}HANCE (NEN Research Products), and modified residues on histone H4 were detected by Western blotting with acetylation-specific antibodies.

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