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The MSL complex: X chromosome and beyond

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X chromosomal regulation is a process that presents systematic problems of chromosome recognition and coordinated gene regulation. In *Drosophila* males, the ribonucleoprotein Male-Specific Lethal (MSL) complex plays an important role in hyperactivation of the X-linked genes to equalize gene dosage differences between the sexes. It appears that X chromosome recognition by the MSL complex may be mediated through a combination of sequence-specificity and transcriptional activities. The resulting transcriptional up-regulation also seems to involve several mechanisms, encompassing both gene-specific and chromosome-wide approaches. Interestingly the histone H4 lysine 16 specific MOF histone acetyl transferase, a key MSL member that hyper-acetylates the male X chromosome, is also involved in gene regulation beyond dosage compensation. A comparison of *Drosophila* and mammalian systems reveals intriguing parallels in MOF behavior, and highlights the multidisciplinary nature of this enzyme.

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Introduction

Eukaryotic species with heterogametic sex chromosomes compensate for differences in gene dosage between the two sexes by a process known as dosage compensation. Rather than simple designed solutions, evolution appears to have produced many different means by which the process is regulated. In mammals and nematodes this involves down-regulation of X-linked genes while in *Drosophila* the single male X chromosome is transcriptionally up-regulated approximately twofold in comparison to the two female X chromosomes (for reviews see [1–4]; see also other reviews in this issue) (Figure 1). In this review we focus on the recent reports concerning *Drosophila* dosage compensation. The emerging view is that dosage compensation is an integrated network of regulatory mechanisms that produces a chromosome-wide system of gene regulation. Moreover, it appears that over the course of evolution, the Male-Specific Lethal (MSL) complex members have evolved to perform additional functions.

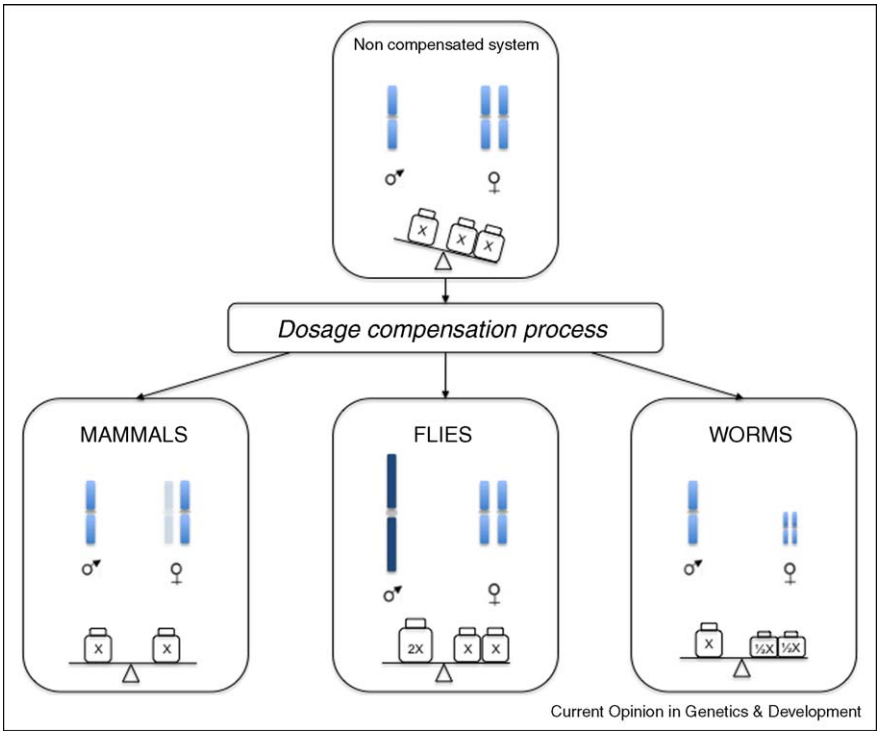
The *Drosophila* MSL complex

The *Drosophila* male X chromosome differs from the female counterparts in several important respects. Cytologically, the width of the single male X chromosome is equal to that of the paired female X chromosomes, revealing an altered chromatin state. The amount of RNA synthesized by individual X-linked genes, or the chromosome as a whole, is also equal between the sexes, indicating that regulation is at the level of gene expression [1]. Classical mutational analyses revealed five proteins (MSL1, MSL2, MSL3, MLE, and MOF) that are required for male survival, which together comprise the MSL complex, also known as the dosage compensation complex (for review see [4]). This complex selectively binds the male X chromosome, it is necessary for both the chromosome puffing and equalization of transcript levels, and is thus the key effector of dosage compensation. The best-characterized consequence of MSL binding is the specific acetylation of histone 4, lysine 16 (H4K16Ac) by the MOF/KAT8 histone acetyl transferase component [5–7]. In addition to the presence of MSL complex and modified chromatin state, the male X chromosome is also coated with two noncoding RNAs, the RNA on the X, *roX1* and *roX2*. The two RNAs appear functionally redundant, but together associate with and help target the MSL complex to the chromosome [8,9]. Several additional cofactors are involved in dosage compensation, most of which also modify chromatin, and have been recently reviewed [2].

X chromosome recognition

Cytogenetic observations show the complex binding to hundreds of distinct locations, which coat the chromosome in a discontinuous fashion (Figure 2). These include the X-linked genes for the two *roX* RNAs, which are exceptionally strong attractors of the MSL complex. When translocated to an autosome, a *roX* gene still mediates MSL binding that can 'spread' up to 1 Mb into the surrounding chromatin [10,11]. The degree of spreading appears to reflect the transcriptional status of the gene itself [12]. That is, very weak transcription, or at endogenous levels, resulted in local spreading of MSL complexes around the transgene site, but under high levels of transcription the MSL complex was only found on the X

Figure 1



Strategies to achieve dosage compensation. Dosage compensation is the mechanism by which species featuring an unequal number of X chromosomes (pictured) between the two sexes balance the expression of X-linked genes. In a noncompensated system, cells belonging to opposite sexes would generate different amount of X-linked gene products. Different organisms compensate for dose following a variety of strategies: mammalian females transcriptionally inactivate one out of the two X chromosomes, *Drosophila* males hyperactivate the single X chromosome, and *Caenorhabditis elegans* hermaphrodites partially repress both of the X chromosomes.

chromosome. This mechanism may affect the distribution of MSL complex between the *roX* genes and the other bound locations on the chromosome.

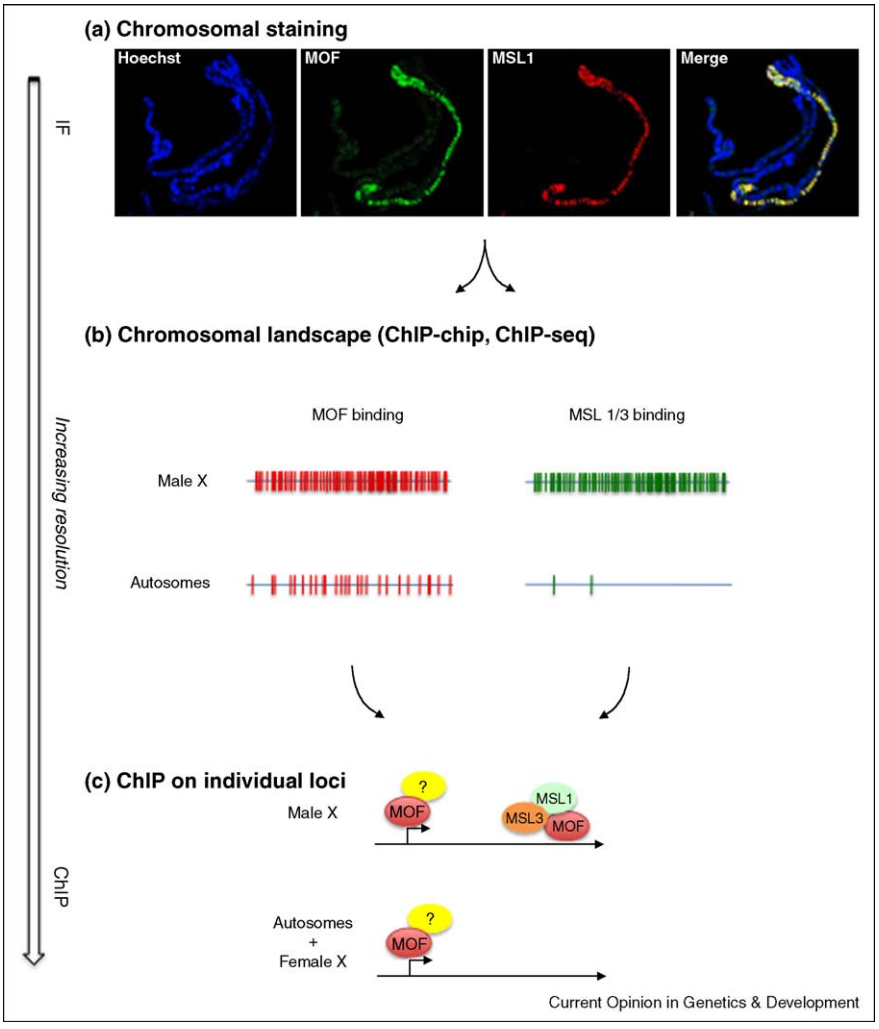
On the basis of classical polytene staining, it was observed that partial complexes lacking MSL3, MLE, or MOF bind a mostly overlapping subset of 60–70 sites, which were termed Chromosome Entry Sites (CES) on account that they may be the sites at which the complex first binds [13]. Alternatively, the sites of highest affinity for the MSL complex can be identified in immuno-precipitates of complex components [14]. Individual CES or 'High affinity Sites' (HAS) differed in their ability to recruit MSL complex, and appeared to share few unifying characteristics (reviewed in [1]).

With the development of ChIP-chip and ChIP-seq methods, the nature of these sites has been further described, and it now appears likely that the HAS encompass those first recognized as chromatin entry sites. With the improved resolution of these techniques, the number of HAS now ranges between 130 and 150 [15••,16••]. However, an objective HAS data set is difficult to describe. The locations bound by partial complexes differ slightly depending on which component is lacking, even at polytene resolution [14]. A detailed comparison of high-resolution profiles of MSL complexes lacking different components is thus similarly desirable. Computational analyses resolved a DNA sequence motif at the heart of the HAS: a nearly perfect repeat of eleven GA dinucleotides that can independently attract functional MSL complex [15••,16••]. These 'MSL Recognition Elements' (MRE) lie predominantly in intronic or noncoding sequences near genes, and are associated with nucleosome depletion. This indicates that the local chromatin state differs from that of the secondary sites of MSL complex binding, and such epigenetic marks should now be characterized. If differences persist between sites obtained from differing tissues and developmental states, then the transcription profile of such sites must also be considered.

Targeting active chromatin

ChIP-chip analyses of wild-type MSL binding reveal that most bound sites differ from those of the highest affinity [17–19,20••]. When at full occupancy, the MSL proteins bind over 700 regions, are almost exclusively within genes rather than intergenic sequences, exons not introns, and prefer coding sequences to un-translated regions. On binding profiles scaled by gene length, MSL proteins

Figure 2



The increasing resolution of techniques improves the description of the *Drosophila* MSL binding activity. The nature of *Drosophila* MSL binding sites is illustrated according to the method used to describe them. **(a)** Immunofluorescent staining of polytene chromosomes shows chromatin binding, with an enrichment on the male X chromosome. Less intense autosomal binding of MOF is also apparent. **(b)** Schematic representation of available genome-wide ChIP-chip and ChIP-seq profiles. The increased resolution of these techniques confirms the polytene observations, and allows precise description of the binding sites. **(c)** Examination of profiles over gene units reveals differential binding of MSL proteins. MSL1, MSL3 and MOF colocalize across the transcribed portion of genes, especially toward the 3′ end. MOF also binds the 5′ regions of genes, peaking at the transcription start site.

peak toward the 3′ end of target genes. About 90% of the target genes are expressed, and clearly bound by RNA PolII; a correlation that hints the MSL complex may be targeted to active chromatin.

Indeed, transcription itself can attract the MSL complex. Random insertions of an inducible Gal4 promoter on the X chromosome attract complex to the newly transcribed sequences, downstream of the promoter [21]. Translocations of X-linked genes, such as the *mof* gene, to an autosome also attract the MSL complex, even when expressed from different promoters or transcribed in an anti-sense direction, but not when lacking a promoter [22]. The complementary approach has also shown MSL binding to depend on gene activation [23]. A large autosomal fragment spanning two active genes was inserted on the X chromosome, where the genes acquired MSL binding, H4K16 acetylation, and compensation. Deletion of the translocated promoter regions abolished MSL binding and reduced H4K16 acetylation, providing further evidence that MSL attraction is transcription-dependent.

The binding profile of MSL3 correlates with that of H3K36me3, a modification that is also enriched in the 3′ region of transcribed genes [24]. Knock-down of the methyl-transferase dSet2 (alias Hypb, KMT3) reduced H3K36me3, bound MSL complex and X-linked

H4K16Ac, and affected compensation of X-linked genes [25•]. The chromodomain of MSL3, thought to bind H3K36me3, was necessary for the establishment of the wild-type binding pattern [26•]. Recombinant MSL3 binds DNA and nucleosomes, prefers Set2-methylated nucleosomes, and exhibits reduced binding to all substrates when lacking the chromodomain [24,26•,27]. However, the active chromatin state must differ from the inactive in many ways besides H3K36me3, and recognition of this epigenetic mark may be merely one of several contributory mechanisms to the identification of active chromatin.

The mechanism by which transcription recruits the MSL complex remains unclear. It is tempting to speculate that since the MSL complex contains RNA binding proteins [28–31], noncoding transcripts originating from the target sites may also contribute toward MSL targeting [32••,33••]. These could either be promoter-associated RNAs or even the transcripts from the dosage compensated genes. However, whether there is any contribution of additional RNAs in X chromosomal targeting, apart from *roX* RNAs, remains to be experimentally determined. It is also important to bear in mind that since transcription occurs genome-wide, although important, it cannot be the only contributor to achieve X chromosome specificity. We anticipate that future studies will once again refine the model of MSL attraction.

Factors contributing to up-regulation of the male X chromosome

The mechanism by which the MSL complex enhances X-linked gene expression remains a mystery. Although the MSL complex preferentially binds active genes, not all active genes on the X are bound by the MSL complex [17–19,20••,34]. Furthermore, expression of unbound genes on the X chromosome can also be affected upon MSL depletion [17,19,34], suggesting that the MSL complex may also have long distance effects on the X chromosome. Moreover, it was recently shown that although integration of HAS on autosomal locations increased transcriptional activity of a reporter gene, there were also exceptions to this rule, suggesting that for some cases sequence-dependent targeting may not be sufficient for up-regulation [16••]. Thus, the mechanism of transcriptional up-regulation remains elusive.

At least one direct effect of MSL binding is the MOF-mediated acetylation of H4K16. Nucleosomes with the H4K16 acetylation resist chromatin compaction, and inhibit nucleosome remodeling [35]. Recently, using *in vitro* chromatin assembly that closely mimicked physiological conditions it was observed that the acetylation specifically inhibits the formation of the 30 nm fiber, to the same degree as lack of linker histone [36]. The decondensed chromatin caused by this acetylation could thus facilitate increased transcription of the male X chromosome.

The binding profile of H4K16Ac resembles that of the MSLs: enriched over active genes on the male X chromosome, peaking toward the 3′ ends of coding regions, and is dependent on MOF [20••,37]. This 3′ bias implies that the process may be regulated at the level of transcription elongation. But a second class of MOF binding is also apparent, and this appears to be distinct from its role in the MSL complex [20••]. ChIP-chip binding profiles of the strongest MOF sites within genes were scaled to gene length, and revealed a bimodal distribution of MOF. In addition to the MSL-type distribution, a strong peak of MOF binding was detected at many promoters, regardless of sex or chromosomal location. A similar but broader profile was also observed for the acetylation also, across the profile of active genes but higher at the 5′ end on autosomes and the female X chromosome. This general 5′ MOF and H4K16Ac remained even after depletion of MSL1, arguing for a role of MOF that is independent of dosage compensation [20••].

However, recent analyses of H4K16Ac profiles also reveal a low general enrichment on the male X chromosome, at all locations irrespective of transcriptional activity [34,38•]. The enrichment was dependent on the MSL complex, even at sites where the complex did not bind [34]. However, since these profiles did not have a complementary MOF profile for comparison, it is difficult to interpret how much is contributed by MOF or from another HAT such as ATAC2 [39•].

In comparison to the profiles of nascent transcripts separated by cell cycle, H4K16Ac enrichment correlated to areas of early DNA replication [38•]. If H4K16Ac does indeed help to specify zones of replication initiation, the global enrichment on the male X chromosome may reflect an additional mechanism of gene regulation beyond chromatin de-condensation. Early replication itself could provide twice the transcript for a longer amount of time, and remove some need for transcriptional up-regulation.

The regulation of subchromosomal regions may also be a strong contributor to dosage compensation. Zones of active transcription associate with nuclear pores, punctuating generally repressive chromatin at the nuclear periphery, suggesting that increased association of X chromosomal regions with nuclear pores may lead to higher transcription levels [40]. Components of the nuclear pores (Mtor and Nup153) copurify with MSL proteins [41]. Furthermore, genome-wide analysis of these components now shows that these proteins bind chromatin in a domain-like fashion encompassing active genes. The X chromosome is enriched in these domains in a male-specific manner [42••]. Taken together with the recent observations in yeast showing that the yeast

nuclear pore component MLP1 mediate gene loops and that these loops enhance transcriptional re-activation of genes [43•], it is tempting to speculate that in *Drosophila* nuclear pores may also mediate gene loops, which could help transcriptional efficiency and aid dosage compensation.

Above we discuss possible scenarios where histone H4 lysine 16 acetylation and the MSL complex are actively involved in regulation of dosage compensation of the male X chromosome. An alternative hypothesis put forward by Birchler and colleagues suggests that the twofold increase in X-linked gene expression is caused by the imbalance of transcription factors in males relative to autosomes [44–46]. According to this model, the MSL complex accumulates on the X chromosome at the expense of the autosomes, and as a consequence sequesters the histone acetyl transferase MOF to the male X chromosome. Hyperacetylation of the male X chromosome as a result of this sequestration is then counteracted by the MSL complex itself, leading to a fine-tuning of gene expression to twofold up-regulation. The MSL complex therefore 'protects' the autosomal genes from becoming hyperactive if MOF is unleashed from the X chromosome. Since MOF is indeed bound to many autosomal sites [20••], future experiments promise to reveal how the balance between X and autosomes is indeed achieved and how MSLs or even the newly identified NSL proteins fine-tune gene expression [41].

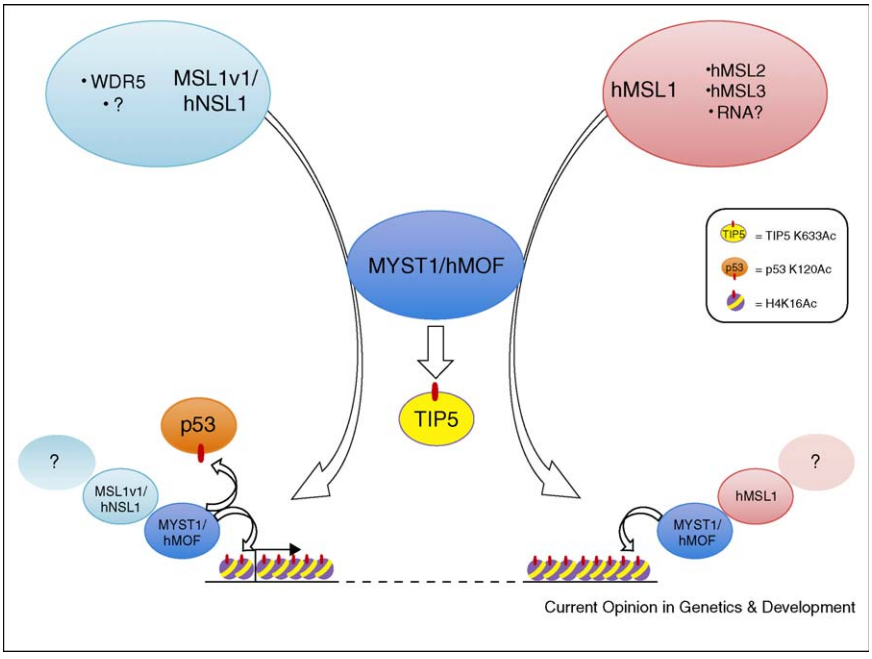
Role of MSL complex members beyond dosage compensation

Accumulating evidence suggests additional functions of the MSL proteins that are still largely unexplored. A prominent example is the global analysis of MOF, which revealed distinct binding behavior on the male X chromosome versus autosomal genes [20••] (Figure 2). Furthermore, *roX* RNAs affect the regulation of genes on the fourth chromosome [47••]. These studies raise the possibility of additional functions for these proteins/RNAs beyond dosage compensation.

Genome-wide profiling of hMOF/MYST1 in human CD4$^+$ T cells has revealed that similar to the *Drosophila* protein, hMOF prevalently binds promoters [48••]. Although not thoroughly addressed yet, single gene studies indicate that hMOF binding may also be detectable at the 3′ end of target genes and in other genomic elements [49,50,51•]. A systematic analysis of the mammalian counterparts of the MSL complex will reveal how prevalent is the contribution of the 3′ enrichment in the genome.

Similarities between the *Drosophila* and mammalian systems go beyond the similarities in MOF binding pattern. MOF-containing MSL complexes are also conserved between *Drosophila* and in mammals [41,52]. So far evidence for noncoding RNAs associated with the mammalian complex is lacking. However, based on extensive

Figure 3



Different roles for the mammalian acetyltransferase MYST1/hMOF. hMOF resides in at least two different complexes in mammalian cells. When recruited in a complex with MSL1v1/hNSL1, hMOF is responsible for the acetylation of both p53 K120 and H4K16 at the 5′ end of target loci. If MYST1 is instead recruited to the mammalian MSL complex via an interaction with hMSL1, the protein specifically acetylates H4K16Ac at the 3′ end of target loci. hMOF also acetylates the Tip5 component of the NoRC chromatin remodeling complex, but the regulation of specificity for this substrate is as yet unknown.

conservation of protein interaction it is quite possible that *roX*-like RNAs are waiting to be discovered even in the mammalian system. Interestingly, it is becoming clear that MSL proteins are not the only proteins that hold the right to interact with MOF. In fact, a number of novel interaction partners have also been copurified in both species [41,52,53]. This analysis clearly indicated that MOF resides in distinct complexes [41,51•].

Further analysis of these complexes has shown that although histone H4 lysine 16 remains one of the major substrate of hMOF, additional substrates exist (Figure 3). In *Drosophila*, MSL3 and MSL1 have been shown to be acetylated by MOF, while in mammals p53 and TIP5 have recently been identified as targets of hMOF [29,31,51,54]. Similar to the *Drosophila* system where acetylation of MSL3 modulates the interaction with *roX* RNAs, in mammals acetylation of TIP5 also affects its interaction with RNAs generated from ribosomal promoters [29,54]. Furthermore, association of hMOF with hMSL1v1/hNSL1 leads to acetylation of p53 and introduction of a bias toward acetylation of H4K16 at the 5′ end of target loci. This bias contrasts with the specificity for the same histone residue at the 3′ end of target loci displayed by the hMSL1–hMOF complex [51•]. Taken together, these findings suggest that the specificity of MOF for different substrates can be controlled through interactions with different chromatin modifying complexes.

Intriguingly, another component of the hMSL complex, hMSL2, has also been shown to perform tasks beyond the ones known in *Drosophila*. Although it remains unclear whether this is an hMSL complex-independent activity, hMSL2 is able to ubiquitinate p53 and promote its cytoplasmic localization without affecting its stability, in an Mdm2-independent fashion [55]. It remains to be verified whether similar parallels also exist in the *Drosophila* system.

Male-specific lethality appears to be a unifying feature of the dosage compensation complex members in *Drosophila*. Nevertheless, here again closer inspection of MOF mutant females has shown that lack of MOF leads to female sterility and reduced life span [34] (Conrad and Akhtar, unpublished results). A complete set of knockout mouse models is missing for MSL proteins. However, some progress has been made recently. hMOF is an essential gene in mice, and its absence leads to early embryonic lethality and severe loss of H4K16 acetylation, indicating that hMOF is the major H4K16 histone acetyl transferase also in mammals [56,57].

Given the increasing importance of epigenetic regulators in oncogenesis, it is perhaps not surprising that global histone modification patterns constitute hallmarks of human cancers [58,59]. Interestingly, H14K16Ac is among the growing list of histone modifications associated with cancer phenotypes and/or prognostic impact [58]. Recent data suggest that abnormal levels of hMOF also correlate with malignant phenotypes [56,57,60,61]. Data from mouse models showed that high levels of hMOF correlate at the cellular level with a faster growth rate, prolonged lifespan and oncogenic transformation [56]. On the other hand, low levels of hMOF have also been reported to correlate with enhanced genomic instability, nuclear polylobulation and cancerogenesis [57,60–62]. Perhaps, a more direct link between hMOF and cancer is represented by the hMOF-dependent acetylation of p53 at K120 [51•,63]. This modification is able to trigger apoptosis upon activation of the proapoptotic p53 target genes BAX and PUMA after induction of DNA damage [51•,63]. Future studies will reveal the mechanisms operating behind these functions and how to separate gene-specific versus global effects of histone H4 lysine 16 acetylation. However, what is already apparent is that MOF plays a mediatory role and with varying outputs depending on which protein networks are being utilized.

Conclusions

The MSL complex provides a well-studied example of a chromatin remodeling complex that regulates the male X chromosome in *Drosophila*. As the resolution of our analysis increases we improve our understanding of the possible mechanisms underlying X chromosomal regulation. In parallel, significant progress is also being made in the study of the mammalian MSL orthologues. Thus a proper appreciation of the dosage compensation process should also encompass the additional functions that the key proteins perform. Evolutionary comparison of these proteins also promises to reveal novel insights that go beyond X chromosome regulation and unveil a broader role in gene regulation.

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