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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*

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Dosage compensation is a regulatory process that insures that males and females have equal amounts of X-chromosome gene products. In *Drosophila*, this is achieved by a 2-fold enhancement of X-linked gene transcription in males, relative to females. The enhancement of transcription is mediated by the activity of a group of regulatory genes characterized by the male-specific lethality of their loss-of-function alleles. The products of these genes form a complex that is preferentially associated with numerous sites on the X chromosome in somatic cells of males but not of females. Binding of the dosage compensation complex is correlated with a significant increase in the presence of a specific histone isoform, histone 4 acetylated at Lys16, on this chromosome. Experimental results and sequence analysis suggest that an additional gene, *males-absent on the first* (*mof*), encodes a putative acetyl transferase that plays a direct role in the specific histone acetylation associated with dosage compensation. The predicted amino acid sequence of MOF exhibits a significant level of similarity to several other proteins, including the human HIV-1 Tat interactive protein Tip60, the human monocytic leukemia zinc finger protein MOZ and the yeast silencing proteins SAS3 and SAS2.
Keywords: dosage compensation/*Drosophila*/histone acetylation/transcription

Introduction

Dosage compensation insures that males with a single X chromosome have the same amount of most X-linked gene products as females with two X chromosomes (Muller, 1932, 1950). In *Drosophila*, this equalization is achieved by a 2-fold enhancement of the level of transcription of the X chromosome in males relative to each X chromosome in females (reviewed by Baker *et al.*, 1994). The products of at least four genes, *maleless* (*mle*) and *male-specific lethal 1*, *2* and *3* (*msl-1*, *msl-2* and *msl-3*) are necessary for the onset or maintenance of dosage compensation. These genes were discovered on the basis of the male-specific lethal phenotype of their loss-of-function alleles (Fukunaga *et al.*, 1975; Belote and Lucchesi, 1980a; Uchida *et al.*, 1981; Lucchesi *et al.*, 1982). This lethality is the result of an abnormally low level of X chromosome transcriptional activity relative to that of the autosomes (Belote and Lucchesi, 1980b; Breen and Lucchesi, 1986). The MLE and MSL proteins produced by these genes are preferentially associated with numerous sites on the X chromosome in somatic cells of males but not of females, and the sex-specific association of any one of these proteins depends on the presence of the others (reviewed by Kelley and Kuroda, 1995). This observation suggests that these gene products must interact and form a complex. Preliminary direct evidence for such an interaction is provided by the observation that MSL-1 and MSL-2 can be co-precipitated with antisera against either protein (Kelley *et al.*, 1995). Binding of MLE and the MSL proteins to the X chromosome in males is correlated with the appearance of histone 4 acetylated at Lys16 (H4Ac16) on the same chromosome (Turner *et al.*, 1992) and at the same sites (Bone *et al.*, 1994).

We have used the phenotype of male-specific lethality to screen the X chromosome of *Drosophila melanogaster* for ethyl methane sulfonate (EMS)-induced mutations, identifying additional genes that may be involved in the regulatory process of dosage compensation. We isolated one such mutation (*mof*) and observed that dying mutant males lack the X-associated isoform of H4Ac16. MOF exhibits the signature motif for the acetyl coenzyme A binding site found in numerous and diverse acetyl transferases, and the *mof* mutation is a single amino acid substitution in the most conserved residue of this motif. This provides evidence that MOF is the histone acetyl transferase (HAT) responsible for the particular histone acetylation involved in the male-specific hypertranscription of X-linked genes.

Results

Mof functions in dosage compensation

Mutant *mof* males can develop to the third larval instar or the prepupal stage but fail to metamorphose and to hatch; the viability of mutant females is unaffected. Two lines of evidence establish that this male-specific lethality is due to a defect in dosage compensation. The first involves the effect of the *mof* mutation on the binding of the other dosage compensation regulatory factors to the X chromosome, as well as its effect on the normal consequences that this binding has on nucleosomal structure. We have determined by immunofluorescence that the association of MSL-1 and MSL-2 with the X chromosome of mutant *mof* male larvae is somewhat reduced, while that of MLE is substantially reduced. The X-specific isoform of histone 4 (H4Ac16) appears to be absent (Figure 1). The apparent reduction in the level of MSL-1

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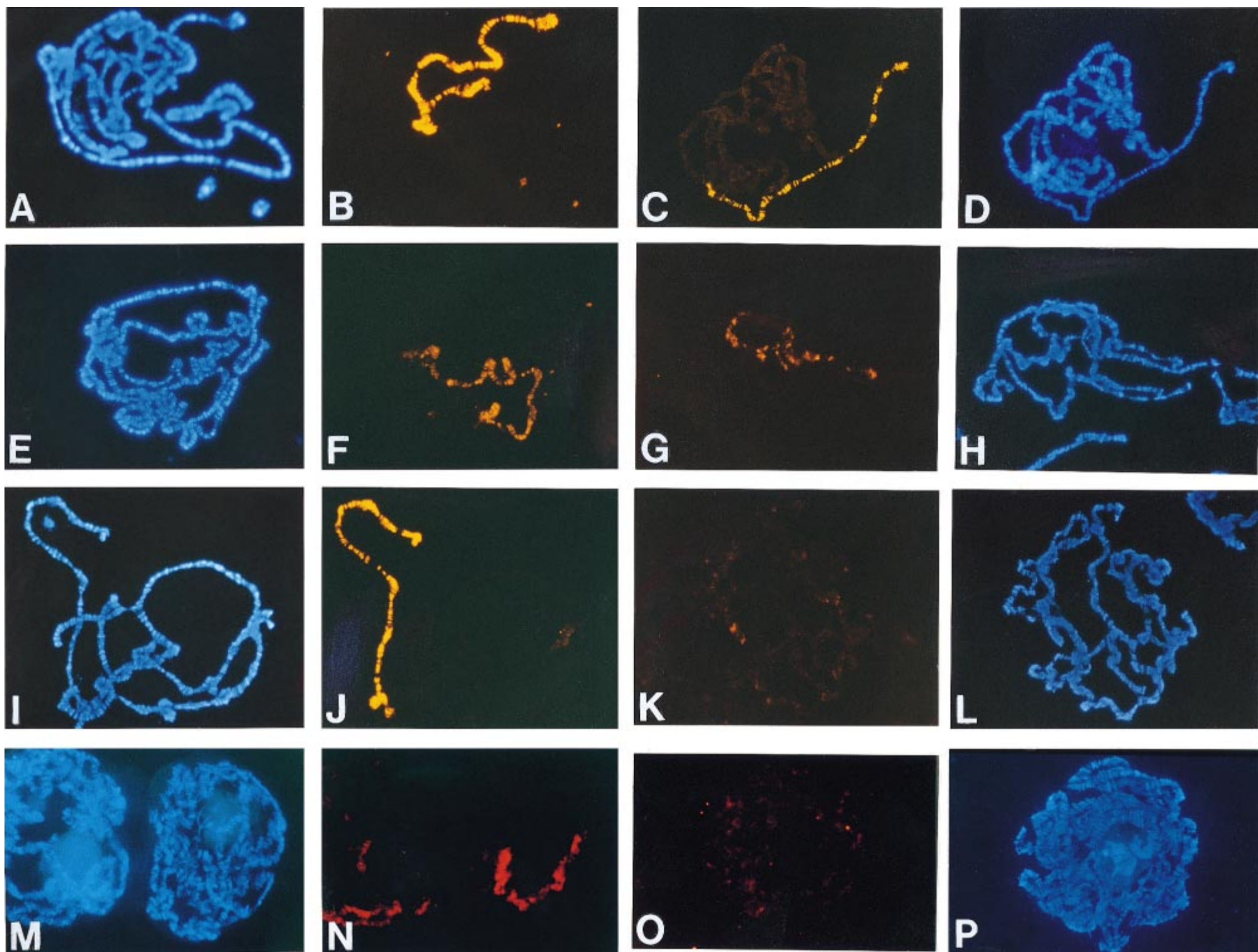


Fig. 1. Immunofluorescence staining of larval salivary gland chromosomes from *mof* mutant males. Panels **A**, **D**, **E**, **H**, **I**, **L**, **M** and **P** are stained with Hoechst 33258; panels **B**, **C**, **F**, **G**, **J**, **K**, **N** and **O** are the same nuclei stained with mouse anti-MSL-1 (B and C), mouse anti-MSL-2 (F and G), rabbit anti-MLE (J and K) or rabbit anti-H4Ac16 (N and O) followed by Texas Red-labeled rabbit anti-mouse or goat anti-rabbit serum. Mutant and control male larvae were distinguished by the use of markers that affect the color of the mouth hooks. In mutant males, the presence of MSL-1 and MSL-2 on the X chromosome is slightly reduced (C and G), the presence of MLE is significantly reduced (K) and there is no evidence of any H4Ac16 (O), in comparison with the respective controls (B, F, J and N).

and MSL-2 bound to the X chromosome may be the indirect result of the poor cytological condition of the salivary glands of moribund mutant male larvae. In contrast, the effect of the mutation on the level of MLE appears significant. As mentioned above, MSL-1 and MSL-2 fail to associate with the X chromosome in male larvae homozygous for loss-of-function *mle* mutations. Yet, RNase treatment of the male X chromosome removes MLE while leaving MSL-1 and MSL-2 undisturbed (Richter *et al.*, 1996). In light of these considerations it may appear that, while MLE is necessary for the initial binding of the dosage compensation complex to the X chromosome in normal males, its association with this chromosome may be stabilized through an interaction with nascent transcripts or with an unidentified RNA component of chromatin (Richter *et al.*, 1996). By interfering with the presence of H4Ac16 on the X chromosome and with hypertranscription, the *mof* mutation may destabilize this interaction.

The second line of evidence demonstrating that *mof* has a functional role in dosage compensation derives from the ability of the *mof* mutation to prevent the lethality caused by the ectopic expression of a particular dosage compensation regulatory factor in females. Females that carry a transduced *msl-2* gene under the control of a heat shock promoter exhibit a very long developmental delay and a significant loss of viability. This is caused by the fact that the presence of MSL-2 is sufficient for the formation of the dosage compensation complex and its association with both X chromosomes, presumably leading to an abnormally high level of X-linked gene products. Normal development is restored in these females by the presence in their genome of *mle* or *msl-3* null alleles in homozygous condition or by the presence of one *msl-1* loss-of-function allele, i.e. of a single dose of the wild-type *msl-1* gene (Kelley *et al.*, 1995). The same level of rescue is achieved by replacing one wild-type copy of *mof* with either a deficiency for the locus (L.Rastelli and M.Kuroda, personal communication) or with the *mof* mutation (Figure 2).

Cloning of the mof gene

We mapped the *mof* mutation by conventional recombination with marked chromosomes to a position on the genetic map corresponding to region 5C on the cytological map of the X chromosome. Using RFLP mapping, we localized *mof* to a fragment on the molecular map of the 5C3 region

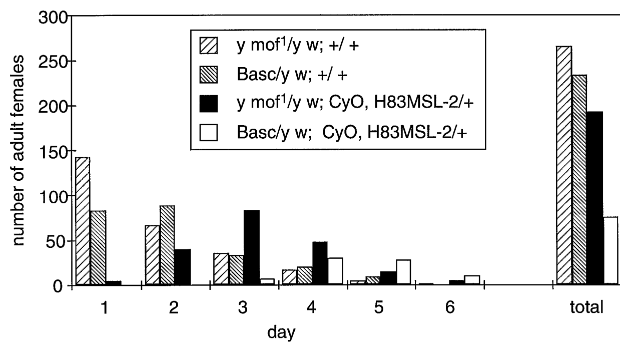
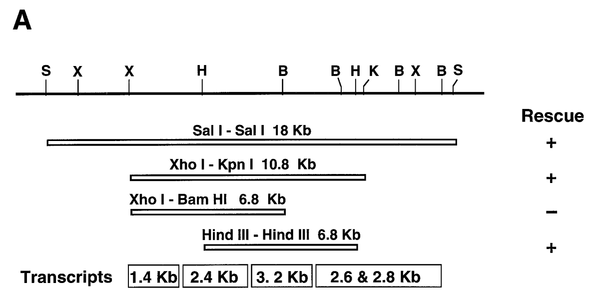
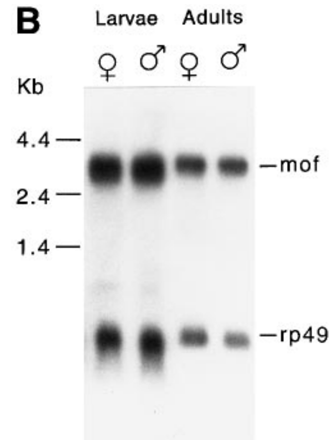


Fig. 2. Effect of the *mof* mutation on the rate of emergence and survival of females bearing an ectopic *msl-2* gene under the control of a heat shock promoter. Females of the genotype *y* mof^1*/Basc*; +/+ were crossed to *y w*/Y; *CyO*, *H83MSL-2*/+ males. The four types of progeny females listed in the Figure are expected to occur in approximately equal numbers. Females bearing the *H83MSL-2* transduced gene (open bars) have a much slower developmental rate and are fewer in number than their sisters lacking this construction (hatched bars); the presence of the mof^1 mutant allele in their genome almost completely rescues these females (solid bars).





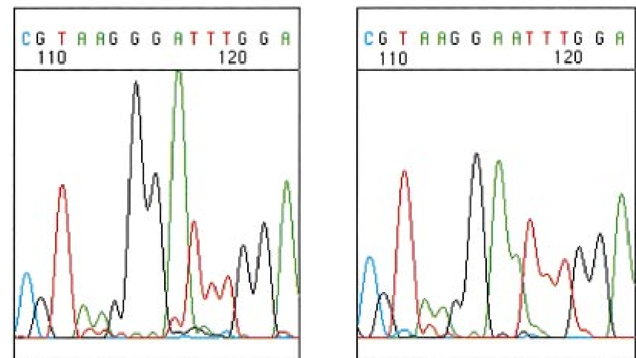


Fig. 3. Molecular mapping of the *mof* gene and its transcript. (**A**) Restriction map of the genomic region defined by RFLP mapping as containing the *mof* mutation. Below the map are represented the fragments that were used for germline transformation and the various transcripts that originate from the region. The effect of the transformation fragments on the male-lethal phenotype of transgenic males bearing the *mof* mutant allele identifies the 3.2 kb transcript as the *mof* product. (**B**) A Northern blot of polyadenylated RNA from male or female third-instar larvae or adults shows that the *mof* mRNA is present in all samples. Normalizing the *mof* RNA with the transcript of *rp49*, an autosomal gene encoding a ribosomal protein, indicates approximately equal amounts of transcript in both sexes. (**C**) Partial sequence of a cDNA from the parental strain used for the mutant search and of the corresponding region of the *mof* mutant. The blue, black, red and green tracings represent C, G, T and A, respectively. Note the G to A base change that results in a glycine to glutamate substitution.

that contains several transcription units. We used the rescue of the male-lethal phenotype by transposon-mediated germline transformation to determine which of these units was *mof* (Figure 3A). We established by Southern blot analysis that *mof* is a single copy gene, and by Northern blot analysis that its transcript is present in larvae and adults of both sexes (Figure 3B). A comparison of the sequences of several *mof* cDNAs with the genomic sequence indicated that this gene has no introns within its coding region and that it encodes a protein of 827 amino acids (DDBJ/EMBL/GenBank accession No. U71219). A comparison of the genomic sequence of the mutant allele with the sequence of the normal gene on the chromosome where the mutation was induced revealed a single amino acid change: a glycine is replaced by a glutamic acid at position 691 (Figure 3C).

Sequence analysis and functional domains

Using BLASTP (Altschul *et al.*, 1990), non-redundant database comparisons indicate a striking similarity of the MOF protein with Tip60, a recently identified human protein that appears to interact with the HIV-1 Tat trans-activator ($P = 4.1\times10^{-104}$). Discovered by means of the yeast two-hybrid selection system, Tip60 has been demonstrated to greatly enhance Tat transactivation of the HIV-1 promoter in transient expression assays (Kamine *et al.*, 1996). MOF also displays extended amino acid homology to MOZ, the human monocytic leukemia zinc finger protein ($P = 8.6\times10^{-90}$). The MOZ gene was recently identified as one of the two breakpoint-associated genes in the translocation found in the M4/M5 subtype of acute myeloid leukemia. The chromosome translocation fuses MOZ in-frame to CBP, the CREB transcriptional factor-binding protein (Borrow *et al.*, 1996). Finally, a significant level of sequence similarity is found between MOF, the *SAS2* ($P = 2.8\times10^{-45}$) and *SAS3* ($P = 3.3\times10^{-65}$) gene products of *Saccharomyces cerevisiae*, and other proteins of yet unidentified function (Figure 4A). In yeast, SAS2 is involved in silencing the telomeres; SAS2 and, to a lesser extent SAS3, are also involved in HMR locus silencing (Reifsnyder *et al.*, 1996). Within this region of homology, which extends for ~250 amino acids (Figure 4B), is present a domain common to many acetyl transferases and shown to be required for the binding of acetyl coenzyme A (Coon *et al.*, 1995). Deduced from

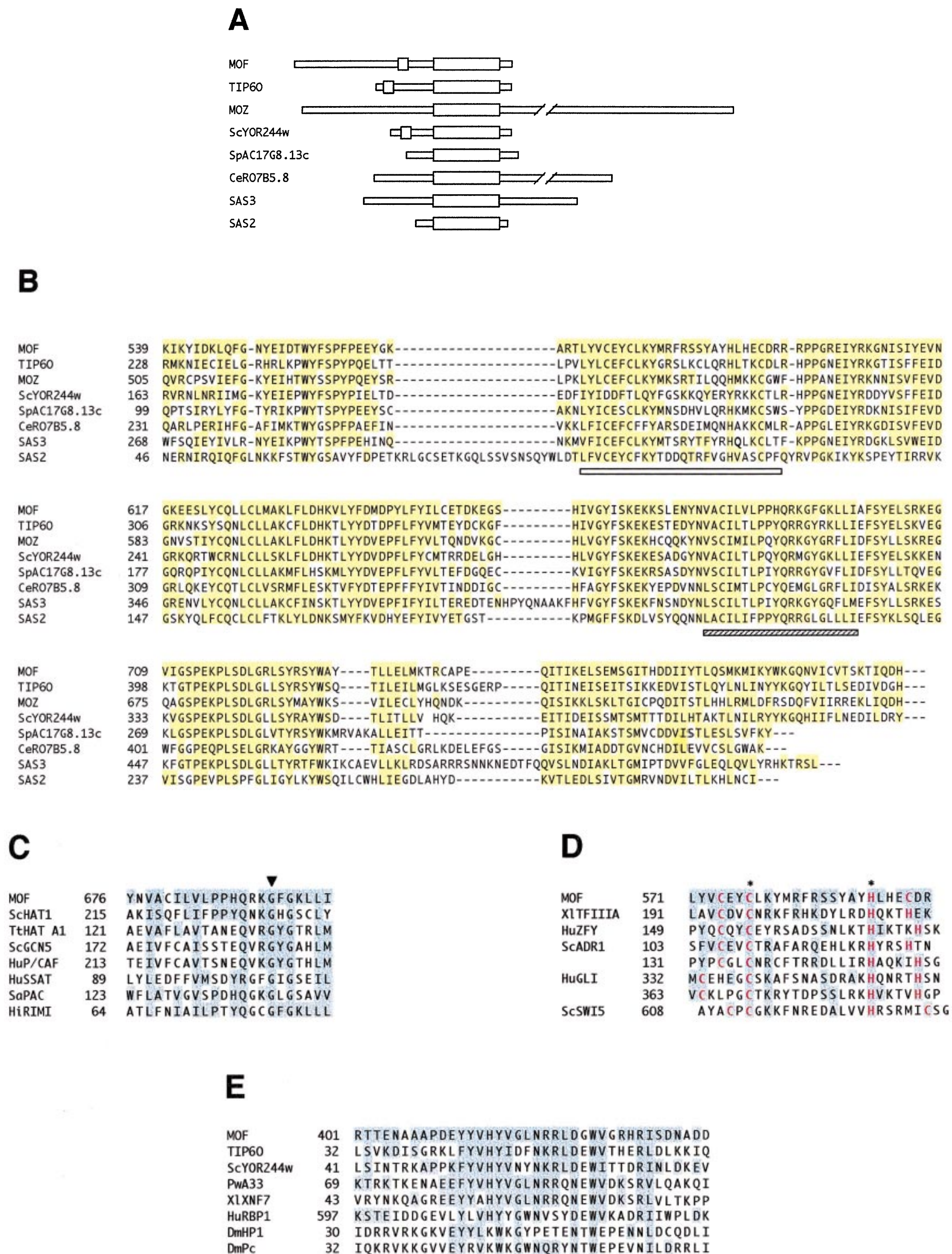


Fig. 4. Similarity of MOF with other proteins. (**A**) The regions of strongest homology are represented as large open boxes. (**B**) Sequence alignment of MOF with Tip60 (accession No. U74667), MOZ (accession No. U47742), ScYOR244w (accession No. Z75152), SpAC17G8.13c (accession No. Z69795), CeRO7B5.8 (accession No. Z72512), SAS3 (accession No. P34218) and SAS2 (accession No. S48299). Identity values range from 53% and 50% with respect to Tip60 and MOZ to 35% with respect to SAS2. The zinc finger and the acetyl coenzyme A putative binding site are indicated by the open or hatched bar, respectively. (**C**) Alignment of the acetyl coenzyme A domain of MOF with ScHAT1, TtHAT A1, ScGCN5, HuP/CAF, HuSSAT, SaPAC (*Saccharomyces alboniger* puromycin *N*-acetyl transferase, accession No. P13249) and HiRIMI (*Haemophilus influenzae* ribosomal-protein-alanine acetyl transferase, accession No. P44305). The arrowhead indicates a conserved glycine residue that is substituted with a glutamate in the *mof* mutant gene product. (**D**) Alignment of the C_2HC zinc finger of MOF with zinc fingers from XlTFIIIA (*Xenopus laevis* transcription factor IIIA, accession No. P17842), HuZFY (human zinc finger Y-chromosomal protein, accession No. P08048), ScADR1 (*S.cerevisiae* alcohol dehydrogenase II regulator protein 1, accession No. P07248), HuGLI (human glioma protein, accession No. P08151) and ScSWI5 (*S.cerevisiae switch 5* gene product, accession No. P08153). The distance between the C and H marked by asterisks is constant in all of the examples listed. (**E**) The colored areas highlight the similarities that exist between the MOF chromo-like domain and those of TIP60, ScYOR244w, PwA33, XlXNF7, HuRBP1, DmHP1 (*D.melanogaster* heterochromatin protein 1) and DmPc (*D.melanogaster* polycomb protein; Paro and Hogness, 1991).

its homology to mammalian spermidine/spermine acetyl transferases and microbial antibiotic acetyl transferases (Lu *et al.*, 1996), this domain is also found in enzymes known to acetylate histones, such as histone acetyl transferase 1 of yeast (Kleff *et al.*, 1995), histone acetyl transferase A of *Tetrahymena* (Brownell *et al.*, 1996) and its yeast homologue Gcn5p (Georgakopoulos and Thieros, 1992), and p300/CBP-associated factor P/CAF (Xiang-Jao *et al.*, 1996) (Figure 4C). A second domain, identified as a C_2HC/H zinc finger found in a variety of transcription factors and in oncogenes (Berg and Shi, 1996), is present in all of the MOF-related proteins with the exception of ScYOR244w. Some examples of proteins in which this type of zinc finger has been studied at the structural level are listed in Figure 4D. An additional region of homology is shared by MOF, Tip60 and the *S.cerevisiae* YOR244w (Figure 4A). This region is very similar to the chromo domain contained within a large number of proteins (Koonin *et al.*, 1995). With respect to this domain, the highest level of similarity with MOF is exhibited by human retinoblastoma-binding proteins RBP-1 and RBP-2 (Fattaey *et al.*, 1993) and the frog XNF7 (Reddy *et al.*, 1991) and newt PwA33 nuclear factors (Figure 4E). The latter is a maternal protein associated with nascent transcripts on the lampbrush chromosome loops of the oocyte (Bellini *et al.*, 1993). The chromo-like domain and the single zinc finger may represent sites of protein–protein interaction, although binding to DNA should not necessarily be ruled out (Pedone *et al.*, 1996).

Discussion

The presence of MOF homologues in organisms as divergent as yeast and humans suggests that these proteins play an important cellular role, presumably in the modulation of transcription. Such a role, documented with respect to Tip60, SAS2 and SAS3, is strongly indicated with respect to MOF by its involvement in dosage compensation. In *Drosophila*, because the *mof* mutation is not lethal in females, the general transcriptional function of the ancestral MOF protein appears to have been appropriated by the dosage compensation mechanism (which is male-specific) and to have been replaced in both males and females by the function of some other factor. The presence of the *mof* transcript in females, in which the MOF product is dispensable, is not an uncommon occurrence in *Drosophila* dosage compensation (Kelley and Kuroda, 1995) and, therefore, should not be interpreted as evidence of MOF function in this sex.

Recently, the study of transcription has been extended from the analysis of the preinitiation complex and its activation to the role played by conformational changes in nucleosome structure and organization. A major aspect of this research has dealt with the role played by the acetylation of the core histones (Brownell and Allis, 1996; Wolffe, 1996; Wolffe and Pruss, 1996). In *Drosophila*, the hypertranscription of the X chromosome in males appears to be directly correlated with the presence of a particular isoform of histone 4, H4Ac16, that is absent or significantly less abundant on the X chromosomes of females, and on the autosomes of both sexes. This feature of the male X-chromosome chromatin appears to be conserved throughout the genus (Bone and Kuroda, 1996; Steinemann *et al.*, 1996). In *D.melanogaster*, the presence of H4Ac16 has been shown to result from the binding to the X chromosome of the complex responsible for the mechanism of dosage compensation (Hilfiker *et al.*, 1994). The specific function of MOF in this regulatory mechanism is indicated by the presence of an apparent acetyl coenzyme A binding site. The functionality of this site is validated by the observation that replacement of Gly104 by an aspartate residue in the human spermidine/spermine acetyl transferase results in a protein with no measurable activity (Lu *et al.*, 1996). Significantly, the glycine in question corresponds to Gly691 in MOF which, when replaced by a glutamic acid, leads to the absence of demonstrable H4Ac16 histone isoform on the X chromosome and to the male-lethal phenotype. These considerations lead us to conclude that MOF may be directly involved in the acetylation of histone 4 at Lys16 on the X chromosome of *Drosophila* males.

It is possible, of course, that the activity of MOF is dependent on its association with other members of the dosage compensation complex or that it is an N-terminal acetyl transferase that exerts its effect on histone acetylation through some intermediary protein. In any case, MOF provides a functional link between this known nucleosomal modification and the transcriptional enhancement that is the basis of dosage compensation. Finally, our results suggest a functional role for two human proteins of significant interest. The sequence similarity of both Tip60 and MOZ to MOF leads to the potentially useful working hypothesis that the two human proteins exert their effect on transcription via acetylation of core histones, especially H4.

Materials and methods

Drosophila stocks and genetic procedures

Flies were cultured at 25°C on standard cornmeal–sugar–yeast–agar medium containing propionic acid and methylparaben as mold inhibitors and seeded with active dry yeast. EMS mutagenesis was performed according to standard procedure (Lewis and Bacher, 1968). A full description of the genetic procedures used to screen the X chromosome for male-specific lethals and to map the *mof* mutation by RFLP is provided elsewhere (A.Hilfiker, D.Hilfiker-Kleiner and J.C.Lucchesi, in preparation). To test for the rescuing effect of the *mof* mutation on females with ectopic expression of MSL-2, females of the genotype *y* mof^1/*Basc*; +/+ were crossed to *y w*/Y; *CyO*, *H83MSL-2*/+ males. The crosses were performed at room temperature, since this promoter has a sufficient level of constitutive activity to affect female viability (Kelley *et al.*, 1995). A full description of the mutants listed and of the *Basc* and *CyO* balancer chromosomes can be found in FlyBase: USA @ Harvard (http://cbbridges.harvard.edu:7081).

Genomic DNA and cDNA library screening

Cosmid clones spanning the 5C3 region of the X chromosome were obtained from the European Genome Project. The following cDNA libraries were screened: male or female third-instar wild-type (Oregon-R) larvae in the NM1149 vector, male or female adults (Oregon-R) in λgt10, mixed late third-instar larvae in λZAP and mixed wild-type adults (Oregon-R) in λEXLX. All the libraries were screened by standard protocols. DNA fragments representing steps of the walk were subjected to restriction endonuclease site mapping; appropriate fragments were subcloned in Bluescript KS(–) for further analysis.

P-element-mediated germline transformation

Genomic fragments were inserted in the CaSpeR4 transformation vector (Pirrotta, 1988) and injected into embryos of the w^{1118} recipient stock by standard procedures (Rubin and Spradling, 1982). The resulting adults were mated to appropriate individuals of the recipient stock and

transformants were detected in their progeny using eye color as a selection marker.

DNA sequencing and database comparisons

Sequencing was performed with an ABI Prizm automated sequencer. Homology searches were performed using BLASTP (Altschul *et al.*, 1990) and sequence alignment blocks were determined with the aid of the MACAW program (Schuler *et al.*, 1991). SeqVu software (Garvan Institute of Medical Research) was subsequently used to refine the multiple sequence alignments. Similarity was determined using the Blosum 62 amino acid substitution matrix (Henikoff and Henikoff, 1992).

RNA isolation and Northern blot analysis

Total RNA was prepared from third-instar larvae or adults by a standard protocol (Ish-Horowicz, 1989) with the sole modification that, in the case of larvae, grinding was performed in homogenization buffer rather than in liquid nitrogen. Poly(A)$^+$ RNA was isolated by oligo(dT) chromatography or by using an Oligotex mRNA kit (Qiagen). Northern blots were performed by separating poly(A)$^+$ RNA on agarose–formaldehyde gels and blotting onto Hybond-N membranes (Amersham). Hybridizations were performed according to the manufacturer's recommendations.

Polytene chromosome immunofluorescence

Mutant and control male larvae were distinguished by the use of markers that affect the color of the mouth hooks. Cytoimmunofluorescence on polytene chromosomes was performed as previously described (Hilfiker *et al.*, 1994). The following antisera were used: mouse anti-MSL-1, mouse anti-MSL-2, rabbit anti-MLE or rabbit anti-H4Ac16. Secondary antisera included Texas Red-labeled rabbit anti-mouse or goat anti-rabbit serum. All chromosome preparations were counter-stained with Hoechst 33258.

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