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The MOF Chromobarrel Domain Controls Genome-wide H4K16 Acetylation and Spreading of the MSL Complex

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SUMMARY

The histone H4 lysine 16 (H4K16)-specific acetyltransferase MOF is part of two distinct complexes involved in X chromosome dosage compensation and autosomal transcription regulation. Here we show that the MOF chromobarrel domain is essential for H4K16 acetylation throughout the *Drosophila* genome and is required for spreading of the male-specific lethal (MSL) complex on the X chromosome. The MOF chromobarrel domain directly interacts with nucleic acids and potentiates MOF's enzymatic activity after chromatin binding, making it a unique example of a chromo-like domain directly controlling acetylation activity in vivo. We also show that the *Drosophila*-specific N terminus of MOF has evolved to perform sex-specific functions. It modulates nucleosome binding and HAT activity and controls MSL complex assembly, thus regulating MOF function in dosage compensation. We propose that MOF has been especially tailored to achieve tight regulation of its enzymatic activity and enable its dual role on X and autosomes.

INTRODUCTION

Covalent modifications of histone tails modify chromatin structure to control transcription in response to environmental cues and developmental programs. Acetylation of lysine 16 on histone H4 (H4K16ac) has the potential to create or obscure binding platforms for chromatin-modifying enzymes and transcriptional activators (Ruthenburg et al., 2011; Zippo et al., 2009). Furthermore, H4K16ac can directly impact on higher order chromatin structure by physically preventing formation of the 30 nm chromatin fiber, thus creating an open, highly accessible chromatin environment (Bell et al., 2010; Shogren-Knaak et al., 2006). Accordingly, H4K16ac is involved in a variety of chromatin-related processes, such as replication timing and transcription (Akhtar and Becker, 2000; Bell et al., 2010).

Global H4K16ac is also hallmark of the dosage compensated male X chromosome in *Drosophila*, where the resulting permissive chromatin structure facilitates spreading of the dosage compensation complex (DCC), also known as the male-specific lethal (MSL) complex, and contributes to the 2-fold transcriptional activation of X-linked genes (Straub and Becker, 2007). Hyperacetylation of the X chromosome is mediated by *Drosophila* MOF, which resides in the MSL complex together with at least four other proteins, male-specific lethal 1–3 (MSL 1–3) and maleless (MLE), as well as two noncoding RNAs on the X (roX1/2). In addition to its role in dosage compensation, MOF has recently been found at hundreds of active promoters across the whole genome in male and female flies, where it is bound as part of the nonspecific lethal (NSL) complex (Kind et al., 2008; Mendjan et al., 2006; Raja et al., 2010). Whereas several studies have found H4K16ac at the 5′end of genes in *Drosophila* (Bell et al., 2007; Kind et al., 2008; Schwaiger et al., 2009), it has remained unclear if MOF is acting as a histone acetyltransferase (HAT) at these sites (Gelbart et al., 2009). Furthermore, it is not known how MOF targeting and activity are differentially regulated and distributed between the NSL complex and the MSL complex.

A common feature of enzymes from the Moz, YBF2, Sas2p, and Tip (MYST) family of HATs, such as *Drosophila* and human MOF, is the presence of a chromo-like "chromobarrel" domain adjacent to the enzymatic part of the protein (Sanjuán and Marín, 2001). Chromodomains are well-known targeting modules that bind to methylated lysine residues. However, chromobarrel domains of the MOF type lack the aromatic cage necessary for methyl lysine binding (Nielsen et al., 2005). Instead, the MOF chromobarrel domain is required for MOF binding to roX RNAs in vitro and in vivo (Akhtar et al., 2000). Surprisingly, however, no biological function has yet been assigned to the MOF chromobarrel domain, and evidence for a direct interaction of the chromobarrel domain with nucleic acids has been missing. In addition to the conserved globular domains, the MOF protein in *Drosophila* species furthermore contains a large, unstructured N-terminal region of unknown function, which is absent in organisms with different dosage compensation systems.

In this study, we show that *Drosophila* MOF is the major H4K16ac-specific HAT across the male and female genome. Strikingly, disruption of the MOF chromobarrel domain leads to

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a genome-wide loss of H4K16ac and compromised MSL targeting to X-linked genes. We find that the MOF chromobarrel domain interacts with nucleic acids (RNA and DNA) and that disruption of its DNA binding capacity abrogates H4K16ac, despite MOF binding to chromatin in vitro and in vivo. We thus reveal the biological role of the chromobarrel domain, which acts as an accessory module to specifically elicit the enzymatic capacity of its associated HAT enzyme toward a nucleosomal substrate. Furthermore, we discovered that the *Drosophila*-specific N-terminal half of the MOF protein controls assembly of the MSL complex on the male X chromosome and regulates MOF's substrate binding and HAT activity. Multiple levels of control therefore reflect the enhanced complexity of MOF functions in flies and the resulting need for increased context dependent regulation of MOF activity.

RESULTS

MOF Is Required for Genome-wide H4K16ac in Both Sexes

Before investigating the in vivo functions of the chromobarrel domain and *Drosophila* specific N terminus of MOF, we wanted to determine the extent and biological significance of MOF activity outside the context of the male X chromosome. To this end, we used antibodies against MOF and H4K16ac in chromatin immunoprecipitations followed by ChIP-sequencing (ChIP-seq) from male and female third-instar larva salivary glands (Table S1, available online). As expected, analysis of the resulting high resolution profiles revealed MOF binding to the entire transcribed regions of male X-linked genes, with peaks of increased binding at the promoter and the 3′-end, whereas on autosomes and generally in females MOF binds only at gene promoters (Figures S1A and S1B). The pattern of H4K16 acetylation closely follows that of MOF binding. Interestingly, outside the context of the male X chromosome, on which H4K16ac is broadly spread around sites of MSL binding (Gelbart et al., 2009), we observe a shift of the H4K16ac signal downstream of gene promoters, most likely reflecting acetylation of the first nucleosome downstream of the transcription start site (TSS). Strikingly, the genome-wide ChIP-seq approach revealed more than three quarters of all active genes as MOF targets on X and autosomes in both sexes (Table S2), signifying the role of MOF for genome-wide gene regulation. Furthermore, most MOF-bound genes were acetylated at H4K16 at the same time in both sexes (Figure S1D), suggesting that MOF is functioning as a HAT also on autosomes. Finally, we found an extremely high overlap of MOF target genes between males and females, especially on autosomes, suggesting that outside the context of the MSL complex MOF functions very similar in male and female flies (Figure S1C). These results confirmed our previous analysis but, because of the increased depth and sensitivity of the ChIP-sequencing method, also revealed that we had substantially underestimated the number of MOF targets (Figure S1E) (Kind et al., 2008).

We were next interested to test if MOF is active as a HAT also outside the context of the male X chromosome. For this purpose, we performed immunostainings with antibodies against MOF, MSL1, and H4K16ac on male and female third-instar larva polytene chromosomes from wild-type (WT) and *mof²* mutant flies that carry a premature stop codon and lack a functional MOF protein (Gu et al., 1998) (Figure 1A). Whereas the male X chromosome, marked by MSL1 staining, appeared enriched for H4K16ac in WT flies, we also detected widespread acetylation across autosomes, as well as the chromocenter (Figure 1B). Likewise, female samples showed widespread H4K16ac but no apparent enrichment of the mark on the X chromosomes, which also lacked MSL1 staining. Strikingly, H4K16ac was entirely lost from all chromosomes in the absence of MOF in male and female *mof²* flies. In the males, this was accompanied by reduced staining of MSL1 on the X chromosome in a pattern most likely corresponding to the previously described high-affinity sites (HAS) and by a delocalization of MSL1 staining to autosomal sites. Importantly, H4K16ac was restored on all chromosomes in both sexes upon expression of an HA-tagged MOF full-length transgene (FL-MOF) in flies of the *mof²* background. We recapitulated the same global MOF dependency of H4K16ac by western blots using extracts prepared from third-instar larvae (Figure 1C). Female WT flies showed about half the amount of global H4K16ac compared to WT males, reflecting the presence of the hyperacetylated male X chromosome. In the absence of endogenous MOF in the *mof²* background, H4K16ac levels were drastically reduced in males and females. Some residual H4K16ac signal was observed in *mof²* females, potentially mediated by maternally contributed MOF present at earlier larval stages. In *mof²* males the H4K16ac signal was below the detection limit. This difference might be explained by slightly reduced sample loading compared to the female sample, as judged by tubulin and H4 signals. Importantly, H4K16ac was again globally restored in both sexes upon expression of FL-MOF. These data clearly demonstrate that MOF is responsible for genome-wide H4K16ac in *Drosophila*.

Since MOF has been identified in a screen for male-specific lethality, the obvious question arises: what is the significance of MOF-mediated H4K16ac for female flies? To address this question we generated homozygous *mof²* females. When compared to control flies, the number of *mof²* females reaching adulthood was reduced by approximately 2-fold, and these flies were mostly sterile (Figure 1C). More strikingly, however, the average lifespan of *mof²* mutant female flies was reduced to an average of 8 days as compared to 37 days in the control flies (Figure 1D). Furthermore, this reduction in lifespan was rescued efficiently by expression of a MOF transgene. This result strongly suggests that MOF-mediated H4K16ac is essential for female survival, although it remains possible that additional MOF functions or a structural role of the MOF protein itself may contribute to this phenotype.

The MOF N Terminus and Chromobarrel Domain Are Required for Male Viability

Having established the general requirement of MOF for genome-wide H4K16ac in male and female flies, we investigated how the MOF chromobarrel domain and N terminus contribute to this activity. Two-point mutations in the MOF chromobarrel domain at Tyr416 (Y416) and Trp426 (W426) were previously shown to disrupt MOF's interaction with roX RNA (Akhtar et al., 2000), and the corresponding residues have subsequently been shown to be required for nucleic acid binding of Esa1, a closely related HAT in yeast (Shimojo et al., 2008). To test if the chromobarrel

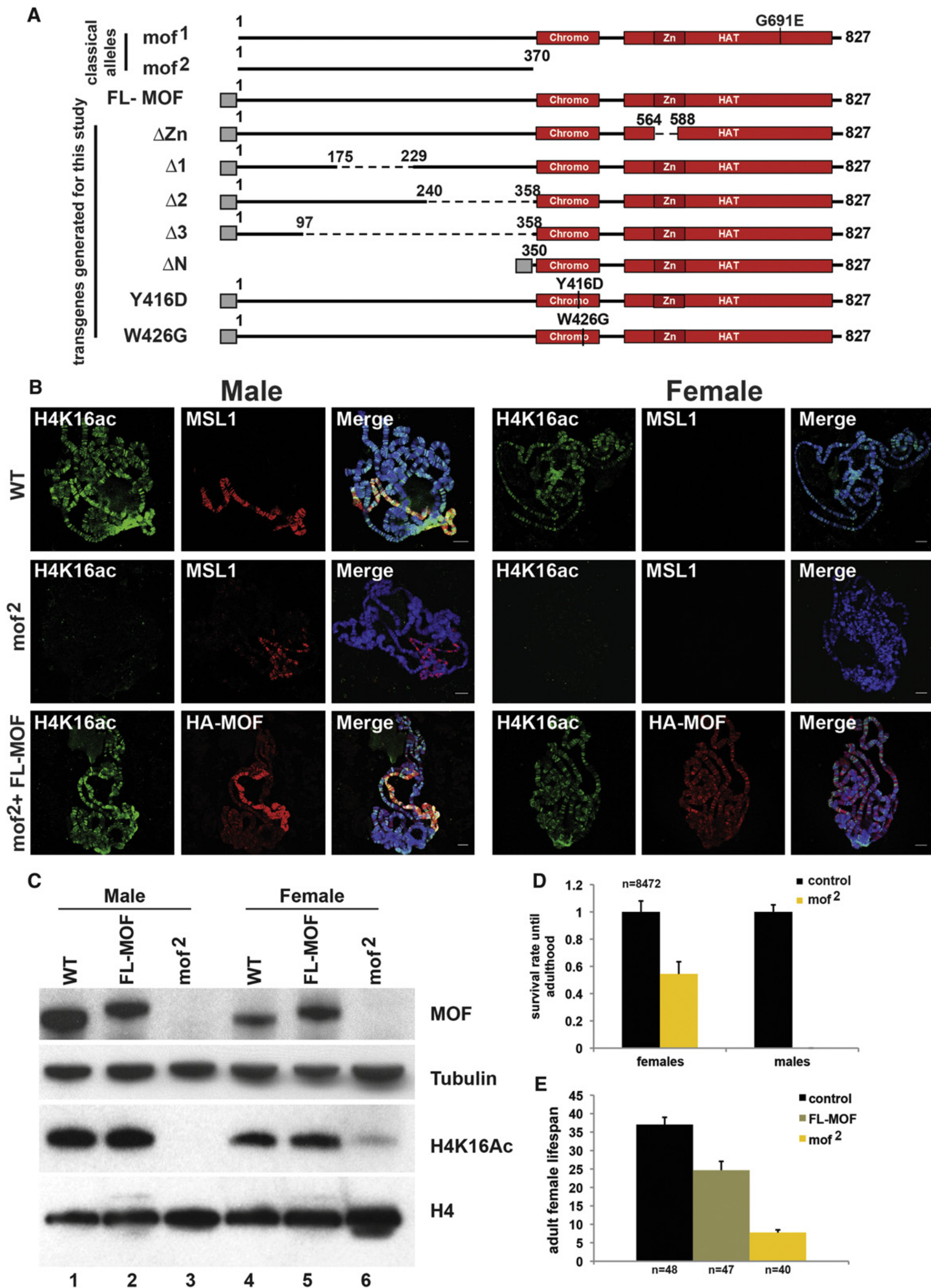

Figure 1. MOF Is the Major H4K16-Specific HAT in the Male and Female Genome

(A) Schematic representation of the domain structure of the known MOF alleles *mof*[1] and *mof*[2], as well as the MOF derivatives generated for this study. Globular domains in the MOF protein are the chromobarrel domain (chromo) and the C-terminal HAT domain (HAT), which contains a zinc finger (Zn). G691 is located in the catalytic center of the MOF HAT domain. Y416D and W426G reside in the chromobarrel domain and disrupt nucleic acid binding. Grey boxes indicate the HA tag.

(B) H4K16ac is lost from all chromosomes in the absence of MOF. Immunostaining of polytene chromosomes from male and female third-instar larva salivary glands, using antibodies against H4K16ac, MSL1, and HA (MOF) as indicated. DNA staining is shown in blue (Hoechst 322).

domain carries essential functions in vivo, we assayed the capability of corresponding mutant MOF derivatives to rescue the male lethal phenotype associated with the loss of endogenous MOF. To this end, we introduced HA-tagged MOF transgenes by p-element-mediated transformation into flies, including a control construct comprising full-length MOF (FL-MOF); a deletion of the zinc finger region, which has been shown to be required for MOF binding to chromatin (Akhtar and Becker, 2001) (ΔZn MOF [Δ565–587]); a series of deletions in the N-terminal half of the MOF protein (Δ1 [Δ 176–228], (Δ 2 [Δ 241–357], Δ 3 [Δ 98–357], and ΔN [Δ 1–349]); and two-point mutations in the MOF chromobarrel domain at Tyr416 and Trp426 (Y416D and W416G) (Figure 1A). All transgenes were expressed to WT levels upon induction with armadillo-Gal4 (Figures S2A–S2H). Full-length HA-tagged MOF completely rescued male lethality (99.7% survival) in this assay when compared to heterozygous *mof*^{2} females resulting from the same cross (Figure 2A), whereas males lacking the MOF zinc finger did not survive the third-instar larva stage, consistent with the crucial role of the zinc finger region for MOF function (Akhtar and Becker, 2001). Male viability was also increasingly compromised by up to 90% upon progressive deletions of the *Drosophila*-specific N-terminal region of the MOF protein, suggesting that vital functions reside in this region. Strikingly, disruption of the chromobarrel domain in Y416D and W426G MOF led to complete male lethality in this assay. This result clearly revealed the essential nature of the MOF chromobarrel domain and N terminus.

The MOF N Terminus Is Required for MSL Complex Assembly on the X Chromosome

Considering the importance of the chromobarrel domain and MOF N terminus for male viability, we were curious to assay for defects in MOF targeting to X-linked genes or autosomes and in MSL complex function. We performed immunostainings of third-instar larva polytene chromosomes from male flies that express MOF transgenes in the *mof*^{2} background. Upon immunostaining with anti-HA antibodies, FL-MOF appeared in a wild-type pattern, showing pronounced enrichment on the X chromosome but also clear targeting to all autosomes (Figure 2B). At the same time, MSL1 staining remained restricted to the X, all together reflecting wild-type MSL complex targeting and function. As expected, ΔZn MOF was no longer detectable on polytene chromosomes, whereas its nuclear localization remained unaffected at the same time, suggesting a general defect in chromatin binding (Figure 2C; Figure S2I) (Akhtar and Becker, 2001; Kadlec et al., 2011). Interestingly, upon disruption of the chromobarrel domain, Y416D and W426G MOF were still detected across all autosomes, whereas MSL spreading on the X chromosome appeared reduced compared to FL-MOF (Figure 2D and see below). Strikingly, however, in the absence of the MOF N terminus, preferential targeting of ΔN MOF to the male X chromosome was entirely lost. Also ΔN MOF staining at autosomal bands appeared reduced (Figure 2E). Furthermore, we detected ectopic binding of MOF to the chromocenter. At the same time MSL1 staining was reminiscent of the patterns that have been observed in the absence of both roX RNAs (Meller and Rattner, 2002), with a nearly complete delocalization of MSLs from the X chromosome, accompanied by severe ectopic binding to autosomal sites and the chromocenter. Indeed, when we measured the levels of roX RNAs in the ΔN MOF mutant background we found a severe depletion of roX2 by more than 98%, indicating that the N terminus of MOF is required for proper incorporation of roXs into the MSL complex (Figure S2J). This phenotype was surprising and suggested that the N terminus of the MOF protein in *Drosophila* species is required for X chromosome and MSL complex specific functions of MOF (Figure S2K). Consistent with retained MSL complex binding at multiple X chromosomal sites, we did not observe the same reduction in roX levels upon disruption of the chromobarrel domain (Figure S2J).

The Chromobarrel Domain Is Required for MSL Spreading on the X Chromosome

It has been observed previously that the resolution provided by polytene chromosome stainings is not sufficient to detect defects in MSL spreading from high-affinity sites (HAS) onto dosage compensated genes (Sural et al., 2008). Therefore, to get a more detailed insight into the MSL binding pattern upon disruption of the chromobarrel domain, we performed ChIP from male third-instar larva to monitor defects in MSL recruitment at higher resolution. Chromatin was immunoprecipitated using antibodies against MSL1 and MSL3. The recovered DNA was measured by quantitative real-time PCR (qPCR). To monitor MSL recruitment to the X chromosome, we used the known HAS at the *roX2* gene and an additional HAS at the cytological location 15A8, which had previously been identified in an MSL3 mutant background (Alekseyenko et al., 2008). We also included sites at the promoter, middle, and 3′end of three X-linked genes, *Rpl22*, *Klp3a*, and *Ucp4a*. The first one of these, *Rpl22*, previously showed some MSL binding in the absence of MSL3 and can thus be described as a medium-affinity site, whereas the remaining two genes are low-affinity MSL targets. As expected, MSL1 binding was retained on the *roX2* high-affinity site but was lost from the body of X-linked genes in the *mof*^{2} background. These defects were restored back to the WT pattern upon expression of FL-MOF but not ΔZn MOF (Figure 3A; Figures S3A and S3B). Interestingly, the 15A8 HAS, which had been identified as a high-affinity site in an MSL3 mutant background, was no longer bound by MSL1 in *mof*^{2}, demonstrating the qualitative differences among high-affinity sites in varying genetic backgrounds (Kadlec et al., 2011). Furthermore, MSL1 and MSL3 binding were lost from dosage compensated genes in the presence of ΔN MOF, confirming

(C) Western blot analysis of extracts prepared from WT, *mof*^{2}, and FL-MOF expressing male and female third-instar larva showing the effect of MOF depletion on H4K16ac.

(D) Number of eclosed *mof*^{2} adults compared to the control carrying a MOF transgene. Whereas males show full lethality, female number is reduced approximately 2-fold. Error bars represent the standard deviation from three independent experiments.

(E) Lifespan of control, *mof*^{2}, and MOF-expressing female flies after eclosion. Error bars represent the standard error of the mean. See also Figure S1 and Tables S1 and S2.

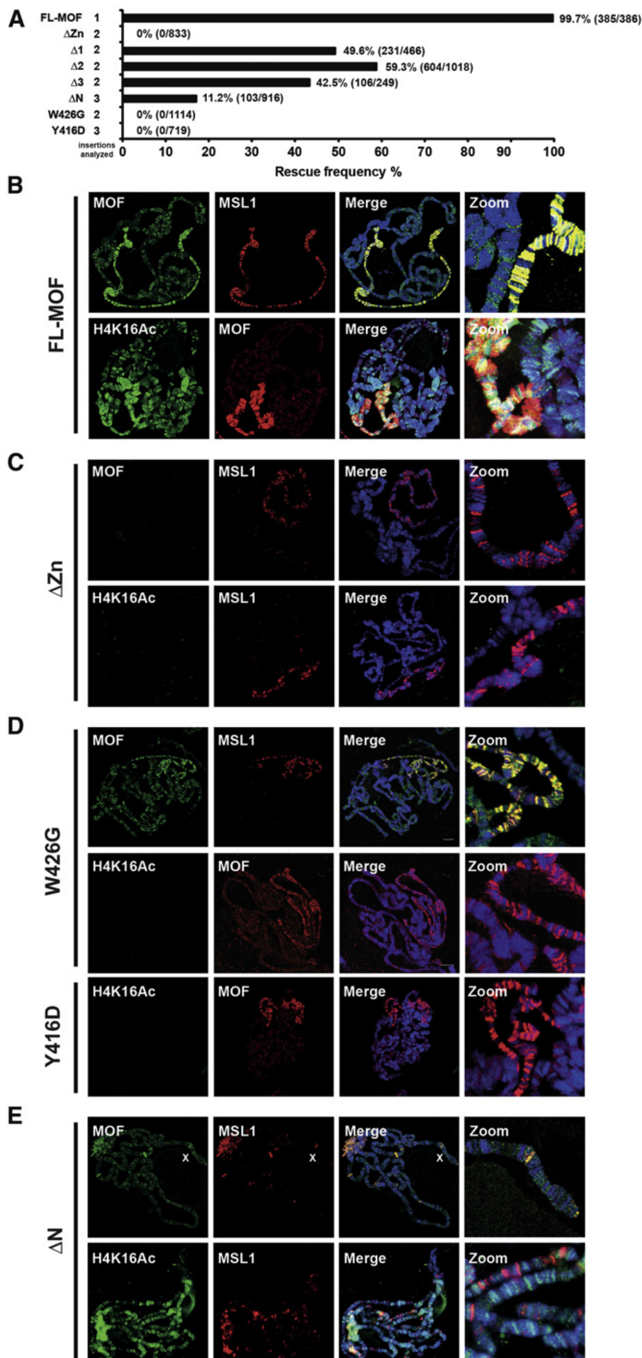

Figure 2. The MOF Chromobarrel Domain and N Terminus Control MOF Function

(A) Male lethality upon disruption of the chromobarrel domain and MOF N terminus. Male viability was assayed upon expression of various MOF transgenes in the *mof²* background as indicated. Percentages refer to the number of *mof²* males compared to the number of heterozygous *mof²* females resulting from the same cross.

(B–E) Immunostaining of polytene chromosomes from *mof²* male third-instar larvae salivary glands, expressing FL (B), ΔZn (C), Y416D and W416G (D), and ΔN MOF (E) transgenes. Antibodies against HA (MOF), MSL1, and H4K16ac were used as indicated in the figure. DNA staining is shown in blue (Hoechst 322). Despite substantial chromatin binding, H4K16ac is lost upon disruption of the MOF chromobarrel domain. Conversely, MSL targeting to the X chromosome is compromised in the presence of ΔN MOF. At the same time, enzymatically hyperactive ΔN MOF causes widespread hyperacetylation despite reduced chromatin binding. See also Figure S2.

immunostainings (Figure 3A). However, in contrast to the *mof²* background, where residual MSL1 and MSL2 containing complexes can bind to HAS, MSL1 binding was also lost from the roX2 HAS in ΔN MOF, most likely as a result of compromised MSL complex assembly.

Surprisingly, upon disruption of the chromobarrel domain in Y416D MOF, we found a dramatic reduction of MSL3 binding at X-linked target sites, particularly in the transcribed region of genes (Figure 3B). At the same time, MSL1 binding was restricted to HAS and gene promoters, reminiscent of the pattern observed in the *mof²* background when MOF is absent. This result was striking and suggested a crucial role of the MOF chromobarrel domain for MSL targeting into the transcribed region of dosage compensated genes, consistent with the male lethality associated with disruption of this domain.

The Chromobarrel Domain Is Required for Genome-wide H4K16ac

In addition to the consequences for MSL spreading on the X chromosome, we next wanted to more specifically address the role of the MOF chromobarrel domain and N terminus for MOF function itself. We therefore performed immunostainings of polytene chromosomes using anti-H4K16ac antibodies. The pattern of H4K16ac in the presence of FL-MOF corresponded to the one observed in the WT, showing widespread acetylation on autosomes and enriched signal on the male X chromosome (Figure 2B). Acetylation was lost genome-wide upon deletion of the zinc finger region in the MOF HAT domain, reflecting defective chromatin targeting of this mutant MOF protein (Akhtar and Becker, 2001) (Figure 2C). To our great surprise, however, we also saw a dramatic loss of the H4K16ac mark in the presence of both chromobarrel domain mutants, and indiscriminately across all chromosomes (Figure 2D). This was particularly striking since MOF binding to chromatin seemed to be much less affected. These data suggested a much more general role of the chromobarrel domain than we had anticipated, which seems to involve the activation of MOFs enzymatic capacity after its recruitment to chromatin, irrespective of the chromosomal context and thus affecting MOF in the NSL, as well as the MSL, complex. In contrast, we detected widespread H4K16ac on all chromosomes in the presence of the ΔN MOF protein, which contains a functional chromobarrel domain (Figure 2E). The observation that at the same time autosomal chromatin targeting of ΔN MOF appeared reduced in immunostainings thus suggested an increased HAT activity of ΔN MOF.

Considering the diverse effects on H4K16ac upon disruption of the MOF chromobarrel domain and N terminus, we wanted to assay for defects in MOF chromatin targeting at higher resolution. To this end, we performed ChIP from mutant MOF expressing male third-instar larva to monitor differences in MOF binding and H4K16ac. In addition to X-linked target sites, we also assayed the promoter regions of the autosomal genes *cg6729*, *cg6884*, *cg31866*, *cg2708*, and *cg7638* and at two

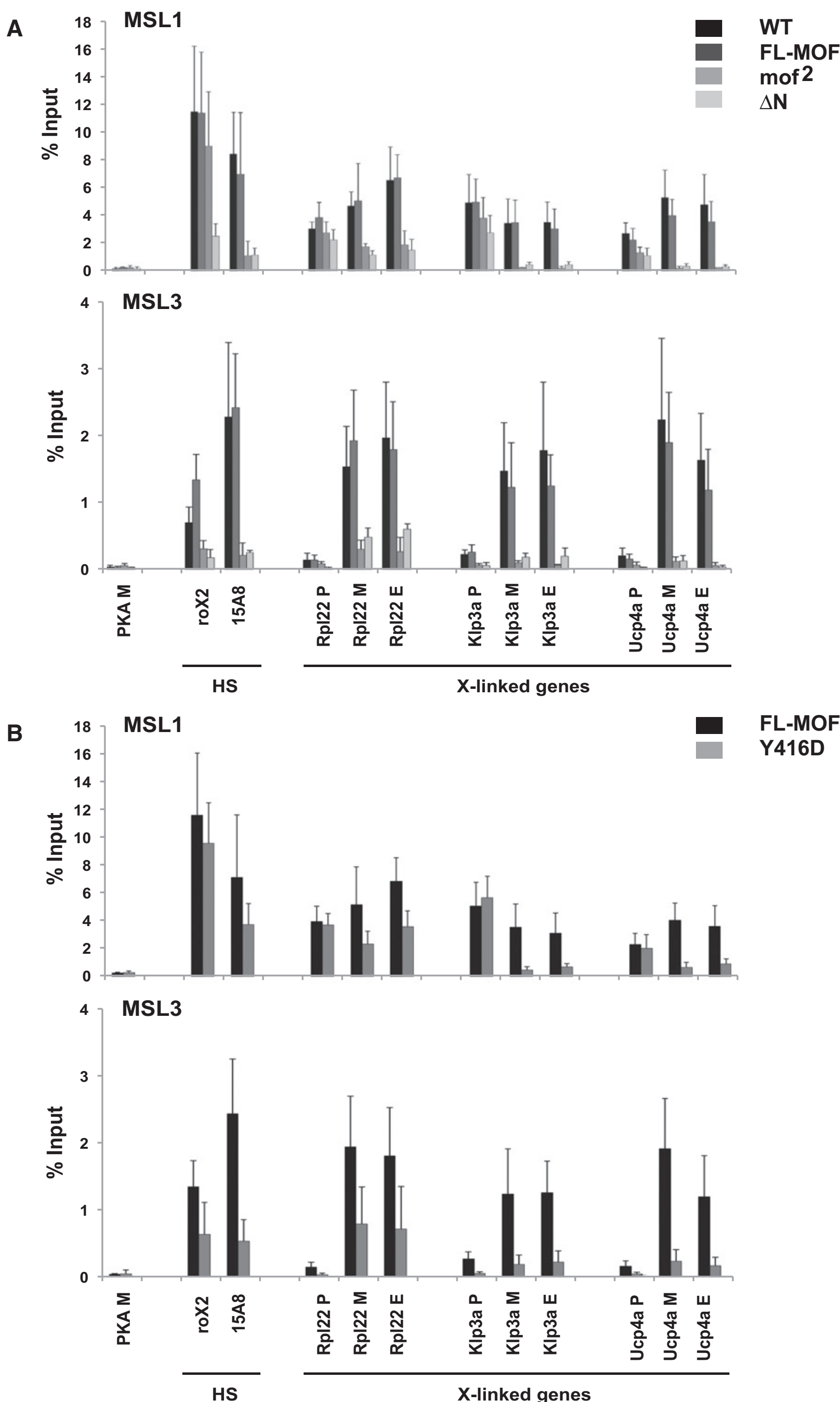

Figure 3. The MOF Chromobarrel Domain Is Required for MSL Spreading

(A) MSL targeting is compromised without the MOF N terminus. ChIP using MSL1 and MSL3 antibodies in WT male third-instar larva as well as *mof*^{2} larva, or *mof*^{2} larva that express FL-MOF or ΔN MOF transgenes. Binding to the X-linked high-affinity sites at the *roX2* gene and location 15A8, as well as the X chromosomal medium-affinity gene *Rpl22*, and low-affinity genes *Klp3a* and *Ucp4a* are shown. Protein kinase A is used as a negative control. Primers were positioned at the promoter (P), middle (M), and end (E) of genes. The exact position of the primers is described in the Supplemental Experimental Procedures. ChIP is shown as percentage recovery of input DNA (% Input). Error bars represent standard deviation (StDev) of three independent experiments.

(B) MSL spreading is lost upon disruption of the chromobarrel domain. Same as in (A), using *mof*^{2} larvae that express FL-MOF or Y416D MOF, respectively.

See also Figures S3 and S4.

nontargets, *bt* and *cg3937*. The pattern of FL-MOF binding in the *mof*^{2} background was indistinguishable from the one observed for endogenous MOF in the WT, with clear binding to autosomal promoters, to the promoter and transcribed region of X-linked genes, and to HAS (Figure 4A). MOF binding was accompanied by H4K16ac at all of these loci in the WT and FL-MOF backgrounds (Figure 4B). Upon removal of endogenous MOF in the *mof*^{2} background alone, MOF binding and H4K16ac were both lost from all sites tested, signifying the general role for MOF also for autosomal gene regulation. Consistent with immunostainings, ΔZn MOF was unable to rescue this phenotype (Figure S3C). The binding pattern of ΔN MOF suggested a general defect in chromatin targeting, showing a pronounced loss from X-linked target sites and a substantial reduction in promoter binding (Figure 4A). At the same time, however, H4K16ac appeared much less affected on the same target sites (Figure 4B). Surprisingly, at target promoters, where ΔN MOF binding was about 2-fold reduced, H4K16ac remained nearly at the levels observed in the presence of FL-MOF. This result was consistent with immunostainings in the presence of ΔN MOF, where H4K16ac appeared widespread throughout the genome and suggested a potentially enhanced acetylation activity of the truncated enzyme.

Disruption of the chromobarrel domain had diverse and contrasting effects on MOF targeting. The Y416D mutant MOF protein showed no difference in binding to gene promoters and the *roX2* HAS (Figure 4C). However, binding to the 15A8 HAS and the transcribed region of *Rpl22* was 2-fold reduced, whereas targeting to the transcribed region of the low-affinity genes *Klp3a* and *Ucp4a* was completely lost. These data indicated a specific defect in chromatin targeting of Y416D MOF as part of the MSL complex, whereas MSL complex independent binding to gene promoters appeared unaffected. We next analyzed

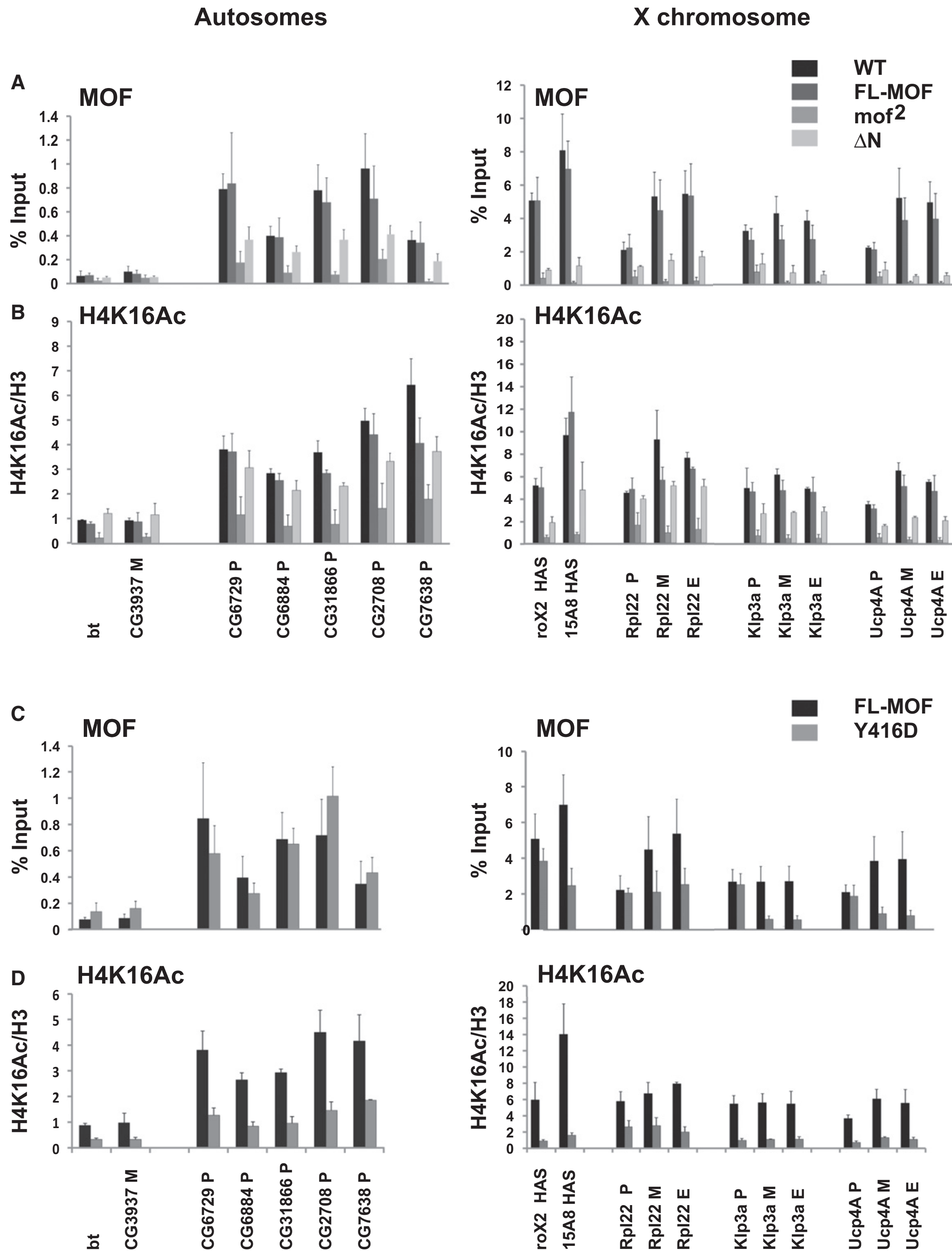

Figure 4. The Chromobarrel Domain Is Required for H4K16ac In Vivo

(A and B) Compromised MOF targeting and H4K16ac in ΔN MOF. ChIP using MOF (A) and H4K16ac (B) antibodies in WT male third-instar larva, as well as *mof*^{2} larva or *mof*^{2} larva that express FL-MOF or ΔN MOF transgenes. Binding to the autosomal genes *bt*, *cg3937*, *cg6729*, *cg6884*, *cg31866*, *cg2709*, *cg7638*, the X-linked high-affinity sites at the *roX2* gene and location 15A8, as well as to the X chromosomal medium-affinity gene *Rpl22* and low-affinity genes *Klp3a* and *Ucp4a* are shown. Primers were positioned at the promoter (P), middle (M), and end (E) of genes. The exact position of the primers is described in the Supplemental Experimental Procedures. ChIP is shown as percentage recovery of input DNA (% Input). Error bars represent standard deviation (StDev) of three independent experiments.

(C and D) H4K16ac is lost upon disruption of the chromobarrel domain, whereas promoter binding of MOF is retained. Same as in (A) and (B), using *mof*^{2} larvae that express FL-MOF or Y416D MOF, respectively.

See also Figure S4.

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H4K16ac across X-linked and autosomal target sites in the presence of Y416D MOF. Confirming immunostainings, H4K16ac was strongly reduced across all sites, including gene promoters and HAS, where the Y416D MOF protein was still readily detected (Figure 4D). This result suggests that the chromobarrel domain serves to trigger MOFs catalytic activity after initial recruitment of MOF to its chromatin targets.

The observed loss of H4K16ac in chromobarrel domain mutants was striking and surprising. We therefore performed western blots from third-instar larva extracts to confirm the global reduction of H4K16ac by an independent method. When probing with HA antibodies, we found that all five MOF transgenes were stably expressed to similar levels (Figure 5A). Antibodies against tubulin and unmodified histone H4 were used as loading controls. FL-MOF efficiently rescued the *mof*^{2}-mediated loss of H4K16ac to WT levels. No H4K16ac was detected upon deletion of the zinc finger in ΔZn MOF, reflecting compromised chromatin targeting of this mutant. Consistent with our previous analysis, substantial amounts of H4K16ac could be detected after deletion of the N terminus in ΔN MOF. However, global H4K16ac levels were dramatically reduced in both chromobarrel domain mutants, confirming the general role of this domain for genome-wide H4K16ac. To control for the specificity of the assay we also probed against H4K5ac, as well as H4K8ac and H4K12ac. Although a recent study has proposed a role of MOF for the acetylation of the H4K5 and K8 residues in the context of the NSL complex in mammals (Cai et al., 2010), the bulk levels of these histone marks remained unchanged in each of the MOF mutants analyzed (Figure 5A and data not shown).

The Chromobarrel Domain Interacts with DNA and Triggers H4K16ac In Vitro

We next wanted to more directly study the requirement of the chromobarrel domain for MOF HAT activity. To this end we performed in vitro HAT assays on purified endogenous nucleosomes, using baculovirus-expressed FL-MOF, Y416D, and W426G MOF that were copurified with MSL1 and MSL3 to yield enzymatically active trimeric complexes (Morales et al., 2004). The stoichiometry of the trimeric complexes was unaffected in all cases (Figure 5C), confirming that MSL protein interactions were not impaired by mutations in the chromobarrel domain, which is consistent with the fact that the sites of MSL1 and MSL3 interaction have been mapped to the HAT domain of MOF (Kadlec et al., 2011; Morales et al., 2004). Consistent with our in vivo observations, H4 directed acetylation activity was approximately 3- to 5-fold reduced in trimeric complexes containing Y416D and W426G MOF, as compared to FL-MOF (Figure 5B and data not shown), demonstrating the crucial role of the chromobarrel domain for nucleosomal acetylation also in vitro. The reduced acetylation activity of chromobarrel domain mutants was specific to nucleosomal substrates, since the activities of the same trimeric complexes toward free histone octamers were similar to FL-MOF or only slightly reduced (Figure 5D). This result again confirmed the formation of functional trimeric complexes by the mutant MOF variants and demonstrated the functionality of the MOF HAT domain in the tested mutants. It has been reported previously that the chromobarrel domain is required for MOF interaction with RNA (Akhtar et al., 2000). Since no direct nucleic acid interactions of the MOF chromobarrel domain have yet been demonstrated, we performed electrophoretic mobility shift assays (EMSA) using highly purified recombinant WT, Y416D, and W426G chromobarrel domain (amino acids 346–448) (Figure 5E). We detected a modest but specific interaction of the chromobarrel domain with a fluorescent-labeled, single-stranded RNA probe (Figure 5F). Importantly, we found a similar interaction with double-stranded DNA, which was again specifically disrupted in both mutant derivatives. Since removal of potentially copurified RNA by RNase treatment had no effect on the in vitro HAT activity of MOF toward nucleosomes (data not shown), our data strongly suggests that the DNA binding capacity of the chromobarrel domain plays an important role in triggering H4K16ac in a nucleosomal context in vitro and in vivo.

The MOF N Terminus Modulates Substrate Binding and HAT Activity of MOF

Having established the central role of the chromobarrel domain for MOF HAT activity, we wanted to further characterize the function of the MOF N terminus. We again performed in vitro HAT assays using trimeric complexes of MSL1 and MSL3 together with FL-MOF and ΔN MOF, as well as with ΔN Y416D and ΔN W426G double mutants. The stoichiometry of trimeric MSL subcomplexes was not affected upon deletion of the MOF N terminus (Figures 5C and 6A). Strikingly, MOF HAT activity appeared substantially enhanced upon deletion of the N terminus (Figure 6B, compare lanes 3 and 5). This result suggests that an autoregulatory function in the N-terminal domain of MOF constrains its enzymatic activity and explains why in vivo levels of H4K16ac remain high in the presence of ΔN MOF, although chromatin targeting of the mutant protein is impaired because of compromised complex assembly. Upon additional mutation of the chromobarrel domain, MOF HAT activity was substantially reduced, even in the absence of the N terminus, suggesting that a functional chromobarrel domain is a general requirement for efficient histone acetylation by MOF (Figure 6B, compare lane 5 with lanes 7 and 9).

We next wanted to ask at which step of initial substrate binding and subsequent acetylation the N terminus and chromobarrel domain function. To address this question we performed EMSA with monomeric baculovirus expressed FL-MOF, ΔN MOF and ΔN Y416D MOF, using fluorescent-labeled reconstituted mononucleosomes as substrate. Although we could not detect a robust nucleosome interaction with the FL-MOF protein at the available protein concentrations, a substantial interaction was detected upon deletion of the N terminus (Figure 6C). These data suggest that the N terminus modulates MOF's substrate interaction, consistent with the increased HAT activity of ΔN MOF. In agreement with the modest DNA interaction that we had observed for the chromobarrel domain, simultaneous mutation of ΔN MOF at Y416D or W426G had no detrimental effect on nucleosome binding. We then performed EMSA with fluorescent-labeled nucleosomes using trimeric complexes containing MOF derivatives, MSL1 and MSL3. Consistent with previous observations (Morales et al., 2004), the trimeric MSL complex bound to nucleosomes with higher affinity compared to MOF alone. This binding appeared again unaffected for both

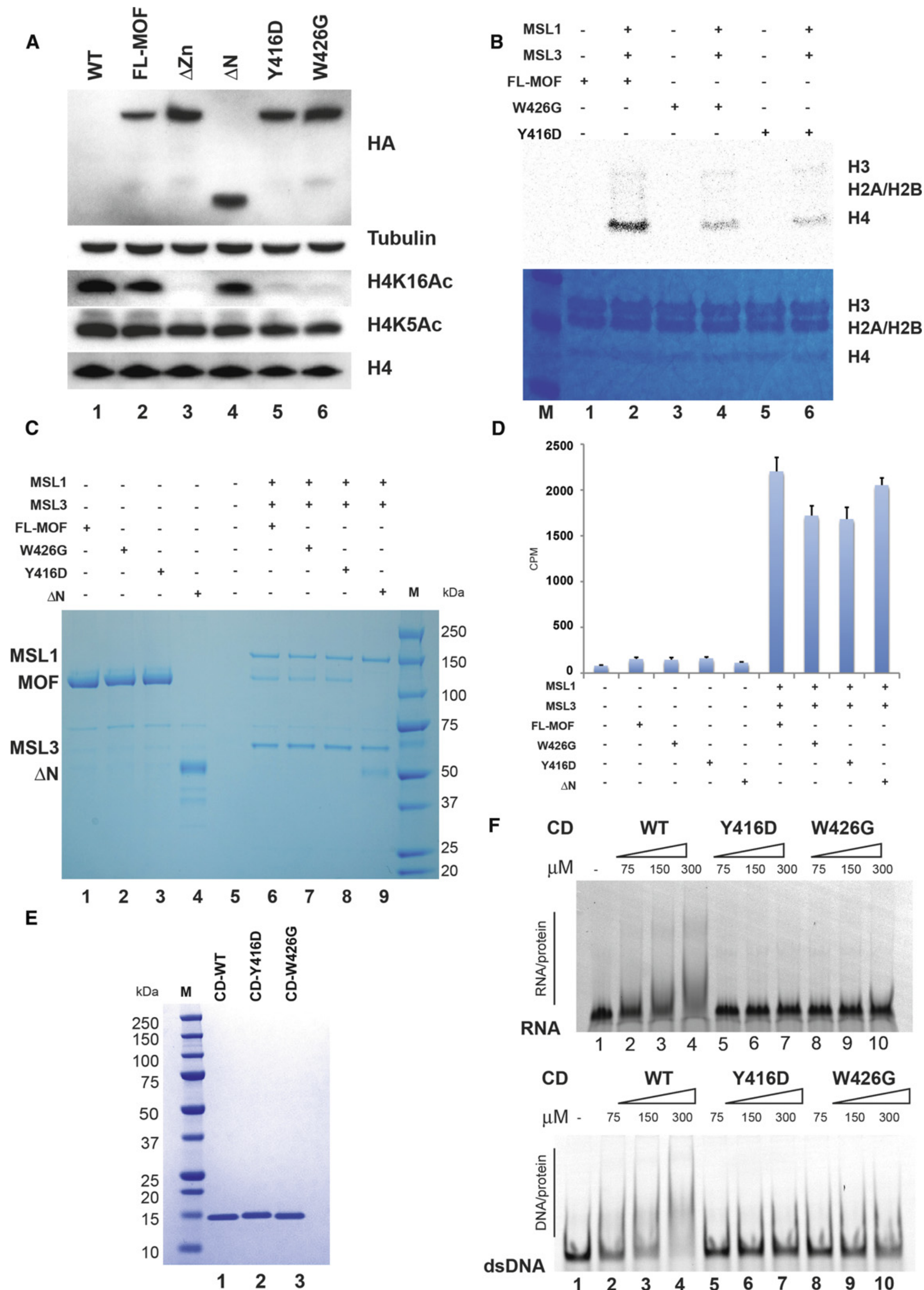

Figure 5. Nucleic Acid Interaction of the Chromobarrel Domain Is Required for Acetylation of a Nucleosomal Substrate

(A) Bulk H4K16ac is lost upon disruption of the chromobarrel domain in vivo. Western blot analysis of extracts from *mof*[2] male third-instar larva expressing the indicated MOF transgenes.

(B) Acetylation assay on native nucleosomes. Each reaction contains C_{14}-labeled acetyl coenzyme A, 0.1 fmol of FL, Y416D, or W426G MOF proteins, with or without MSL1 and MSL3, respectively, and 1.5 μg of native nucleosomes purified from MCF-7 cells.

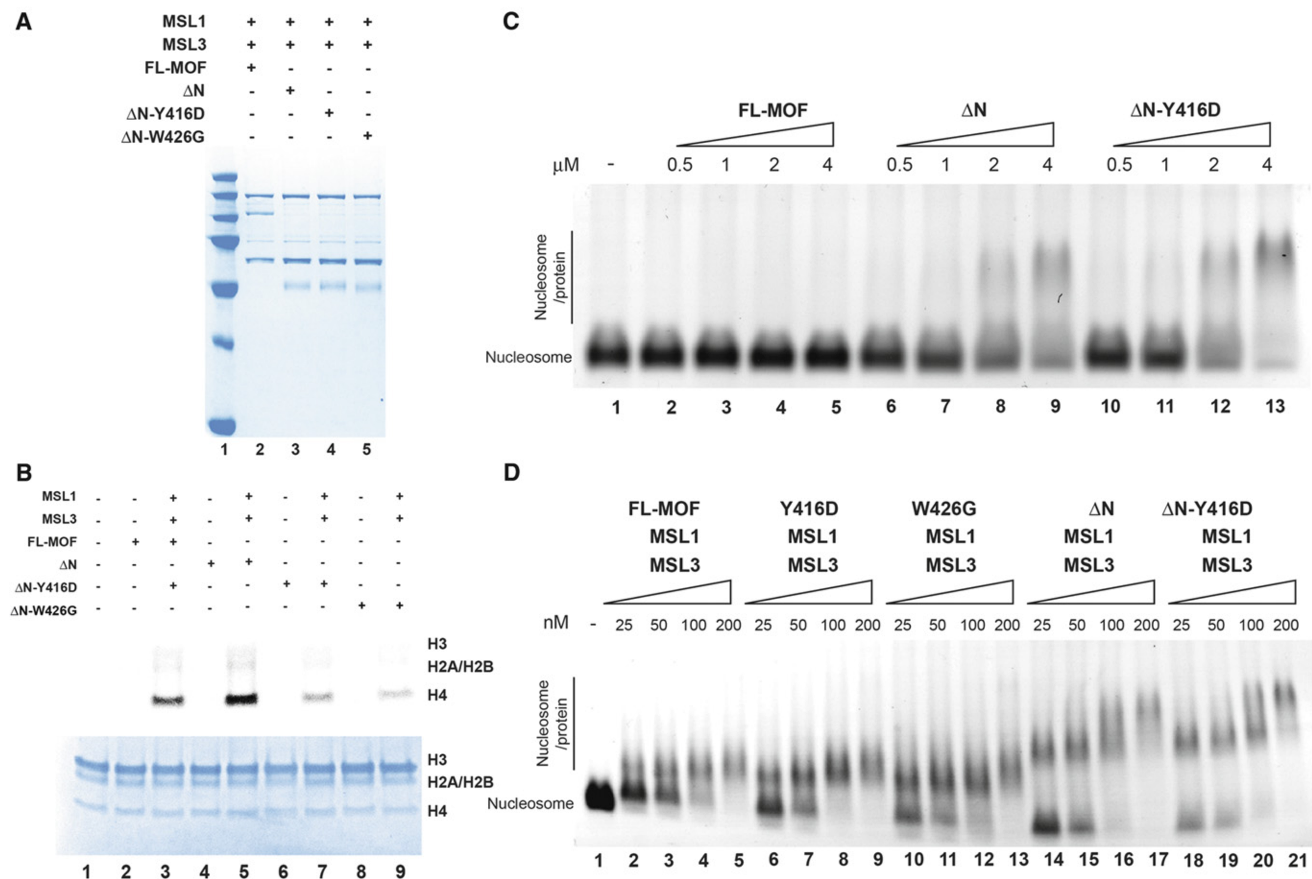

Figure 6. The MOF N Terminus Controls Substrate Binding and HAT Activity of MOF

(A) Purification of FL-MOF, ΔN MOF, ΔN-W426G, and ΔN-Y416D MOF proteins as trimeric complexes containing MSL1 and MSL3.

(B) MOF's HAT activity is enhanced upon deletion of the N terminus but still dependent on a functional chromobarrel domain. Each reaction contains C_{14}-labeled acetyl coenzyme A, 0.1 fmol of FL-MOF, ΔN MOF, ΔN-W426G, and ΔN-Y416D MOF proteins, with or without MSL1 and MSL3, respectively, and 1.5 μg of native nucleosomes purified from MCF-7 cells.

(C) The N terminus constrains substrate binding of MOF. EMSA using fluorescent-labeled mononucleosomes together with indicated amounts of FL-MOF, ΔN MOF, ΔN-W426G, and ΔN-Y416D MOF proteins.

(D) The N terminus constrains substrate binding of the trimeric complex. Same assay as in (C) using indicated amounts of FL-MOF, ΔN MOF, ΔN-W426G, and ΔN-Y416D MOF proteins together with MSL1 and MSL3.

chromobarrel domain mutants. However, nucleosome binding of the trimeric complex was substantially increased upon deletion of the MOF N terminus, or in the presence of the ΔN Y416D double mutant (Figure 6D). The affinity of the MOF HAT domain for the nucleosomal substrate is thus controlled by the MOF N terminus. This binding is independent of a functional chromobarrel domain, whose DNA contact is subsequently required to trigger acetylation of the H4 tail.

DISCUSSION

MOF Is the Major H4K16-Specific HAT in *Drosophila*

Since the recent discovery that MOF resides at autosomal gene promoters as part of the NSL complex, the full extent of MOF function at these sites has remained elusive (Gelbart et al., 2009; Kind et al., 2008; Raja et al., 2010). Disruption of other NSL complex members leads to lethality in males and females

(C) The stoichiometry of trimeric MSL complexes is unaffected by mutations in the chromobarrel domain or upon deletion of the N terminus. Purification of FL, W426G, Y416D, ΔN MOF proteins either alone (lanes 1–4) or as trimeric complexes containing MSL1 and MSL3 (lanes 6–9).

(D) Acetylation assay on free histone octamers. 0.4 fmol of the respective recombinant MOF-protein or trimeric complexes were incubated with C^{14} labeled acetyl coenzyme A and 1,5 μg of recombinant (*Xenopus*) histone octamer, as indicated in the figure. The reaction was then applied on a P81 filter paper, air-dried, washed, and counted in scintillation liquid. Error bars represent the standard deviation from three independent experiments.

(E) Highly purified bacterial expressed WT, Y416D, and W426G MOF chromobarrel domain (CD) that was used to study nucleic acid interactions.

(F) EMSA using a fluorescent-labeled 84bp ssRNA (top) or dsDNA probe (bottom) together with indicated amounts of MOF chromobarrel domain derivatives from (E).

See also Figure S4.

(Mendjan et al., 2006). This suggested that MOF is not strictly necessary for NSL complex function, since adult female flies can be recovered in the absence of MOF (Hilfiker et al., 1997). However, the catalytically impaired *mof[1]* allele that was identified as male lethal in that study has later been shown to retain approximately 10% of its enzymatic activity (Akhtar and Becker, 2001), and defects in female fitness were not addressed. By using the *mof[2]* allele, which completely eliminates the MOF protein, we show that MOF is indeed required for survival in both sexes, since the number of females reaching adulthood, and especially female lifetime, are reduced in the absence of MOF. Furthermore, our data demonstrate that, in addition to its role in X chromosome dosage compensation, MOF is responsible for the bulk of H4K16ac at gene promoters in males and females, suggesting that MOF is the major H4K16ac-specific HAT in *Drosophila*. This result is in contrast to a recent report that did not detect a substantial reduction of H4K16ac at promoters in the absence of MOF (Gelbart et al., 2009), which might be attributable to residual levels of maternally contributed MOF protein and the small number of target genes assayed in that study. However, since recent work showed that ATAC2 is contributing to bulk H4K16ac during embryonic development (Suganuma et al., 2008), the possibility remains that additional enzymes might have the capacity to mediate H4K16ac in certain developmental stages or tissues. Interestingly, H4K16ac is also strongly diminished in human cells upon RNAi and in mutants of the close homolog hMOF (Gupta et al., 2008; Smith et al., 2005; Taipale et al., 2005; Thomas et al., 2008), and it has subsequently been shown that hMOF is targeted to thousands of gene promoters across the human genome (Wang et al., 2009). It is therefore highly likely that the pattern of MOF binding that we observe at promoters across male and female autosomes and on the female X chromosome in *Drosophila*, is indeed reflecting the most ancient mode of MOF function.

The MOF Chromobarrel Domain Controls Genome-wide H4K16ac

An earlier study claimed that the MOF chromobarrel domain had a minor role to play in dosage compensation (Morales et al., 2004). However, this study only tested the capacity of MOF to target to the X chromosome by simply overexpressing MOF variants in a male cell line so that essential defects would have been complemented for by the presence of the endogenous protein. Indeed, demonstrating the advantages of the in vivo system, we were able to reveal the crucial role of the MOF chromobarrel domain for MOF function. Upon disruption of its nucleic acid binding properties, MSL spreading to X-linked genes is compromised, leading to a defect in dosage compensation (Figure 7). This is reminiscent of impaired MSL spreading upon deletion of the chromobarrel domain of MSL3 (Sural et al., 2008). However, the MSL3 chromobarrel domain is thought to contribute to MSL targeting via its binding to the H3 tail trimethylated at K36 (Larschan et al., 2007). Also an interaction with the H4 tail monomethylated at K20 has been proposed (Kim et al., 2010; Moore et al., 2010). In contrast, the MOF chromobarrel domain lacks the aromatic cage required for binding to methylated lysine residues (Nielsen et al., 2005). Considering the weak affinity of the chromobarrel domain-nucleic acid interaction, reduced MSL spreading in MOF chromobarrel domain mutants might not be the result of a direct chromatin binding defect, consistent with the fact that the binding of MOF to gene promoters in vivo and to nucleosomes in vitro is unaffected upon disruption of the chromobarrel domain. The most dramatic and unexpected consequence upon disruption of the chromobarrel domain was the loss of genome-wide H4K16ac, which affected MOF in the NSL as well as MSL complex. Importantly, in this mutant background, H4K16ac was also lost from the HAS on the X chromosome, despite MSL complex binding at the same sites (Figure 7). It is clear from previous work that compromised H4K16ac alone is sufficient to disrupt MSL spreading into X-linked chromatin. Upon mutation of the catalytic site of the MOF enzyme in the *mof[1]* allele, MSL binding is restricted to high-affinity sites in a pattern similar to the one observed in chromobarrel domain mutants (Gu et al., 1998). Indeed, the in vivo binding pattern of MSL3 upon mutation of the MOF chromobarrel domain precisely mirrors the pattern observed in the *mof[1]* background (Figure S4A). It is therefore likely that reduced MSL spreading upon disruption of the chromobarrel domain is primarily caused by the concomitant reduction in X-linked H4K16ac.

We have provided evidence for a direct interaction of the MOF chromobarrel domain with RNA and DNA. Interestingly, we had not observed such an interaction in an earlier study. However, previous assays were performed using a shorter fragment of the chromobarrel domain (aa 367–454; Figure S4B) (Nielsen et al., 2005). A recent study on yeast Esa1 has shown that, in the presence of a short N-terminal extension, a minor conformational change occurs in the core of the homologous Esa1 tudor domain, triggering its RNA/DNA-binding activity (Figures S4C and S4D) (Shimojo et al., 2008). Accordingly, we have now assayed nucleic acid binding of a chromobarrel domain construct that includes the corresponding additional amino acids at its N terminus (comprising amino acids 346–448). Although the detected nucleic-acid-binding affinities of the isolated chromobarrel domain are low, it appears that the high local DNA concentrations within the context of a fully assembled MSL-nucleosome complex could confer biological significance to the weak DNA-chromobarrel domain interaction. This idea is strongly supported by the severe phenotypes that correlate with the disruption of this interaction in vitro and in vivo. Interestingly, the structure of the chromobarrel domain of MSL3 has recently been determined in complex with dsDNA and the N-terminal tail of histone H4 monomethylated at H4K20 (Kim et al., 2010). In the MSL3 structure, Trp66 corresponding to MOF chromobarrel domain Trp426 (W426) is directly involved in the interaction with DNA (Figures S4F and S4G). Although the aromatic residues that are critical for interaction of the MSL3 chromobarrel domain with the methylated lysine side chain of the H4 peptide are not well conserved, it remains unclear whether the MOF chromobarrel domain could also bind unmodified histone tail residues (Figure S4E). One could potentially envisage that a mode of action similar to MSL3 may also exist for the MOF chromobarrel domain, and that interactions with nucleosomal DNA and the unmodified H4 tail might direct the tail toward acetylation by the MYST domain. Interestingly, mutations in the related tudor domain in yeast Esa1 affect its HAT activity in vitro, although the significance of this observation for Esa1 function in vivo remained unclear (Selleck et al., 2005). Since the chromobarrel domain is also conserved in human MOF it is furthermore likely

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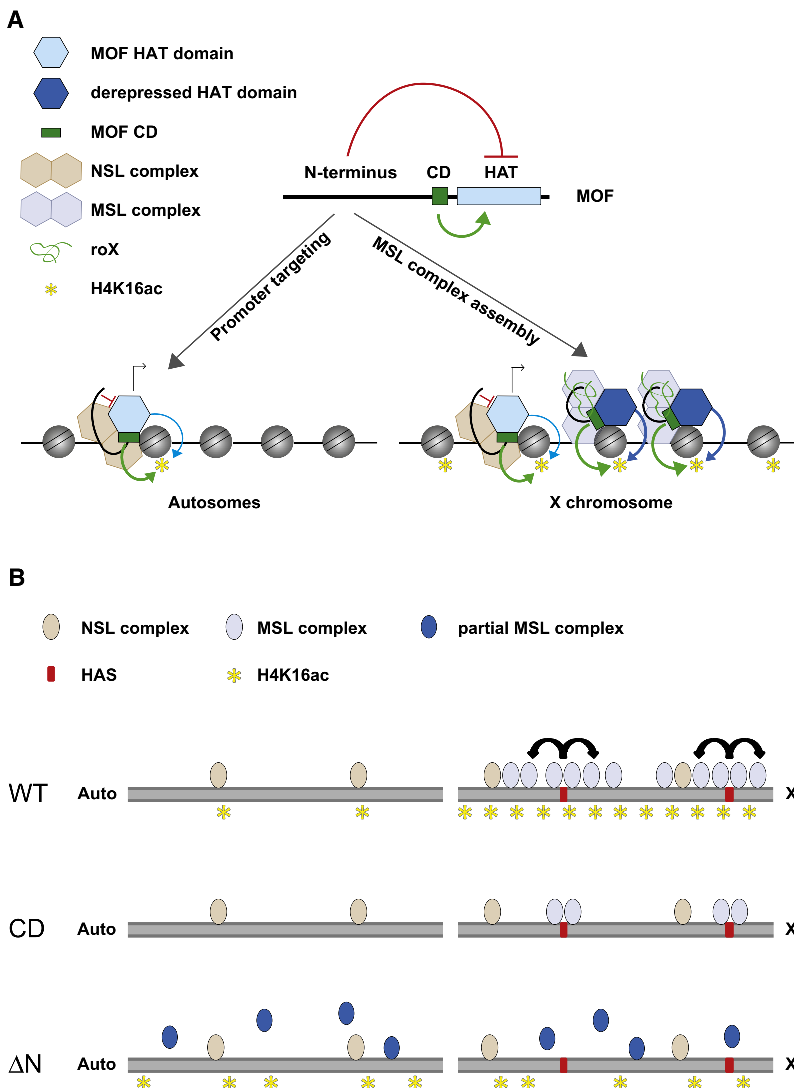

Figure 7. MOF Function Is Controlled by Its N Terminus and Chromobarrel Domain

In addition to hyperacetylation of the X chromosome, MOF is responsible for the majority of H4K16ac at gene promoters in male and female flies. MOF activity is tightly regulated to achieve these diverse tasks.

(A) The *Drosophila*-specific N-terminal half of the protein is required for the assembly of functional MSL complexes in vivo and for efficient targeting of MOF to gene promoters as part of the NSL complex. The N terminus furthermore modulates the affinity of the MOF HAT domain for its nucleosomal substrate, thus regulating MOF HAT activity. We propose that this *Drosophila*-specific level of regulation serves to restrict strong hyperacetylation to the male X chromosome. DNA contact of the chromobarrel domain is generally required to trigger acetylation of the H4 tail after chromatin binding of MOF containing complexes. The chromobarrel domain thus acts as an accessory module to direct the enzymatic activity of MOF toward H4K16.

(B) In the WT situation, MSL complexes are attracted by high-affinity binding sites (HAS) throughout the X chromosome (X), from where they spread onto the transcribed regions of genes in surrounding chromatin (depicted in A, right). MOF in the MSL complex is enzymatically hyperactive and acetylates chromatin beyond MSL complex binding sites. In contrast, MOF-mediated acetylation on autosomes (Auto) is restricted to NSL complex binding sites (WT, top). Upon mutation of the chromobarrel domain, H4K16ac is lost genome-wide, preventing the spreading of MSL complexes from HAS (CD, middle). In the absence of the MOF N terminus, MSL complex assembly is compromised. However, enzymatically hyperactive ΔN MOF containing subcomplexes mediate substantial levels of unspecific H4K16ac throughout the genome during transient substrate interactions (ΔN, bottom).

that MOF's acetylation activity is controlled in a similar manner in humans.

MOF Controls Dosage Compensation via Its N-Terminal Domain

Another striking observation during this study was that the N-terminal part of the MOF protein is required for MSL complex assembly and, at the same time, regulates substrate binding and HAT activity of MOF (Figure 7). The presence of a large MOF N-terminal region is a prominent feature of *Drosophila* species and absent in all other MOF homologs from yeast to human (Figure S2K). Addition of this domain correlates with the evolution of the *Drosophila* dosage compensation system, involving the targeting of MSLs and H4K16ac to the male X chromosome. It seems thus plausible that the N-terminal domain has added functions that utilize the evolutionarily ancient transcriptional regulator MOF for the more recently evolved task of dosage compensation. Strikingly, upon deletion of the MOF N terminus, MSL targeting to the X chromosome was completely abolished. This also included high-affinity sites (HAS), which are otherwise resistant to MOF depletion (Gu et al., 1998). Aberrant MSL targeting was reminiscent of the phenotypes observed in roX1 and roX2 double mutants (Meller and Rattner, 2002), and indeed roX levels were reduced by 98% in the absence of the MOF N terminus. This result suggests that, although interaction with MSL1 and MSL3 is mediated by the zinc finger region of MOF (Morales et al., 2004), the N terminus is required to assemble the core MSL subunits together with roX RNAs into a functional MSL complex. At the same time, the N terminus is also required for efficient targeting of MOF to gene promoters, where it resides as part of the NSL complex.

MSL protein domains required for MSL complex assembly and targeting, as well as their cognate DNA binding sequences, have been shown to undergo rapid adaptive coevolution in *Drosophila melanogaster* (Bachtrog, 2008; Rodriguez et al., 2007). Also roX RNA sequences are highly divergent throughout *Drosophila* species (Park et al., 2007). Likewise, the N-terminal domain of MOF shows huge variation in size and amino acid sequence between *Drosophila* species, suggesting ongoing selective pressure by other MSLs or roX RNA (Figure S2K). Another striking feature of the N-terminal domain is its intrinsic disorder, according to computational secondary structure

prediction. It is a recently emerging concept that protein function is not necessarily linked to fixed secondary and tertiary structures (Chouard, 2011). Functional disordered regions have been particularly suggested for domains in hub proteins that control a variety of biological processes and mediate interactions to multiple interaction partners (Oldfield et al., 2008). We therefore propose that the unstructured N-terminal domain integrates the multiple functions of MOF in dosage compensation and for genome-wide H4K16ac (Figure 7).

A further intriguing finding was that the N terminus regulates the affinity of the HAT domain for its nucleosomal substrate and thus MOF's enzymatic activity in vivo and in vitro. Although chromatin targeting of ΔN MOF was impaired in vivo, owing to compromised complex assembly, widespread acetylation appeared to be mediated by short-lived chromatin interactions of the hyperactive enzyme. Again, the necessity for the additional level of regulation imposed by the MOF N terminus might arise from the dual function that MOF has adopted in *Drosophila*. H4K16ac displays much higher baseline levels on the male X chromosome as in any other chromosomal context (Figure S1B). Furthermore, MOF-mediated H4K16ac extends beyond regions of MSL binding on the male X chromosome. We do not observe the same phenomenon on male autosomes or in females, where lower levels of H4K16ac are restricted to sites of MOF binding at gene promoters. The activity level of MOF thus differs to a large degree, depending on the chromosomal context. It is known that MOF requires interaction with MSL1 and MSL3 to be active as a HAT in vitro. However, our data suggest that an additional constrain is imposed on MOF's enzymatic activity by an autoinhibitory function residing in its N terminus. Interestingly, our data show that H4K16ac spreads from sites of ΔN MOF binding into neighboring regions, even in the absence of other MSLs. We therefore propose that in the wild-type situation, the N terminus may control or restrict H4K16ac spreading around autosomal MOF binding sites, and this constrain is only released in the presence of a fully assembled MSL complex on the male X chromosome for more extensive acetylation.

In previous experimental setups, loss of MSL binding has been accompanied by simultaneous loss of MOF-mediated H4K16ac from the transcribed regions of X-linked genes (Gelbart et al., 2009; Gu et al., 1998; Hilfiker et al., 1997; Kind et al., 2008). In the presence of hyperactive ΔN MOF, we now detected substantial amounts of H4K16ac in the transcribed regions of X-linked genes despite concomitant loss of MSL from the same sites (Figures 4A and 4B). Intriguingly, about 11% of males escaped lethality in this background. This finding supports the view that H4K16ac alone is sufficient to upregulate the transcription of X-linked genes and that the main function of the MSL complex is to direct H4K16ac to the male X chromosome.

In summary, in this study we have revealed the function of the MOF chromobarrel domain, which is to elicit the activity of its associated HAT enzyme on the chromatin target. The huge degree of sequence conservation of the MOF protein between flies and mammals suggests that a similar mode of operation might be present in mammalian MOF. Intriguingly, MOF's enzymatic activity toward a nonnucleosomal substrate did not require a functional chromobarrel domain (Figure 5D), which may potentially allow an experimental uncoupling of MOF's chromatin-related functions from the MOF-mediated acetylation of nonhistone substrates. Our findings thus have important implications for the study of this enzyme in humans, where it is implicated in a wide range of processes, like transcription, DNA repair, and cancer (Rea et al., 2007; Wang et al., 2009; Zippo et al., 2009). In contrast to the chromobarrel domain, the N-terminal part of the MOF protein is specific to *Drosophila* species and controls the sex-specific function of MOF during dosage compensation. Accordingly, our work also highlights how a HAT has been adopted through evolution to carry out distinct functions on X chromosomal and autosomal genes.

EXPERIMENTAL PROCEDURES

ChIP from Salivary Glands

ChIP-seq experiments were performed from third-instar larva salivary glands; ChIP from MOF mutants was performed on whole third-instar larva as described in the Supplemental Experimental Procedures.

Fly Stocks and Crosses

All stocks were maintained on standard medium at 25°C. To assay for female viability, we conducted the cross *y/mof*^{2}*;sb/P{w*$^{+}$ *UAS-HA-MOF}* x *mof*^{2}*/mof*^{2}*;P{w*$^{+}$ *UAS-HA-MOF}/tm3* and the control cross *y/w*$^{-}$*;sb/+* x *w*$^{-}$*/w*$^{-}$*;+/tm3* and compared the proportion of *mof*^{2}*/mof*^{2}*;sb/t3* or *w*$^{-}$*/w*$^{-}$*;sb/t3* in the respective offspring. Leaky expression from UAS-MOF was sufficient to rescue male viability in this assay. For the complementation test, female flies of the genotype *mof*^{2}*/fm7;P{w+ UAS-HA-MOF}* were crossed to *y/fm7;P{armadillo-GAL4}* males to induce transgene expression, and the ratio of male *Y/mof*^{2} *to* female *mof*^{2}*/fm7* offspring was scored as relative male survival. Fly strains are listed in the Supplemental Experimental Procedures.

For qRT-PCR, RNA and corresponding genomic DNA were simultaneously isolated from salivary glands to determine absolute expression per gene copy as described in the Supplemental Experimental Procedures.

Immunostaining of Polytene Chromosomes and Confocal Microscopy

Immunostainings were performed as previously described (Raja et al., 2010). For details see the Supplemental Experimental Procedures.

Generation of Protein Extracts and Western Blotting

Protein levels were determined from adult fly heads and third-instar larva by western blotting. For details see the Supplemental Experimental Procedures.

Expression and Purification of *Drosophila* Recombinant Proteins with the Baculovirus System

Recombinant proteins for in vitro enzymatic and binding assays were produced using the baculovirus system as described in the Supplemental Experimental Procedures.

HAT Assay on Nucleosomal Templates and Histone Octamers

HAT assays were performed on nucleosomes from MCF-7 cells or on recombinant histone octamers using baculovirus produced recombinant proteins as described in the Supplemental Experimental Procedures.

Recombinant Protein Expression

Recombinant chromobarrel domains were expressed and purified from bacteria as described in the Supplemental Experimental Procedures.

Electrophoretic Mobility Shift Assay

Binding reactions were carried out in 20 μL EMSA buffer (20 mM HEPES [pH 7.6], 73mM NaCl, 3mM MgCl2, 0.01% NP40, 2.5% Ficoll 400, 1mM DTT, and 0.1mg/ml BSA) containing 50 nM of a 84 bp ssRNA or dsDNA probe or 5 nM mononucleosomes, 5′ labeled with Alexa647. Proteins were used in concentrations as indicated in the figures. The mixture was incubated on ice for 15 min, and 10μl were run on a 4% native polyacrylamide gel in 0.5% TBE for 90 min at 120 V to visualize chromobarrel domain-nucleic acid complexes or on 0.6% agarose gels for 1 hr at 60 V to resolve MOF and trimeric

MSL complexes bound to mononucleosomes. The gels were scanned on a FLA5000 scanner (Fujifilm, Tokyo, Japan).

ACCESSION NUMBERS

ChIP-seq data are available under the accession number E-MTAB-911, and the gene expression data are available under the accession number E-MEXP-3506.

SUPPLEMENTAL INFORMATION

Supplemental Information includes four figures, two tables, and Supplemental Experimental Procedures and can be found with this article online at doi:10.1016/j.devcel.2011.12.016.

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REFERENCES

Akhtar, A., and Becker, P.B. (2000). Activation of transcription through histone H4 acetylation by MOF, an acetyltransferase essential for dosage compensation in *Drosophila*. Mol. Cell *5*, 367–375.

Akhtar, A., and Becker, P.B. (2001). The histone H4 acetyltransferase MOF uses a C2HC zinc finger for substrate recognition. EMBO Rep. *2*, 113–118.

Akhtar, A., Zink, D., and Becker, P.B. (2000). Chromodomains are protein-RNA interaction modules. Nature *407*, 405–409.

Alekseyenko, A.A., Peng, S., Larschan, E., Gorchakov, A.A., Lee, O.K., Kharchenko, P., McGrath, S.D., Wang, C.I., Mardis, E.R., Park, P.J., and Kuroda, M.I. (2008). A sequence motif within chromatin entry sites directs MSL establishment on the *Drosophila* X chromosome. Cell *134*, 599–609.

Bachtrog, D. (2008). Positive selection at the binding sites of the male-specific lethal complex involved in dosage compensation in *Drosophila*. Genetics *180*, 1123–1129.

Bell, O., Wirbelauer, C., Hild, M., Scharf, A.N., Schwaiger, M., MacAlpine, D.M., Zilbermann, F., van Leeuwen, F., Bell, S.P., Imhof, A., et al. (2007). Localized H3K36 methylation states define histone H4K16 acetylation during transcriptional elongation in *Drosophila*. EMBO J. *26*, 4974–4984.

Bell, O., Schwaiger, M., Oakeley, E.J., Lienert, F., Beisel, C., Stadler, M.B., and Schübeler, D. (2010). Accessibility of the Drosophila genome discriminates PcG repression, H4K16 acetylation and replication timing. Nat. Struct. Mol. Biol. *17*, 894–900.

Cai, Y., Jin, J., Swanson, S.K., Cole, M.D., Choi, S.H., Florens, L., Washburn, M.P., Conaway, J.W., and Conaway, R.C. (2010). Subunit composition and substrate specificity of a MOF-containing histone acetyltransferase distinct from the male-specific lethal (MSL) complex. J. Biol. Chem. *285*, 4268–4272.

Chouard, T. (2011). Structural biology: Breaking the protein rules. Nature *471*, 151–153.

Gelbart, M.E., Larschan, E., Peng, S., Park, P.J., and Kuroda, M.I. (2009). Drosophila MSL complex globally acetylates H4K16 on the male X chromosome for dosage compensation. Nat. Struct. Mol. Biol. *16*, 825–832.

Gu, W., Szauter, P., and Lucchesi, J.C. (1998). Targeting of MOF, a putative histone acetyl transferase, to the X chromosome of Drosophila melanogaster. Dev. Genet. *22*, 56–64.

Gupta, A., Guerin-Peyrou, T.G., Sharma, G.G., Park, C., Agarwal, M., Ganju, R.K., Pandita, S., Choi, K., Sukumar, S., Pandita, R.K., et al. (2008). The mammalian ortholog of *Drosophila* MOF that acetylates histone H4 lysine 16 is essential for embryogenesis and oncogenesis. Mol. Cell. Biol. *28*, 397–409.

Hilfiker, A., Hilfiker-Kleiner, D., Pannuti, A., and Lucchesi, J.C. (1997). mof, a putative acetyl transferase gene related to the Tip60 and MOZ human genes and to the SAS genes of yeast, is required for dosage compensation in *Drosophila*. EMBO J. *16*, 2054–2060.

Kadlec, J., Hallacli, E., Lipp, M., Holz, H., Sanchez-Weatherby, J., Cusack, S., and Akhtar, A. (2011). Structural basis for MOF and MSL3 recruitment into the dosage compensation complex by MSL1. Nat. Struct. Mol. Biol. *18*, 142–149.

Kim, D., Blus, B.J., Chandra, V., Huang, P., Rastinejad, F., and Khorasanizadeh, S. (2010). Corecognition of DNA and a methylated histone tail by the MSL3 chromodomain. Nat. Struct. Mol. Biol. *17*, 1027–1029.

Kind, J., Vaquerizas, J.M., Gebhardt, P., Gentzel, M., Luscombe, N.M., Bertone, P., and Akhtar, A. (2008). Genome-wide analysis reveals MOF as a key regulator of dosage compensation and gene expression in *Drosophila*. Cell *133*, 813–828.

Larschan, E., Alekseyenko, A.A., Gortchakov, A.A., Peng, S., Li, B., Yang, P., Workman, J.L., Park, P.J., and Kuroda, M.I. (2007). MSL complex is attracted to genes marked by H3K36 trimethylation using a sequence-independent mechanism. Mol. Cell *28*, 121–133.

Meller, V.H., and Rattner, B.P. (2002). The roX genes encode redundant male-specific lethal transcripts required for targeting of the MSL complex. EMBO J. *21*, 1084–1091.

Mendjan, S., Taipale, M., Kind, J., Holz, H., Gebhardt, P., Schelder, M., Vermeulen, M., Buscaino, A., Duncan, K., Mueller, J., et al. (2006). Nuclear pore components are involved in the transcriptional regulation of dosage compensation in *Drosophila*. Mol. Cell *21*, 811–823.

Moore, S.A., Ferhatoglu, Y., Jia, Y., Al-Jiab, R.A., and Scott, M.J. (2010). Structural and biochemical studies on the chromo-barrel domain of male specific lethal 3 (MSL3) reveal a binding preference for mono- or dimethyllysine 20 on histone H4. J. Biol. Chem. *285*, 40879–40890.

Morales, V., Straub, T., Neumann, M.F., Mengus, G., Akhtar, A., and Becker, P.B. (2004). Functional integration of the histone acetyltransferase MOF into the dosage compensation complex. EMBO J. *23*, 2258–2268.

Nielsen, P.R., Nietlispach, D., Buscaino, A., Warner, R.J., Akhtar, A., Murzin, A.G., Murzina, N.V., and Laue, E.D. (2005). Structure of the chromo barrel domain from the MOF acetyltransferase. J. Biol. Chem. *280*, 32326–32331.

Oldfield, C.J., Meng, J., Yang, J.Y., Yang, M.Q., Uversky, V.N., and Dunker, A.K. (2008). Flexible nets: disorder and induced fit in the associations of p53 and 14-3-3 with their partners. BMC Genomics *9* (*Suppl 1*), S1.

Park, S.W., Kang, Y.Ie., Sypula, J.G., Choi, J., Oh, H., and Park, Y. (2007). An evolutionarily conserved domain of roX2 RNA is sufficient for induction of H4-Lys16 acetylation on the *Drosophila* X chromosome. Genetics *177*, 1429–1437.

Raja, S.J., Charapitsa, I., Conrad, T., Vaquerizas, J.M., Gebhardt, P., Holz, H., Kadlec, J., Fraterman, S., Luscombe, N.M., and Akhtar, A. (2010). The nonspecific lethal complex is a transcriptional regulator in *Drosophila*. Mol. Cell *38*, 827–841.

Rea, S., Xouri, G., and Akhtar, A. (2007). Males absent on the first (MOF): from flies to humans. Oncogene *26*, 5385–5394.

Rodriguez, M.A., Vermaak, D., Bayes, J.J., and Malik, H.S. (2007). Species-specific positive selection of the male-specific lethal complex that participates in dosage compensation in *Drosophila*. Proc. Natl. Acad. Sci. USA *104*, 15412–15417.

Ruthenburg, A.J., Li, H., Milne, T.A., Dewell, S., McGinty, R.K., Yuen, M., Ueberheide, B., Dou, Y., Muir, T.W., Patel, D.J., and Allis, C.D. (2011). Recognition of a mononucleosomal histone modification pattern by BPTF via multivalent interactions. Cell *145*, 692–706.

Sanjuán, R., and Marín, I. (2001). Tracing the origin of the compensasome: evolutionary history of DEAH helicase and MYST acetyltransferase gene families. Mol. Biol. Evol. *18*, 330–343.

Schwaiger, M., Stadler, M.B., Bell, O., Kohler, H., Oakeley, E.J., and Schübeler, D. (2009). Chromatin state marks cell-type- and gender-specific replication of the *Drosophila* genome. Genes Dev. *23*, 589–601.

Selleck, W., Fortin, I., Sermwittayawong, D., Côté, J., and Tan, S. (2005). The Saccharomyces cerevisiae Piccolo NuA4 histone acetyltransferase complex requires the Enhancer of Polycomb A domain and chromodomain to acetylate nucleosomes. Mol. Cell. Biol. *25*, 5535–5542.

Shimojo, H., Sano, N., Moriwaki, Y., Okuda, M., Horikoshi, M., and Nishimura, Y. (2008). Novel structural and functional mode of a knot essential for RNA binding activity of the Esa1 presumed chromodomain. J. Mol. Biol. *378*, 987–1001.

Shogren-Knaak, M., Ishii, H., Sun, J.M., Pazin, M.J., Davie, J.R., and Peterson, C.L. (2006). Histone H4-K16 acetylation controls chromatin structure and protein interactions. Science *311*, 844–847.

Smith, E.R., Cayrou, C., Huang, R., Lane, W.S., Côté, J., and Lucchesi, J.C. (2005). A human protein complex homologous to the *Drosophila* MSL complex is responsible for the majority of histone H4 acetylation at lysine 16. Mol. Cell. Biol. *25*, 9175–9188.

Straub, T., and Becker, P.B. (2007). Dosage compensation: the beginning and end of generalization. Nat. Rev. Genet. *8*, 47–57.

Suganuma, T., Gutiérrez, J.L., Li, B., Florens, L., Swanson, S.K., Washburn, M.P., Abmayr, S.M., and Workman, J.L. (2008). ATAC is a double histone acetyltransferase complex that stimulates nucleosome sliding. Nat. Struct. Mol. Biol. *15*, 364–372.

Sural, T.H., Peng, S., Li, B., Workman, J.L., Park, P.J., and Kuroda, M.I. (2008). The MSL3 chromodomain directs a key targeting step for dosage compensation of the *Drosophila melanogaster* X chromosome. Nat. Struct. Mol. Biol. *15*, 1318–1325.

Taipale, M., Rea, S., Richter, K., Vilar, A., Lichter, P., Imhof, A., and Akhtar, A. (2005). hMOF histone acetyltransferase is required for histone H4 lysine 16 acetylation in mammalian cells. Mol. Cell. Biol. *25*, 6798–6810.

Thomas, T., Dixon, M.P., Kueh, A.J., and Voss, A.K. (2008). Mof (MYST1 or KAT8) is essential for progression of embryonic development past the blastocyst stage and required for normal chromatin architecture. Mol. Cell. Biol. *28*, 5093–5105.

Wang, Z., Zang, C., Cui, K., Schones, D.E., Barski, A., Peng, W., and Zhao, K. (2009). Genome-wide mapping of HATs and HDACs reveals distinct functions in active and inactive genes. Cell *138*, 1019–1031.

Zippo, A., Serafini, R., Rocchigiani, M., Pennacchini, S., Krepelova, A., and Oliviero, S. (2009). Histone crosstalk between H3S10ph and H4K16ac generates a histone code that mediates transcription elongation. Cell *138*, 1122–1136.