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LETTER TO THE EDITOR

Structural insight into the regulation of MOF in the male-specific lethal complex and the non-specific lethal complex

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Dear Editor,

Dosage compensation of the male X-chromosomal genes in *Drosophila* results from acetylation of histone H4 Lys16 (H4K16) along the male X-chromosome by the MSL (*m*ale-*s*pecific *l*ethal) complex [1]. The MSL complex comprises five proteins (MSL1, MSL2, MSL3, MLE and MOF), as well as two non-coding RNAs (roX1 and roX2). The enzymatic activity of histone acetyltransferase (HAT) MOF (*m*ales-absent *o*n the *f*irst) is tightly regulated by MSL1 and MSL3 [2-4]. MSL1 tethers MOF with the regulatory factor, the MRG domain of MSL3, through two adjacent regions at its C-terminus [4, 5]. Only in complex with MSL1 and MSL3, MOF is capable of specifically acetylating nucleosomal H4K16 [4, 5].

In addition to the MSL complex, MOF also resides in an NSL (*n*on*s*pecific *l*ethal) complex that plays an important role in genome-wide chromatin modification and transcriptional regulation [6, 7]. Besides MOF, the NSL complex includes six other components (NSL1, NSL2, NSL3, MCRS2, MBD-R2 and WDS). MOF is directly associated with an MSL1-like protein, NSL1 [7]. Compared with the MSL complex, although an MSL3-like factor is absent from the NSL complex, MOF and NSL1 alone are sufficient for acetylating nucleosomal H4K16 [6-8]. It remains unclear how the nucleosomal HAT activity of MOF is regulated in both complexes.

The two MOF-containing complexes have conserved mammalian orthologs, which are essential for genome-wide H4K16 acetylation [8, 9]. We found that human $MSL1_{473\text{-}520}$ and $NSL1_{885\text{-}934}$ are the minimal MOF-binding motifs (MBM) of MSL1 and NSL1, respectively (Figure 1A and Supplementary information, Figure S1A). Sequence alignment of $MSL1_{MBM}$ and $NSL1_{MBM}$ across species reveals two highly homologous regions, indicating that MSL1 and NSL1 might interact with MOF in similar manners (Figure 1B).

In the MSL complex, the nucleosomal HAT activity requires the MRG domain of MSL3 ($MSL3_{MRG}$) [10]. $MSL3_{MRG}$ shares substantial similarities with other MRG family proteins (Supplementary information, Figure S1C); $MSL3_{MRG}$ can be superimposed onto many other MRG domains with root-mean-square deviation values of less than 2.0 Å. Notwithstanding the high degree of overall structural conservation, the connecting loop regions have great variations in $MSL3_{MRG}$ compared with other MRG domains. For example, $MSL3_{MRG}$ has a long loop (~150 residues) between helices α4 and α5, whereas helices α4 and α5 of other MRG domains are connected by a short four-to-five-residue turn (Supplementary information, Figure S1C). Deletion of this loop greatly impaired the nucleosomal HAT activity of the MSL complex (Figure 1D), suggesting that it plays a crucial role in HAT activity regulation.

MSL1 utilizes two separate regions to bind to the HAT domain of MOF (MOF_{HAT}) and $MSL3_{MRG}$; MSL1 residues 550-591 (MSL3 binding motif; $MSL1_{M3BM}$) C-terminal to $MSL1_{MBM}$ mediate the interaction with $MSL3_{MRG}$ (Figure 1A) [4, 10]. $MSL1_{473\text{-}591}$ that includes both $MSL1_{MBM}$ and $MSL1_{M3BM}$ associates with MOF_{HAT} and $MSL3_{MRG}$ simultaneously to form a stable ternary complex that is capable of acetylating nucleosomal substrates (Figure 1C and Supplementary information, Figure S1B). Notably, a hybrid molecule, in which $MSL3_{MRG}$ was directly connected to the C-terminus of $MSL1_{MBM}$ by an 18-amino-acid linker – $(GlyGlySer)_6$, was sufficient to stimulate the activity of MOF_{HAT} (Figure 1E), suggesting that in the MSL complex MSL1 likely functions as a scaffold to tether $MSL3_{MRG}$ and MOF_{HAT} together for optimal enzymatic activity regulation.

Our previous studies indicated that NSL1 alone is sufficient to support the HAT activity of MOF on nucleosomal substrates [8]. Further mapping narrowed the necessary region of NSL1 down to a short fragment ($NSL1_{850\text{-}932}$) that only contains $NSL1_{MBM}$ and 35 amino acids immediately N-terminal to $NSL1_{MBM}$ (Figure 1F). As $NSL1_{MBM}$ itself did not activate the nucleosomal HAT activity of MOF_{HAT} (Figure 1F), the observed stimula-

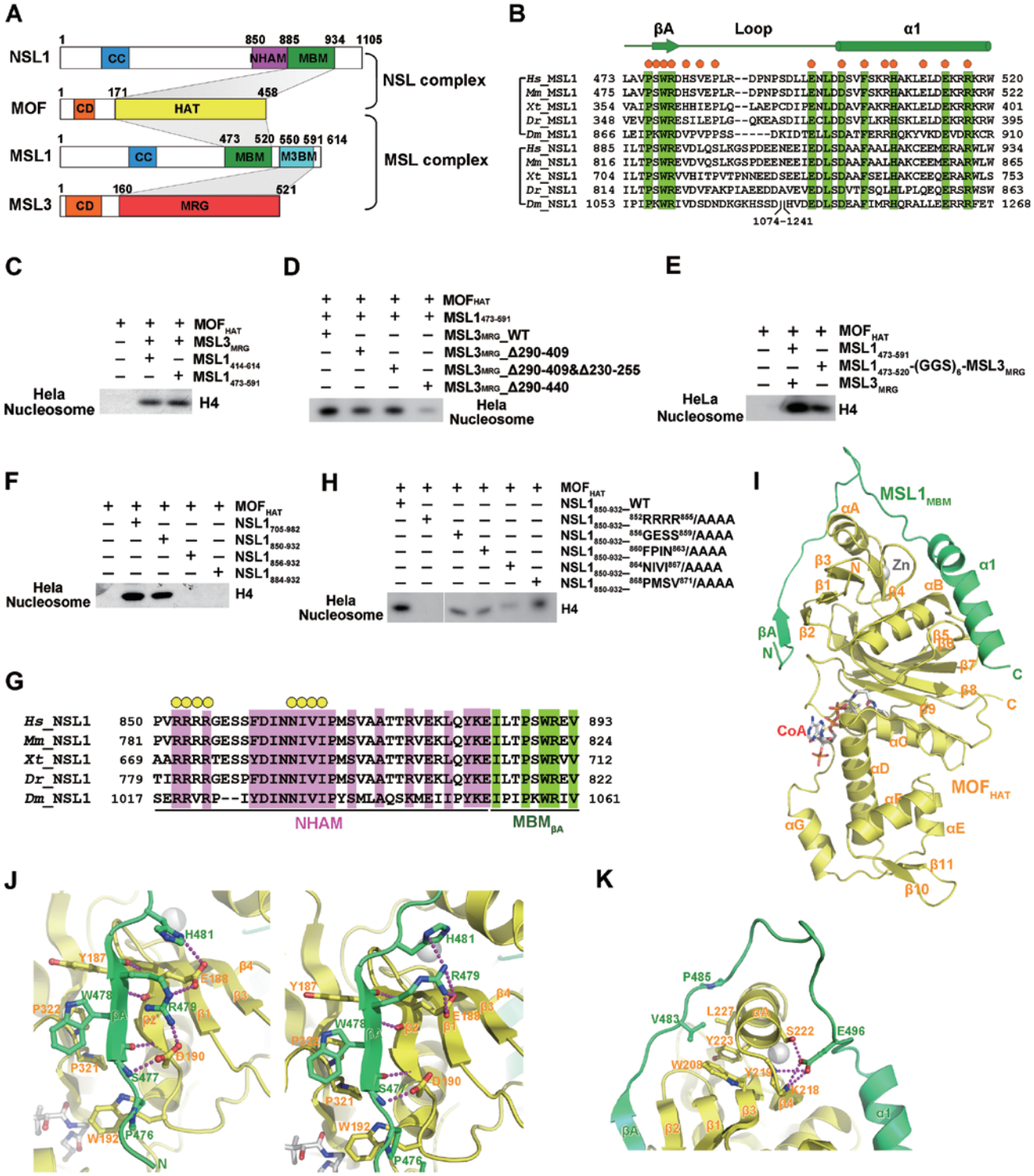

Figure 1 Structural and functional analysis of the MSL and NSL complexes. **(A)** Domain organization of the MSL and NSL complexes. Numerals indicate residue numbers at the boundaries of subdivisions. Protein interactions are indicated with gray-shaded areas. CC, coiled coil; NHAM, nucleosomal HAT activation motif; MBM, MOF-binding motif; CD, chromodomain; M3BM, MSL3-binding motif. **(B)** Sequence alignment of $MSL1_{MBM}$ and $NSL1_{MBM}$ across species. Structure-based secondary structure assignments are shown as cylinders (α helices) and arrows (β strands). Conserved residues are highlighted in green and the MOF-interacting residues are denoted with orange circles. **(C)** *In vitro* HAT assays were performed with MOF_{HAT} and the MSL complexes composed of MOF_{HAT}, $MSL3_{MRG}$ and different MSL1 fragments. **(D)** *In vitro* HAT assays were performed with the MSL complexes composed of MOF_{HAT}, $MSL1_{473-591}$ and several deletion mutants of $MSL3_{MRG}$. **(E)** *In vitro*

tory effect of $NSL1_{850-932}$ likely resulted from $NSL1_{850-884}$. Hereafter, we will refer to $NSL1_{850-884}$ as the *n*ucleosomal *H*AT *a*ctivation *m*otif of NSL1 ($NSL1_{NHAM}$) (Figure 1A and 1G). Mutagenesis analysis of $NSL1_{NHAM}$ revealed that substitution of the four N-terminal arginine residues with alanines completely abolished the HAT activity of the NSL complex on HeLa nucleosomes. This result is consistent with the observation that the MOF_{HAT}–$NSL1_{856-932}$ complex does not possess nucleosomal HAT activity (Figure 1F and 1H). In addition, alanine substitution of resides $_{864}NIVI_{867}$ in $NSL1_{NHAM}$ also led to decreased activities (Figure 1H). Notably, both the arginine residues and $_{864}NIVI_{867}$ are highly conserved across species (Figure 1G), consistent with their crucial roles in MOF activation.

To examine the structural basis of how the nucleosomal HAT activity of MOF is regulated in the MSL and NSL complexes, we determined the crystal structure of MOF_{HAT} in complex with $MSL1_{MBM}$ at a resolution of 2.05 Å (Supplementary information, Table S1). The formation of the MOF_{HAT}–$MSL1_{MBM}$ complex involves an extensive set of interactions and causes the burial of 1,580 $Å^2$ of surface area at the interface. $MSL1_{MBM}$ adopts an extended conformation and surrounds the N-terminal half of MOF_{HAT}, with both termini close to the central catalytic site of MOF_{HAT} (Figure 1I). Comparison with the MOF_{HAT} structure complexed with cofactor CoA (PDB: 2PQ8) suggests that the MOF_{HAT}–$MSL1_{MBM}$ interaction does not interfere with MOF_{HAT} binding to CoA (Figure 1I). $MSL1_{MBM}$ can be divided into three binding modules. From the N- to the C-terminus, $MSL1_{MBM}$ contains a β strand (residues 477-480), an extended region (residues 481-499), and an α helix (residues 500-520) (Figure 1I). Both the β-strand and α-helix modules form extensive contacts with MOF_{HAT}. In contrast, the middle loop region makes limited contribution to the interaction (Figure 1I). This is consistent with the observation that both the length and the sequence of the loop region are less conserved than the β strand and α helix modules (Figure 1B). Of note, *Drosophila* $NSL1_{MBM}$ contains a large 168-residue loop region (Figure 1B), explaining the failure to detect MBM in *dm*NSL1 by bioinformatics [10].

The interface between MOF_{HAT} and the α-helix module of $MSL1_{MBM}$ is almost identical to that of a recently published crystal structure of the MOF-MSL1 complex [10] (Supplementary information, Figure S2). However, although a similar MSL1 construct was used in both studies, the N-terminal conformation of $MSL1_{MBM}$ is completely absent in their structure, possibly resulting from crystal packing effect (Supplementary information, Figure S3). Our structure clearly demonstrates that the N-terminus of $MSL1_{MBM}$ forms an antiparallel intermolecular β-sheet with strand β2 of MOF_{HAT}, resulting in a twisted five-stranded β sheet extending over both molecules (Figure 1J). In addition to these main-chain contacts, the intermolecular β sheet is further stabilized by electrostatic contacts, as well as van der Waals interactions (Figure 1J). On one side of the β sheet, the side chains of $MSL1_{MBM}$Arg479 and His481 adopt two different conformations within an asymmetric unit, but both participate in hydrogen-bonding interactions with the side chain of MOF_{HAT}Glu188. Another hydrogen bond is formed between the side chain of MOF_{HAT}Asp190 and the backbone of Ser477 from $MSL1_{MBM}$ (Figure 1J). In contrast, the interaction between MOF_{HAT} and $MSL1_{MBM}$ is highly hydrophobic on the other side of the intermolecular β sheet. The aromatic side chain of $MSL1_{MBM}$Trp478 sits snugly on a hydrophobic surface formed by Tyr187, Pro321, and Pro322 of MOF_{HAT}, while $MSL1_{MBM}$Pro476 packs against the side chain of MOF_{HAT} Trp192 (Figure 1J). Deletion mutant of the β-strand of $MSL1_{MBM}$ failed to form a stable MSL complex (Supplementary information, Figure S4A), underscoring the importance of the N-terminal interaction of $MSL1_{MBM}$ with MOF. As residues 888-892 of $NSL1_{MBM}$ are almost identical to residues 476-480 of $MSL1_{MBM}$ (Figure 1B), the same interface should also mediate the MOF-NSL1 interaction. Indeed, NSL1 with a polyalanine substitution for the N-terminal

HAT assays were performed with MOF_{HAT} in complex with a hybrid protein, $MSL1_{473-520}$-$(GGS)_6$-$MSL3_{MRG}$. **(F)** *In vitro* HAT assays were performed with the NSL complexes composed of MOF_{HAT} and different NSL1 fragments. **(G)** Sequence alignment of the NHAM motif of NSL1 across species. Conserved residues of $NSL1_{NHAM}$ and $NSL1_{MBM}$ are highlighted in magenta and green, respectively. Yellow circles denote the $NSL1_{NHAM}$ residues that are crucial for activating the nucleosomal HAT activity of MOF. **(H)** *In vitro* HAT assays were performed with the NSL complex comprising MOF_{HAT} and different alanine-substituted mutant $NSL1_{850-932}$ fragments. **(I)** Overall structure of the MOF_{HAT}-$MSL1_{MBM}$ complex. MOF_{HAT} and $MSL1_{MBM}$ are colored in yellow and green, respectively. CoA is superposed from the MOF_{HAT}-CoA structure (PDB: 2PQ8). **(J)** Detailed interactions between MOF_{HAT} and the β-strand module of $MSL1_{MBM}$. Two slightly different conformations within an asymmetric unit are shown separately. Interacting residues of $MSL1_{MBM}$ and MOF_{HAT} are shown in ball-and-stick models. Hydrogen-bonding interactions are denoted with magenta dashed lines. **(K)** The middle loop region of $MSL1_{MBM}$ makes less contribution to the MOF_{HAT}-$MSL1_{MBM}$ interaction. Interacting residues are shown in ball-and-stick models, and hydrogen-bonding interactions are denoted with magenta dashed lines.

β-strand cannot form a stable complex with MOF_{HAT} (Supplementary information, Figure S4B).

The middle extended region of $MSL1_{MBM}$ surrounds the zinc finger motif of MOF_{HAT} with few direct contacts. Val483 and Pro485 of MSL1 packs on a hydrophobic patch on one side of the MOF_{HAT} αA helix (Figure 1K). On the other side, the side chain of Glu496 forms hydrogen-bonding interactions with Ser222, Lys218 and Tyr219 of MOF_{HAT} (Figure 1K). This region of $MSL1_{MBM}$ is highly variable across species in both sequence and length (Figure 1B), mostly likely serving as a flexible linker between the β-strand and α-helix modules of $MSL1_{MBM}$.

From our structural and functional analyses, it is clear that $NSL1_{NHAM}$ and $MSL3_{MRG}$ are functional counterparts in the two MOF-containing complexes. Notably, the tie-like conformation of the MOF-binding motif suggests that $NSL1_{NHAM}$ and $MSL3_{MRG}$ likely are placed close to the active site of MOF_{HAT} from opposite directions by the N-terminal β strand or the C-terminal α helix, respectively. Although the detailed mechanisms of the activation of MOF_{HAT} on nucleosomal substrates by $NSL1_{NHAM}$ and $MSL3_{MRG}$ remain to be explored, our results presented here provide a basic structural insight into the regulation of MOF in the MSL and NSL complexes.

Accession numbers

Atomic coordinates and structure factors have been deposited with the Protein Data Bank accession code 4DNC.

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Jing Huang[1, 2], Bingbing Wan[1, 2], Lipeng Wu[3], Yuting Yang[2], Yali Dou[2, 3], Ming Lei[1, 2]

[1]Howard Hughes Medical Institute, University of Michigan Medical School, 1150 W. Medical Center Drive, Ann Arbor, MI 48109, USA, [2]Department of Biological Chemistry, University of Michigan Medical School, 1150 W. Medical Center Drive, Ann Arbor, MI 48109, USA, [3]Department of Pathology, University of Michigan Medical School, 1150 W. Medical Center Drive, Ann Arbor, MI 48109, USA

Correspondence: Ming Lei
Tel: +1-734-647-5839
E-mail: leim@umich.edu

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