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The MSL complex: juggling RNA–protein interactions for dosage compensation and beyond

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The Male Specific Lethal (MSL) complex provides an exquisite example of an epigenetic modulator that is involved in chromosome-wide as well as individual gene regulation in flies and mammals. In this review, we discuss the recent advances in biochemical and structural understanding of the MSL complex modules and how they function in X chromosome regulation in flies. Moreover, we describe possible conserved and dosage compensation-independent functions of the MSL complex with a particular focus on mammalian systems.

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Introduction

Sex determination mechanisms in different organisms are extraordinarily diverse and in many instances involve chromosomal differences between the two sexes. In *Caenorhabditis elegans*, *Drosophila* and mammals, males are heterogametic (XY), whereas females are homogametic (XX) [1]. Interestingly, parallel mechanisms operating on a chromosome-wide level have evolved to ensure equal gene expression from sex chromosomes. Already three decades ago, Male-Specific Lethal (MSL) mutants have been identified and characterized in the fruit fly *Drosophila melanogaster*, leading to the notion that in flies, dosage compensation manifests in males [2,3]. Since then, dosage compensation has become a paradigm to study chromosome-wide transcription regulation by epigenetic mechanisms.

Biochemically, at least five proteins, MSL1, MSL2, MSL3, MOF (males-absent-on-the first) and MLE (maleless) as well as two non-coding RNAs roX1 and/or roX2 (RNA on the X) form a complex known as the MSL complex [4,5,6•,7•,8]. The MSL complex assembles exclusively in male flies, as translation of the *msl-2* mRNA is inhibited in females by the RNA binding protein sex-lethal (*sxl*) [9,10]. It mediates global acetylation of histone H4 lysine 16 (H4K16ac) on the single male X chromosomes, which causes an upregulation of transcription [11,12••,13,14,15••,16••].

Notably, apart from MSL2, other MSL complex members are also expressed in female flies and orthologs exist in many species, where dosage compensation mechanisms are absent or fundamentally different. This suggests that the MSL complex members also function outside of the dosage compensation machinery, a property that is likely to be mediated by the different enzymatic and protein-interaction modules found in these proteins. For example, MOF additionally resides in the Non-Specific Lethal complex (NSL complex), which is involved in global transcription regulation of housekeeping genes in both sexes [17,18]. Here, we review MSL complex function in dosage compensation in flies with a particular focus on recent structural and biochemical work. On the basis of this, we discuss possible conserved, dosage compensation-independent, functions focussing on mammalian systems.

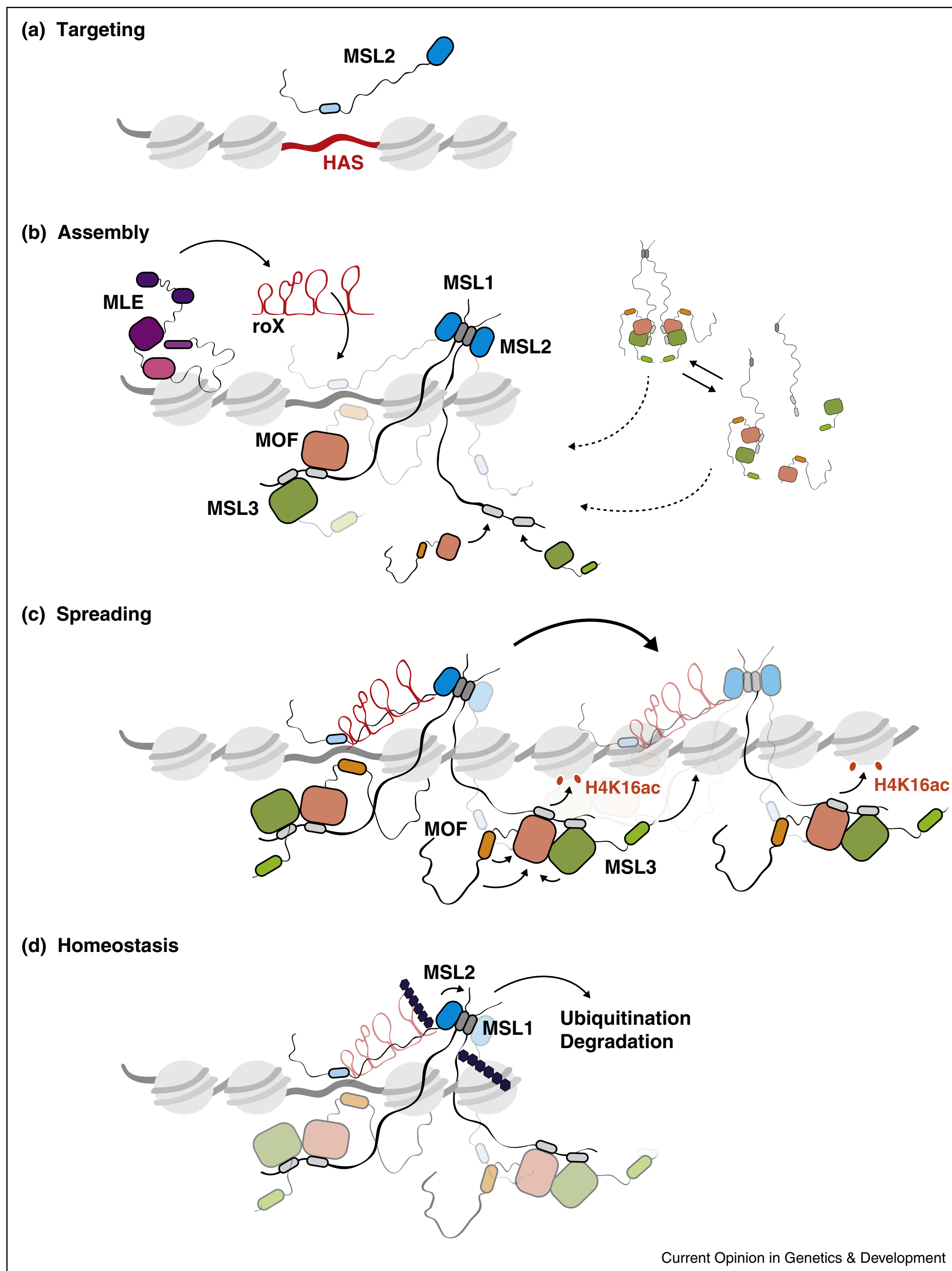
Structural analyses of MSL2 revealed the targeting principles of the MSL complex

The MSL complex orchestrates dosage compensation on the male X chromosome in a multistep process (Figure 1). Firstly, the complex is targeted to numerous high-affinity sites (HAS) on the X, following its complete assembly [19,20•,21]. Then, it spreads from HAS to the rest of the X establishing chromosome-wide H4K16ac. This results in upregulated transcription on the X chromosome, which is stably maintained and requires tight control of MSL complex levels. To accomplish these complex events, the core MSL complex contains several enzymatic and multiple adaptor modules.

The fact that MSL2 expression is inhibited in females, underscores that MSL2 is probably the most central regulator of dosage compensation [7•]. The MSL2 protein functions in targeting of HAS on the X chromosome (Figure 1a), MSL complex assembly (Figure 1b) and control of functional MSL complex levels (Figure 1d).

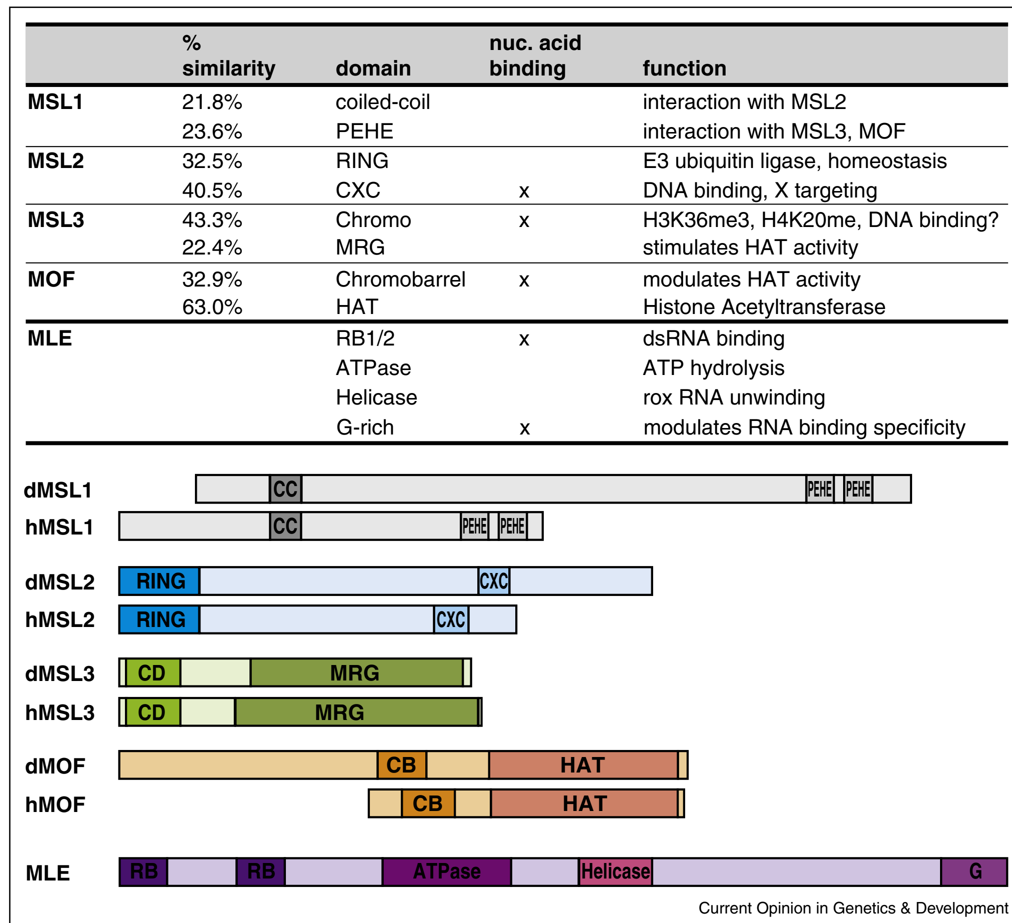
HAS targeting is probably mediated by the MSL2 CXC domain and might involve nucleic acid binding [22] (Figure 2). It occurs before full MSL complex assembly, as in the absence of MOF, a partial MSL complex

Figure 1



Stepwise establishment of dosage compensation in *Drosophila* via the MSL complex. **(a)** Targeting: MSL2 (blue) via its CXC domain recognizes high affinity sites (HAS) (red) on the male X-chromosome. Nucleosomes are visualized in grey. **(b)** Assembly: Dimerization of MSL1 (black/grey) provides an interaction surface for the MSL2 RING domain and is a first important step in complex assembly. It is possible that before the interaction with MSL2, MSL1 is preassembled with MOF (red/orange) and MSL3 (green) in a trimer or hexamer, already. Alternatively, MOF association occurs in a second step after MSL1/MSL2 interaction. Because in ChIP experiments, MSL3 association with HAS is minimal, it is also possible that MSL3 incorporation occurs later. Lastly, rox1/2 ncRNA (red) integration is catalysed by the RNA helicase MLE (pink). MLE can only be found at high affinity sites by ChIP and its association with the complex is transient. Possibly, these events lead to conformational changes rendering the complex in a spreading competent form **(c)**. (c) Spreading: Once the complex is fully assembled, the complex is thought to spread from HAS to actively transcribed regions in a chromosome-wide manner. The exact mechanism of transition from assembly at HAS to spreading is unknown; however, MSL3 seems to have a key role in this process. Ultimately, this leads to H4K16 hyperacetylation of the entire

Figure 2



	% similarity	domain	nuc. acid binding	function
MSL1	21.8%	coiled-coil		interaction with MSL2
	23.6%	PEHE		interaction with MSL3, MOF
MSL2	32.5%	RING		E3 ubiquitin ligase, homeostasis
	40.5%	CXC	x	DNA binding, X targeting
MSL3	43.3%	Chromo	x	H3K36me3, H4K20me, DNA binding?
	22.4%	MRG		stimulates HAT activity
MOF	32.9%	Chromobarrel	x	modulates HAT activity
	63.0%	HAT		Histone Acetyltransferase
MLE		RB1/2	x	dsRNA binding
		ATPase		ATP hydrolysis
		Helicase		rox RNA unwinding
		G-rich	x	modulates RNA binding specificity

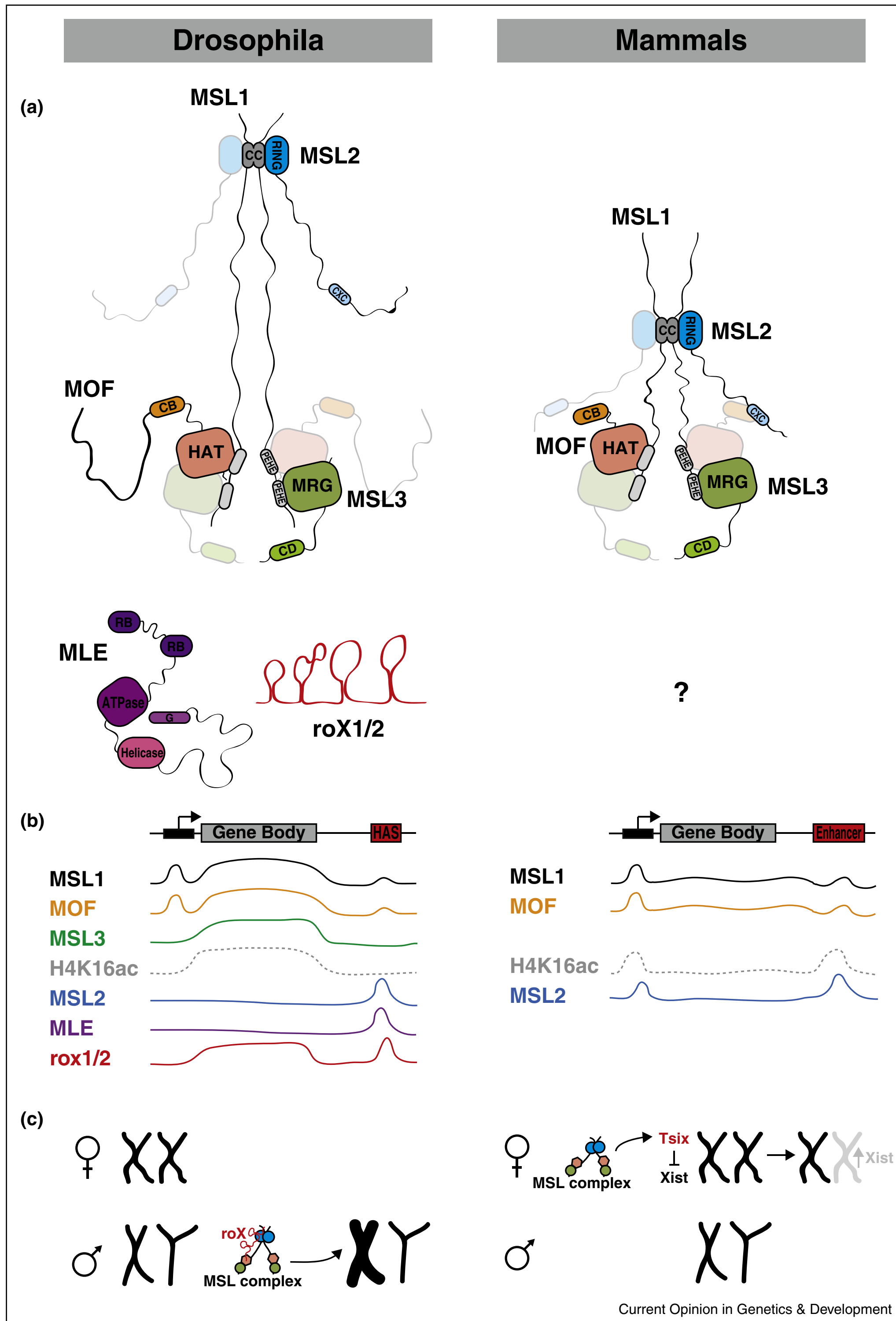
Overview of the domain architecture and functions of the *Drosophila* and human MSL complex proteins. The core MSL complex members MSL1, 2, 3 and MOF have distinct domains, which each are responsible for different functions. Percent similarity to the human proteins was calculated using the CLUSTALO program. The domain architecture of the complex members is remarkably similar between *Drosophila* and mammals, however note, that most of the mammalian proteins are considerably smaller. The PEHE and CXC domains are named after the amino acids, which are characteristic for these domains: proline (P), glutamate (E), histidine (H), glutamate (E) for PEHE and cysteines (C) intervened by any amino acid (X) for CXC [86]. CC: coiled-coil, RING: really interesting new gene, CD: chromodomain, MRG: morf-related gene, CB: chromobarrel domain, HAT: histone acetyltransferase, RB: double-stranded RNA binding domain, G: glycine-rich C-terminus.

consisting of MSL2, MLE and to some degree MSL1 resides at HAS [23]. Furthermore, it is possible that MSL complex targeting by MSL2 is aided by the presence of other co-factors [24,25]. Interestingly, on the one side targeting appears to be dynamic, as inhibition of transcription leads to loss of the MSL complex members on the X chromosome [26]. On the other hand, a FRAP (fluorescence recovery after photobleaching) study revealed remarkably stable MSL2 association with the X chromosome [27]. Therefore, a combination of dynamic and stable interactions helps in establishing dosage compensation.

MSL complex assembly and control of its protein levels is mediated by the MSL2 RING domain (Figure 1). Overexpression of MSL2 results in inappropriate MSL complex binding to autosomes [28]. By contrast, depletion results in destabilization of MSL3 and MLE and thereby disintegration of the MSL complex [29]. MSL2 also negatively controls MSL1 levels, as MSL1 mutants that

(Figure 1 Legend Continued) male X. **(d)** Homeostasis: Homeostasis of functional MSL levels on chromatin occurs via MSL2-mediated ubiquitination and degradation of MSL2 and MSL1. As MSL1 forms the integral scaffold of the complex, degradation of MSL1 will lead to complex disassembly. Most probably, homeostasis is required to prevent accumulation of MSL complexes, which might lead to unwanted MSL complex association with autosomes.

Figure 3



Comparison of the architecture and function of the *Drosophila* and mammalian MSL complexes. **(a)** The core *Drosophila* and mammalian MSL complexes adopt the same overall architecture consisting of a MSL1 dimer, which is bridging interactions with MSL2 at its N-terminus, as well as MOF and MSL3 at its C-terminus. Note, however, that the unstructured region between the MSL1 N-terminus and C-terminus is smaller in mammals, resulting in reduced complex size. The RNA helicase MLE and the ncRNAs roX1/2 are important functional components of the *Drosophila* MSL complex. Whether an RNA component is part of the mammalian MSL complex is not known. **(b)** Schematic chromatin binding profiles of the MSL complex and H4K16ac on the male X chromosome. Association with promoters (black box with arrow), gene bodies (grey) and

fail to interact with MSL2 can be expressed to much higher levels than the wild-type MSL1, *in vivo*. Furthermore, interaction of MSL1 with MSL2 is essential for dosage compensation [30•].

Recent biochemical and structural studies revealed the molecular basis for this, showing that the MSL2 RING domain acts both as an enzyme as well as a protein–protein interaction module [30•,31]. Two MSL2 alpha helices interact with an MSL1 dimer formed through an N-terminal coiled-coiled region in a 2:2 stoichiometry. These findings were unexpected and showed, that the core MSL complex is most probably an octamer consisting of two molecules of each MSL1, MSL2, MSL3 and MOF (Figure 3a).

The MSL2 RING finger itself contains seven absolutely conserved cysteine residues coordinating two zinc atoms and does not participate in the interaction with MSL1. It mediates E3 ubiquitin ligase catalytic activity and interestingly, shows an unusual conformation of the putative E2 interaction surface possibly reflecting an autoinhibited state. Previous studies have demonstrated that MSL2 interaction with the complex significantly enhances its enzymatic activity [30•]. Apart from autocatalytic activity, MSL2 ubiquitinates MSL1, which probably results in the buffering of MSL complex levels by proteasomal degradation (Figure 1d) [31,32]. Whether MSL2 has other substrates in *Drosophila* is not known and will be the matter of future investigations.

The architecture of the MOF HAT domain enables its function as enzymatic and protein–protein interaction module

After recognizing and binding HAS, the MSL complex fully assembles and spreads from these docking sites to the rest of the X chromosome resulting in chromosome-wide H4K16ac (Figure 1c). H4K16ac is catalysed by the MYST-family histone acetyltransferase (HAT) MOF [12••,33,34]. *In vivo*, *mof* mutation results in a loss of H4K16ac from MSL-target genes [35••]. However, a partial complex consisting of MSL2, MLE and to some degree MSL1 remains at HAS, demonstrating that MOF participates in downstream events after initial targeting of the X chromosome and complex assembly [23,36••,37•].

X-ray crystallography revealed that the HAT domain of MOF uses a catalytic glutamate residue to transfer the acetyl moiety from CoA to the acceptor lysine, probably in a one-step catalytic mechanism [38]. A cysteine-rich zinc-binding module embedded in the N-terminus of the HAT domain is important for substrate recognition [39]. Furthermore, enzymatic activity is enhanced in the presence of MSL1 and MSL3 [5] and is modulated by the N-terminus of MOF, which is unique to *Drosophila* MOF [40]. Indeed, this property is crucial for dosage compensation and spreading into gene bodies of X-linked genes, in contrast to the autosomal binding in both *Drosophila*, as well as mammalian cells, where MOF seems to be restricted to promoters (Figure 3b) [41•].

The MOF HAT domain, apart from its enzymatic function, is also responsible for interaction with the core MSL complex. The interaction interface is formed between the MOF HAT domain and an alpha helix in the C-terminal PEHE domain of MSL1 and involves multiple hydrogen bonds and salt-bridges [38]. The MSL1 residues responsible for these contacts are highly conserved, and interestingly, they are also found in the PEHE domain of the NSL complex member NSL1. This common mode of interaction explains, why association of MOF with the MSL and the NSL complexes is mutually exclusive [17] and suggests, that through such interactions, MOF might be associated with complexes other than MSL and NSL complexes. As such, MOF might acetylate many more proteins than previously anticipated.

Accordingly, MOF binds to autosomal gene promoters in both male and female cells independently of the MSL complex [35••] and is the major HAT in both sexes [40]. Furthermore, it has been demonstrated that mammalian MOF acetylates a number of substrates other than the H4 tail, MSL3 [29] and p53 in the context of the NSL complex in mammalian cells [42].

MSL1 forms the dimeric 'heart' of the MSL complex

MSL1 serves as an integral scaffold protein of the MSL complex and is responsible for the formation of the MSL octamer (Figure 3a). Its N-terminal coiled-coil dimer mediates interaction with MSL2 [30•]. The C-terminal PEHE domains interact with MOF and MSL3 [38]. Between the N-terminal coiled-coiled and the C-terminal PEHE domains, MSL1 contains a large stretch of putatively unstructured amino acids (152–885). Indeed,

(Figure 3 Legend Continued) high affinity sites (HAS, red box) is distinct for the individual members. This suggests that complex assembly might be dynamic and intrinsically allow the formation of subcomplexes possibly reflecting the different stages of dosage compensation (targeting, assembly, spreading, homeostasis). Note, that some binding, for example MSL1 association with promoters, is also independent of dosage compensation. In mammals, some MSL complex members, instead of high affinity sites, bind to enhancers (red box). **(c)** Comparison of dosage compensation systems in *Drosophila* and mammals with respect to the MSL complex. In flies, the MSL complex and its integral ncRNA roX, physically associate with the single male X chromosome resulting in chromosome-wide transcriptional upregulation by H4K16ac. In mammals, dosage compensation is achieved by inactivation of one of the two X chromosomes during female development. In mouse embryonic stem cells, the MSL complex targets the regulatory region of the ncRNA *Tsix* (Xist antisense gene), which plays a central role in regulating levels of *Xist* at the onset of X inactivation. Ultimately, during the process of differentiation one of the two *Xist* alleles becomes hyperactivated producing a ncRNA, which coats the entire X chromosome in *cis* and triggers chromosome-wide silencing.

MSL1 also exhibits an unexpected behaviour *in vivo*, as it associates with promoters independently of the other MSL complex members and/or a functional dosage compensation pathway [30•]. Whether the unstructured amino acids and/or novel interaction partners are involved in this binding and whether promoter association is required for a more specific aspect of transcription regulation remains elusive till date.

MSL3 is an adaptor protein bridging multiple chromatin interactions

MSL3 contains two adaptor modules: the N-terminal chromodomain (CD) and the C-terminal MRG domain. Earlier data indicated that the CD is involved in H3K36me3 recognition [43,44,45]. H3K36me3 chromatin is preferentially found towards the 3′ end of actively transcribed genes and its reduction results in a X-specific depletion of H4K16ac [46]. These data are consistent with a model, in which the MSL complex through the MSL3 CD-H3K36me3 interactions spreads on actively transcribed, X-linked genes independently of the actual gene sequence (Figure 1c). However, the above model was brought into question as the structural analyses of the MSL3 CD revealed an unusual polar surface, which surprisingly makes up a ternary complex together with DNA and H4K20 monomethylated histone tails [47,48]. Such a binding does not occur, if H4 is acetylated at K16. How H4K20me, a mark that has been involved in DNA damage, DNA replication and higher order chromatin architecture, relates to dosage compensation *in vivo* is currently an unsolved question. Again, it is possible that MSL3 and its CD function outside the dosage compensation pathway and in this context, H4K20 monomethylation might be important.

The MRG domain of MSL3 is responsible for interaction with MSL1 and is required to stimulate HAT activity of MOF [4]. The MRG-mediated interaction between MSL1 and MSL3 occurs via highly conserved phenylalanine residues of MSL1, which insert into several hydrophobic pockets of MSL3 [38]. Point mutations of these residues result in dissociation of MSL3 from the MSL complex and, consequently, in compromised dosage compensation. How the MRG domain stimulates HAT activity of MOF is unknown. Indeed, the widespread roles of MRG domain proteins, for example in RNA splicing [49], suggest that the regulatory potential of MSL3 and its MRG domain has not been fully elucidated, yet.

Nucleic acid-binding domains within the MSL complex

Apart from the DNA-binding MSL3 CD (see above), the core MSL complex contains two additional nucleic acid binding domains (Figure 2). Firstly, the MOF chromobarrel domain is an RNA binding module [50]. Originally considered a regular CD, later structural studies revealed that it adopts a beta-barrel structure that is distinct from the classical CD [51]. Mutations of residues essential for RNA binding (Tyr416 and Trp426) result in the complete absence of male progeny. Biochemical assays revealed, that the main function of the chromobarrel domain is to control enzymatic activity of MOF [40].

Secondly, the MSL2 CXC domain is a nucleic acid binding module and this plays a critical role in MSL complex targeting to the X chromosome (Figure 1a). The CXC domain is required, but not sufficient for MSL2 binding to DNA [22]. The solution structure of the CXC domain has been recently determined by nuclear magnetic resonance (NMR) [52]. It contains a cluster of nine strictly conserved cysteine residues, which coordinate three zinc ions. This suggests that the domain has maintained DNA binding properties throughout evolution.

Tethering experiments, however, revealed, that in *Drosophila*, MSL2 requires a co-factor to specifically recognize HAS sequences on the X chromosome and initiate dosage compensation. The recently identified protein CLAMP might provide such a link. However, since CLAMP is bound throughout the genome, its exact contribution towards dosage compensation requires further work [24].

roX RNAs contain hotspots for MSL complex assembly

The identification of the non-coding RNAs (ncRNAs) Xist and roX1/2 involved in dosage compensation in mammals and *Drosophila*, respectively, have pioneered a whole field working on chromatin-associated ncRNA activities [53]. The two functionally redundant ncRNAs roX1 and roX2 are integral components of the MSL complex in *Drosophila* [6•,36••,50,54,55] (Figure 3). It is fascinating that the roX1 and roX2 genes itself are encoded on the X and contain a HAS, suggesting that they provide unique entry sites for the MSL complex. Indeed, MSL complex assembly will only be efficient, if it occurs in association with the X chromosome [19].

Incorporation of roX1/2 into the MSL complex is catalysed by the RNA helicase MLE and involves transient RNA-mediated interactions with the core MSL complex (Figure 1). Chromatin isolation by RNA purification (ChIRP) showed, that roX2 associates with male X-linked gene bodies and peaks at HAS, reflecting the pattern of the core MSL complex and in particular MSL2 [56,57]. The interplay between roX1/2 and MLE has been recently explored in greater detail [57–59]. *In vivo*, individual-nucleotide resolution UV crosslinking and immunoprecipitation (iCLIP) analysis revealed that MLE and MSL2 bind distinct stem-loop structures within roX1 and roX2, which cooperate to provide functional platforms for MSL complex assembly and spreading. Interestingly, MLE remodels these stem-loop structures and thereby, integrates roX1/2 into the MSL complex. Within the

complex, ncRNA is most probably handed over from MLE to MSL2, as both proteins bind to the same roX1/2 regions forming double-stranded RNA domains. Indeed, association of MLE with both the complex as well as with roX1/2 seems to be transient and requires co-factors such as UNR [60]. In this context, it is interesting to note, that MLE has a role in splicing of the *para* RNA, a gene encoding a sodium channel [61]. This reinforces the notion, that many of the MSL complex members might play vital roles outside the core complex.

Transcription regulation by the MSL complex and H4K16ac

The ultimate outcome of the MSL complex action on the male X chromosome is upregulated transcription, independently of the actual gene sequence and length. MSL-mediated H4K16ac might inhibit chromatin compaction directly [62] or influence nucleosome remodelling and spacing [63], for example in the context of trans-tail histone modification patterns [64,65]. Which stage of the RNA Polymerase II (RNA Pol II) transcription cycle (initiation, pause-release, elongation or termination) is affected during dosage compensation has been extensively studied over the past years. Firstly, elevated H4K16ac might enhance accessibility at the promoter, where transcription factor binding might occur more frequently [66]. In agreement with this model, RNA Pol II is significantly enriched at male X-linked promoters compared to autosomes or females [15••]. On the other hand, GRO-seq experiments [67] mapping nascent RNA production in male tissue culture cells showed that transcriptional elongation appears to be enhanced on X-linked versus autosomal genes [16••]. Furthermore, direct nascent RNA sequencing (DnRS), a method that captures the actual position of RNA Pol II at steady-state, showed increasing Pol II along the gene body towards the 3′ end of the X-linked genes in comparison to autosomes in male S2 cells[68]. Indeed, H4K16ac is preferentially enriched on gene bodies of active X-linked genes [37•]. Taken together, the MSL complex most likely not only facilitates early promoter events such as Pol II recruitment and pause release but also facilitates RNA Pol II processivity and could also ensure efficient termination [69] of X-linked genes.

Importantly, all the methods used to date capture an average over a population of events, involve extensive sample preparation and lack temporal resolution. We therefore envision that single cell and kinetic analyses will finally allow dissecting, at which steps the MSL complex and H4K16ac globally affect the transcription machinery on the male X chromosome. Such studies should aim at visualizing individual rounds of transcription in a time-resolved manner rather than looking at averages of cells.

MSL complex function in mammals

Despite the fact that dosage compensation in mammalian cells is fundamentally different compared to *Drosophila* [70], at least the core MSL complex consisting of MSL1, 2, 3 and MOF is conserved in mammalian species (Figures 2 and 3) [71•,72]. This provides a unique opportunity to study the MSL complex independently of the dosage compensation system. Indeed, two important regulators of dosage compensation, an RNA helicase homologous to MLE or a ncRNA component such as roX have not been identified in the mammalian complex, so far [72,73•]. Interestingly, since MLE and MSL2 bind to relatively small stem loop structures within roX RNAs *in vivo*, it is possible that if the orthologues interact with ncRNAs, the overall size of such ncRNAs could also be variable.

Recently, H4K16ac and the core mammalian MSL complex have been studied genome-wide in mammalian cells and revealed a remarkable functional complexity. Firstly, the MSL complex seems to co-operate with the NSL complex in regulating housekeeping genes through promoter association in a cell-type invariant manner [41•,74]. Indeed, association with the NSL complex seems to be the dominant function of MOF, at least on a genome-wide level. Interestingly, a very small fraction of genes showed exclusive enrichment for the MSL complex, including the regulatory region of *Tsix*, a non-coding transcript that is critically involved in orchestrating X inactivation in rodents [75]. Therefore, the MSL complex is also required for efficient *Tsix* expression and, in consequence, determination of transcription and accumulation of *Xist* in differentiating female murine embryonic stem cells. Remarkably, there is also evidence for a function of MOF and/or the MSL complex in upregulating the active X chromosome, which is currently a matter of active investigation [76–79]. Certainly, additional studies will be essential for clarifying the role of the MSL complex in regulating mammalian X inactivation as well as activation.

Interestingly, mammalian MSL complex members also appear to bind chromatin individually, suggesting that they might carry regulatory potential independent of the core MSL complex. Particularly, MSL2 binds to a large number of genomic locations independently of the MSL complex. Secondly, MSL2 and to a certain extent also MOF associates with tissue-specific enhancers. Because H4K16ac has been found at enhancers, while surprisingly not affecting chromatin accessibility, it is possible that MOF and/or MSL2 regulate enhancers in a completely novel manner than appreciated from earlier studies in *Drosophila* [80]. One possibility is that they might regulate transcription of enhancer RNAs, which have been recently identified as crucial regulators of enhancer function [81].

Conclusions

Structural, biochemical and genome-wide studies performed in the recent years have shed light on the highly

modular architecture of the MSL complex, which has evolved to function as a male-specific transcription regulator on the *Drosophila* X-chromosome. Although these studies advanced our understanding of the MSL complex modules, we are currently missing the bigger picture. How do these modules play together in the full complex? How does the MSL complex achieve such a remarkable precision in targeting as well as its impact on gene expression? And how do the chromatin binding profiles relate to biochemically defined (sub)complexes exerting different MSL complex functions: targeting, assembly, spreading, homeostasis? These compelling questions still await their answer. We envision, that structural analyses combined with studies focusing on complex dynamics using novel single-molecule and imaging techniques might provide important insights, which will finally help to understand this highly complex interplay of the MSL complex members in dosage compensation in flies.

On the other hand, the modular principle and the high degree of MSL complex conservation suggest that many of the members function also outside of dosage compensation. This has become particularly evident in the recent studies in mammals, revealing that we have probably only scratched the surface in understanding the regulatory potential of the MSL complex and its individual members.

Indeed, we currently lack in depth proteomic studies of the MSL complex members in other species than *Drosophila*. Considering the rapid developments in genome editing technologies, it will be feasible to perform such studies in an endogenous context and in different cell types in the near future. This will allow us to biochemically define individual pathways and functions, in which the MSL complex is acting. Looking at the MSL complex in a different light, it will be equally important to study MSL complex isoforms. Differential isoform expression is prevalent in mammalian systems, and in addition to different interaction partners, isoforms might explain the multiple facets of the MSL complex.

Furthermore, it is important to note that the full repertoire of substrates of the two enzymes, MOF and MSL2, is probably not fully elucidated, yet. For example, MSL2 ubiquitinates p53 and thereby promotes p53 translocation to the cytoplasm [82]. In addition, MOF acetylates p53, which might explain its role in DNA damage repair [83]. Identification of novel MOF and MSL2 substrates, in the context of the MSL complex and other complexes, will therefore be important jigsaw pieces in understanding MSL complex function.

Lastly, future studies will have to address the mechanism of MSL complex-mediated transcription regulation. For example, human MSL1/2 has been involved in H2BK34 mono-ubiquitination, which results in crosstalk with other histone modifications and enhanced processivity of RNA Pol II via PAF1 and pTEFb [84,85]. How the MSL complex affects the transcription machinery directly, both during dosage compensation and in other processes, is an outstanding question in the field. Altogether, these studies will help to understand the multiple facets of the MSL proteins, which function in many essential processes, dosage compensation and beyond.

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