Role of the male specific lethal (msl) Genes in Modifying the Effects of Sex Chromosomal Dosage in Drosophila

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ABSTRACT

Immunostaining of chromosomes shows that the male-specific lethal (MSL) proteins are associated with all female chromosomes at a low level but are sequestered to the X chromosome in males. Histone-4 Lys-16 acetylation follows a similar pattern in normal males and females, being higher on the X and lower on the autosomes in males than in females. However, the staining pattern of acetylation and the mof gene product, a putative histone acetylase, in msl mutant males returns to a uniform genome-wide distribution as found in females. Gene expression on the autosomes correlates with the level of histone-4 acetylation. With minor exceptions, the expression levels of X-linked genes are maintained with either an increase or decrease of acetylation, suggesting that the MSL complex renders gene activity unresponsive to H4Lys16 acetylation. Evidence was also found for the presence of nucleation sites for association of the MSL proteins with the X chromosome rather than individual gene binding sequences. We suggest that sequestration of the MSL proteins occurs in males to nullify on the autosomes and maintain on the X, an inverse effect produced by negatively acting dosage-dependent regulatory genes as a consequence of the evolution of the X/Y sex chromosomal system.

Dosage compensation is the phenomenon by which the expression of genes on chromosomes at different doses is equalized. For the sex chromosomes of Drosophila, the X chromosome in males (XY;AA) is hypertranscribed so that its output equals that of the two X chromosomes of females (XX;AA) (Müller 1932; Lucchesi and Manning 1987; Baker et al. 1994; Kelley and Kuroda 1995; Birchler 1996; Arkhipova et al. 1997). The products of a set of genes identified in screens for male specific lethal (msl) mutations (maleless, mls-1, mls-2, mls-3, and mof) together with RNAs from rox1 and rox2 are preferentially associated with the male X chromosome and have been postulated to double its transcription rate (Kuroda et al. 1991; reviewed in Baker et al. 1994; Kelley and Kuroda 1995; Amrein and Axel 1997; Hilfiker et al. 1997; Meller et al. 1997). Furthermore, association of the male-specific lethal (MSL) proteins enriches acetylated histone H4 isoform Lys-16 residues on the male X (Turner et al. 1992; Bone et al. 1994).

A relationship between the mutational effect of the maleless (mle) gene and the association of MSL proteins on the X chromosome was proposed by immunostaining and chromosomal autoradiography experiments (Lucchesi and Manning 1987; Baker et al. 1994; Kelley and Kuroda 1995). In earlier studies, the reduced ratio of X:autosomal autoradiographic signals had been interpreted as a lowered expression for X-chromosomal transcription in mle/ mle males (Bolote and Lucchesi 1980). However, a study of the effects of the maleless mutation on the phenotypic, enzyme activity or specific transcript level of 39 genes indicated a general trend for maintenance of dosage compensation of the X and an overall doubling of the autosomal expression (Hiebert and Birchler 1994), when particular attention to absolute levels of gene expression was made. That is, hyperactivation of X-linked genes was retained in the mle/ mle mutant males but the unlinked autosomal loci were generally increased in expression.

This situation is analogous to the changes in gene expression that are found in chromosomal dosage series. When large chromosomal segments are reduced in dosage from two to one, many of the genes on the varied segment exhibit dosage compensation, but unlinked genes are usually doubled in expression (Birchler 1979; Devlin et al. 1982, 1988; Birchler et al. 1990; Guo and Birchler 1994). For compensation to occur, the twofold elevation of gene expression, operating in trans but affecting linked structural genes, cancels the twofold reduction in output from the target genes on the varied segment (Birchler 1981). The negative correlation between target gene expression and chromosomal dosage is referred to as an inverse dosage effect, because the response is within the range of one dose doubling expression and three doses reducing it to two-thirds relative to the normal diploid (see Figure 1). The predominant effect of changes in chromosomal dosage

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is an inverse correlation between the dose and the expression of the target genes (Birchler 1979; Devlin et al. 1988; Birchler et al. 1990, 1994; Rabinow et al. 1991; Guo and Birchler 1994; Bhadra et al. 1997a,b; Frolov et al. 1998). Many transcriptional regulators or chromatin components are dosage dependent (e.g., Dorn et al. 1993; Henikoff 1996). To date there are published accounts of six genes that will produce an inverse dosage effect upon the white eye color gene under at least some circumstances (Rabinow et al. 1991; Birchler et al. 1994; Bhadra et al. 1997a,b; Frolov et al. 1998) and numerous others have been identified (J. A. Birchler, unpublished results). Molecularly defined examples include RegA, an orthologue of a member of the NOT global negative regulatory complex of yeast (Frolov et al. 1998); Ultrafemale overexpression (=lola), a DNA-binding transcription factor (Giniger et al. 1994; Bhadra et al. 1997a); and apterous, a LIM family transcription factor (Cohen et al. 1992; U. Bhadra, M. Pal-Bhadra and J. A. Birchler, unpublished results). Despite a large number of such genes, multiple combinations varied in dosage together do not usually produce a response greater than the inverse range (Bhadra et al. 1998).

Because the expression of X and autosomal genes in mle mutant males is strongly similar to the dosage effects predicted for a large monosomic situation (Birchler 1979; Hiebert and Birchler 1994), we considered the possibility that the MSL localization evolved to modify the effect of transcriptional regulators or chromatin constituents that produce a negative dosage effect (Birchler 1996). Because the X chromosome composes 20% of the genome, the dosage effects of transcriptional regulators/ chromatin components can account for the dosage compensation of the X and the approximately twofold increase in unlinked autosomal expression as found with the mle mutation. The present experiments were performed to understand the relationship of MSL binding to the X chromosome, the changes in H4 acetylation, and the msl mutational effects on gene expression.

**MATERIALS AND METHODS**

**Drosophila stocks:** Flies were raised at 25°C. All stocks not specifically referenced in the text are described in FLYBASE (http://morgan.harvard.edu/fb.html/).

**Genetic crosses:** X-chromosomal genes on the X and A. Genetic crosses combined different white or yellow insertions noted in results with the mle 

\[ MLE \] mutation. To generate segregating populations, \[ w^{1118}/w^{1118}, mle^{Em}/mle^{Em} \] females were crossed to +/Y; T(2;3) CyO, Cy Tm/ TM3, 3r males. The Curly Tubby males from this cross were then crossed to \[ w^{1118}/w^{1118}, mle^{Em}/mle^{Em}, w^{+}/w^{+} \] females. The progenies are as follows: (a) \[ w^{1118}/Y, mle^{Em} \]

\[ mle^{Em}; w^{+}+/+, \]

(b) \[ w^{1118}/w^{1118}, mle^{Em}/mle^{Em}, w^{+}+/+, \]

(c) \[ w^{1118}/y, mle^{Em}/T(2;3) CyO, Cy Tb; w^{+}+/+, \]

and (d) \[ w^{1118}/w^{1118}, mle^{Em}/T(2;3) CyO, Cy Tb; w^{+}+/+. \]

For X-chromosomal insertions, \[ w^{1118}/w^{+}/w^{1118}, mle^{Em}/mle^{Em} \] females were crossed to +/Y; T(2;3) CyO, Cy Tm/ TM3, 3r males. The \[ w^{1118}/y, mle^{Em}/T(2;3) CyO, Cy Tb \] males in the progeny were then mated to \[ w^{1118}/w^{1118}, mle^{Em}/mle^{Em}, mle^{Em}/mle^{Em} \] females. The final progenies are as follows: (e) \[ w^{1118}/Y, mle^{Em}/mle^{Em}, mle^{Em}/mle^{Em} \]

(f) \[ w^{1118}/w^{1118}, mle^{Em}/mle^{Em}, mle^{Em}/mle^{Em} \]

(g) \[ w^{1118}/Y, mle^{Em}/T(2;3) CyO, Cy Tb, \]

and (h) \[ w^{1118}/w^{1118}, mle^{Em}/mle^{Em}, mle^{Em}/T(2;3) CyO, Cy Tb. \] These males and females (a-h) were used for RNA analysis. Similar genetic crosses were used to analyze yellow transformants in a background of DF1(ly) ac- on the X (Roseman et al. 1995).

To combine the two yellow inserts (one on the X and the other on chromosome 3) with H83M2, females from each stock [DF(1)ly ac- w^{1118}; SUPor-P-210-1 or SUPor-P-822-1] were
separately crossed with Df(1)~r1- ac w1118 Y; H 83 M 2/ T M 6, Tb males. The following segregating classes were examined from the X insert class: (a) y y' Y; H 83 M 2/ +, (b) y y' Y; T M 6, Tb +, (c) y y' y w 1118; H 83 M 2/ +, and (d) y y' y w 1118; T M 6, Tb +. The classes from the autosomal insert class are as follows: (e) y' Y; H 83 M 2/ +, (f) y' Y; T M 6, Tb y', (g) y y' y w 1118; H 83 M 2/ +, and (h) y y' y w 1118; T M 6, Tb y'.

A autosomal gene on the X and A; Genetic crosses were performed to determine the localization of the MSL proteins and dosage effects. Two different Adh transformant stocks were generated: Adh 'y' / Adh 'ry'; Adh 'bs' mle 'e' / Adh 'bs' mle 'e' and (b) Adh 'bs' y' / Adh 'ry'; Adh 'bs' mle 'e' / Adh 'bs' mle 'e' females (b). In another set of crosses, the Adh 'bs' y'/ Y; Adh 'bs' mle 'e' / Adh 'bs' mle 'e' females to generate (c) the Adh 'y' / Y; Adh 'bs' mle 'e' / Adh 'bs' mle 'e' males and (d) Adh 'ry' / Adh 'ry'; Adh 'bs' mle 'e' / Adh 'bs' mle 'e' females. These three proteins are co-localized on the polytene nuclei of the wild-type male larvae. The progeny are as follows: (e) + / Y; Adh 'bs' mle 'e' / Adh 'bs' mle 'e'; Adh 'ry' / Adh 'ry'; y/ Y, (f) + / +, Adh 'bs' mle 'e' / Adh 'bs' mle 'e'; Adh 'ry' / Adh 'ry'; y/ Y, and (h) + / Y; Adh 'bs' mle 'e' / Adh 'bs' mle 'e'; Adh 'ry' / Adh 'ry'; y. The (a), (b), (c), (d) and (e), (f), (g), (h) larvae were used to examine Adh transcripts.

**RESULTS**

**Binding of MSL proteins:** On full-length X-derived transgens: The polytene chromosomes of wild-type male larva were stained with anti-MLE, MSL-1, and MSL-2 antibodies. These three proteins are co-localized on the general cytological regions of the X (1B and 3C) surrounding the endogenous yellow and white genes (Figure 2A, arrows). We selected white and yellow transgene stocks to examine the association of MSL proteins at autosomal locations.

The white transgene construct P(w)B contains a 14.3-kb segment of the wild-type white sequence (Hazelrigg et al. 1984). Immunofluorescent staining using MLE, MSL-1, and MSL-2 antibodies of the polytene nuclei from two such white transformant stocks that have integrations at the 92BC [P{w}B B1-1] and 89A [P{w}B B1-1] regions was conducted. Neither of the integrated sites shows any trace of staining in contrast to the X chromosome in the same nuclei (Figure 2B). We did, however, detect binding at a few autosomal sites, which have been noted in earlier studies (Kuroda et al. 1991; Baker et al. 1994). Also, no binding was found when we examined a yellow construct that was inserted at the 41F site (data not shown). The yellow construct is 5.2 kb in length, containing 2.8 kb of 5' and 0.13 kb of 3' flanking DNA (Roseman et al. 1995). These results indicate that the MSL proteins are associated with the white and yellow regions on their normal X-chromosomal locations but not with their transposed autosomal sites despite the fact that they carry sufficient cis-acting sequences for normal expression and dosage compensation (see below).

on mini-white transgens flanked by su(Hw)-binding regions: Autosomally integrated mini-white genes are fully dosage compensated when flanked by the su(Hw)-binding sites (Roseman et al. 1995), which act as chromatin insulators. This construct also contains a yellow gene 5' to mini-white. Thus, two X-linked genes act as a combined target for MSL binding in this case. We have examined two such inserts present at 23A (SUPor-P-613-1) and 83B (SUPor-P-35-1) (Figure 2B, e, f, h, and i; Roseman et al. 1995). Similar to the full-length single transgenes, neither of the double transgenic constructs shows any
Figure 2.—MSL antibody probing of transgenes. (A) MLE antibodies are associated at the 1B and 3C regions surrounding the yellow and white genes on the male X chromosome. (a) DNA stain of male X chromosomal tip and (b) anti-MLE staining of the same chromosomal segment. Arrows indicate locations of the yellow and white genes. Bars, 10 μm. (B) Anti-MSL-1 staining for selected white transgenes. (a) Schematic diagram of a full length white containing transposon. The insertion site of each transgene is noted in parentheses. (b) DNA stain (Hoechst 33258) of chromosomes from third instar larvae carrying the white transgene. (c) Anti-MSL-1 stain (Texas red) of the same nucleus. (d) SuPor-P-It transposon (Roseman et al. 1995) carrying the mini-white gene flanked by su(Hw)-binding sites (triangles). This transposon contains a yellow gene upstream of mini-white. Black striped box represents eye enhancer sequences. (e) DNA stain of polytene chromosomes carrying this transposon. (f) Anti-MSL-1 staining of the same nucleus. (g) SuPor-P-It transposon, in which the eye enhancer is located outside the su(Hw)-binding
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Figure 3.—MSL-1 protein association with large chromosomal segments. (A) MSL-1 antibody probing of the salivary gland polytene chromosome from TE89 larvae using confocal microscopy. (a) The chromosomes of TE89 larvae stained with PI (red). (b) Same nucleus stained with anti-MSL-1 antibodies (green). (c) A superimposed image (yellow in the stained regions) of the first two figures. Bars, 10 μm. (Top right) An enlarged view of the insertion site from the merged image (box in c) showing the X-chromosomal bands (in brackets) at the 98F region illustrating the lack of anti-MSL-1 staining. (Bottom right) The same 3R chromosomal region from a wild-type nucleus. The arrow shows the point of insertion. (Extreme right) The tip of the X chromosome from a wild-type male larva. The bracket shows that the 3C2-3C5 region of the X chromosome, which is transposed to the autosomes in TEs, is strongly associated with MSL-1 antibodies on the X. (B) (a) Confocal image of the polytene chromosomes from third instar larvae of the Dp(1;3)w167k stock stained with PI. (b) MSL-1 antibody staining of the same nucleus showing that MSL-1 protein is associated with the X-chromosomal segment within the autosome. (Top right) Enlarged view of the boxed region in B (a), (middle right) anti-MSL-1 binding, and (bottom right) the merged image are shown. The large brackets show the limits of the X-chromosomal translocated segment. The small brackets (opposite side of the chromosome) represent the 3C2-3C5 region, the transposed segment in TEs, which is strongly associated with MSL-1 antibodies within the large translocation. Bars, 10 μm.

Evidence of detectable MSL binding (Figure 2B, f and i). The insulator sequences do not promote MSL binding on yellow and mini-white.

On the X-chromosomal bands in TEs: TE is a transposable element that carries the segment between the white and roughest loci flanked by Foldback element sequences (Ising and Block 1981). The distance between white and roughest is ~179 kb (Davis and Judd 1994), indicating that the TE carries a portion of the X chromosome greater than that length.

The strains TE16, TE89, and TE25 transpose the X-chromosomal segment 3C2-3C5, which is cytologically visible, to the 30C, 98F, and 100A regions, respectively. Immunostaining of chromosomes with MLE, MSL-1, and MSL-2 antibodies was carried out using male larvae of all three strains. In examination of whole nuclei, the MSL proteins are found to be localized on the X chromosomes along with a few sharp bands on the autosomes, but staining is not found in any of the transposable element integration sites using standard fluorescence analysis (data not shown). We therefore examined the sites using confocal microscopy to distinguish any low level of staining (Figure 3A). No trace of MSL proteins was found at these sites, although the X-chromosomal bands can be cytologically distinguished within the autosome (Figure 3A, top right). In comparison, the 3C region of the wild-type male X chromosome exhibits MSL binding (see Figure 3A, extreme right). Thus, this >179 kb of X-chromosomal material alone is not sufficient to initiate MSL protein association.

X;A insertion of 3A to 3E: A stock [Dp(1;3)w167k] that carries a large segment encompassing region 3A to 3E and inserted at the 87E region on the left arm of chromosome 3 (Datta and Kankel 1992) was examined. Immunofluorescent staining of polytene nuclei using region (Roseman et al. 1995). (h) DNA stain of polytene chromosomes containing mini-white and yellow genes. (i) Anti-MSL-1 staining of the same nucleus. MSL-1 is associated with many sites on the X chromosome, but not on the white, yellow, or mini-white insertional sites. Arrows indicate the location of the transposons. Arrowheads show single autosomal bands normally associated with anti-MSL-1 proteins. Bars, 10 μm.
Effect of the maleless mutation on expression of selected transgenes. The relative quantitative assay of each transgene is represented by the bar diagrams above the Northern blot panels. β-Tubulin transcripts act as a gel loading control. The effect of mle on white transgenes (A and C), yellow transgenes (B and D), and Adh transgenes (E) is shown. The X-derived transgenes (w, y) are homozygous in females and hemizygous in males for the X insertions. Autosomal insertions are present in one copy in both sexes. Adh transgenes are present in two copies for autosomal insert sites. The X-linked insertion is hemizygous in males and homozygous in females. The chromosomal locations of the transgenes are noted on the panel. An asterisk denotes values that are significantly different from that of the mle/1 male control at the 95% confidence level as determined by triplicate measurements. The male values denoted with a circle are significantly different from that of the control female.

MLE, MSL-1 (data not shown), and MSL-2 proteins show that they are associated along the length of the autosomally inserted X-chromosomal segment (Figure 3B, a and b). This result confirms that labeling is present at the w region in general. Moreover, the labeling is associated with the included 3C2–3C5 region, from which the TEs described above are derived. Therefore, the TE segment alone is not sufficient for MSL binding in the autosomal sites; whereas when flanking sequences are present, the MSL complex encompasses this region (Figure 3B, right, small bracket).

Effect of the maleless mutation: On transgene expression: To determine the relationship between the effect of the maleless mutation on gene expression and MSL binding, we used homozygous males of the null mutation, mlepml 8 F [P{(w)B}1-2] positions on the X produces an equal amount of white mRNA in both genotypes (Figure 4, A and C, respectively). However, integration of the white construct at the 10D [P{(w)AR}4-10] or 17D-F [P{(w)B}1-2] positions on the X produces an equal amount of white mRNA in both genotypes (Figure 4, A and C, respectively). We next examined the effect of mle on yellow transposons at 9CD (SUPor-P-822-1) and at endogenous loci of the tested transgenes. Parallel to the immunostaining, third instar larvae were also used for Northern analyses.

The X-linked genes, white and yellow, are partially dosage compensated at numerous sites on the autosomes (Hazelrigg et al. 1984; Qian and Pirrotta 1995; Roseman et al. 1995). The autosomally derived Alcohol dehydrogenase (Adh) locus exhibits dosage compensation when inserted into the X chromosome (Laurie-Ahlberg and Stam 1987). To determine the effect of mle on individual transgenes, we have combined the mlepml 8 mutation with Adh and representative yellow and white transgenes used for the tests of MSL binding.

The mle mutation elevates the expression of white in the autosomal locations 92BC (P{(w)B}2-1) and 89A (P{(w)B}1-1), relative to heterozygous brothers (Figure 4, A and C, respectively). However, integration of the white construct at the 10D [P{(w)AR}4-10] or 17D-F [P{(w)B}1-2] positions on the X produces an equal amount of white mRNA in both genotypes (Figure 4, A and C, respectively). We next examined the effect of mle on yellow transposons at 9CD (SUPor-P-210-1) and 18E (SUPor-P-73-1) on the X and on the third chromosome at an unknown location (SUPor-P-822-1) and at 82E (SUPor-P-F2-3). This transgene is unaffected on the
X, but is significantly elevated in the autosomal sites (Figure 4, B and D, respectively).

The autosomally derived Adh transposon carries an 8.6-kb Sad/Clai Adh fragment including 5.4 kb 5’ and 0.8 kb 3’ flanking DNA that is integrated at the 1CD (P-Adh1-215-4) region on the X and 67A (P-Adh1-257-1) region on chromosome 3 (Laurie-Ahlberg and Stamat 1987). The mlep mutation reduces the Adh transcripts at the 1CD region but elevates them at the autosomal site (Figure 4E). The autosomal expression of the Adh transgene in males is greater than females in some developmental stages (Pal-Bhadra et al. 1997). The mlep mutation makes this sexual dimorphism greater, suggesting that the effect of mlep on the Adh transgene intensifies the sex-specific expression (Figure 4E).

The results suggest that, similar to the mlep mutation (Hiebert and Birchler 1994), mlep8 modulates the transgenes at various locations, but affects the same construct differently depending on an X or autosomal location. The two X-derived genes, white and yellow, are unaffected by mlep in their normal location on the X (Hiebert and Birchler 1994) or as a transgene on the X. On the autosomes, however, both are elevated in expression. The autosomal gene Adh is reduced on the X but increased when inserted in an autosome.

On TE89: The transposition TE89 was spontaneous in origin and is present in a stock that carries a deletion [y Df(1) w sex Y; Tp(Y; 1;3) TE89] of the entire white locus. TE89 contains the whiteapricot mutation in which a copia element is inserted in the second intron of white. A copia-initiated readthrough transcript that can be distinguished from the other copia transcripts (Hiebert and Birchler 1992) and the normal white message were measured (Figure 5A).

Northern blots of total RNA extracted from w; mlep and TE89; mlep larvae were hybridized with the white exon 4-5 probe. This experiment allows one to compare w on the X where the MSLs bind and on the third chromosome where there is no MSL binding. The 2.6-kb white-initiated and 7.9-kb copia-initiated transcripts were analyzed (Figure 5B). A Northern profile shows that white transcripts are not affected in mlep males and females in the original X-chromosomal site relative to their mlep/+ brothers (Figure 5B) but are significantly elevated in TE89 (Figure 5B). White is dosage compensated in both locations although to a lesser degree in the autosomal site; that is, males with one copy show a greater expression than females with equal copy number. With the mlep mutation, dosage compensation for white-initiated transcripts is maintained in both the autosomal and X positions but the expression of the autosomal insertion is significantly increased.

The copia-initiated readthrough transcripts (7.9 kb) are present in both strains (Figure 5B). They are not dosage compensated in w; mlep/+, because males and females with one copy are equally expressed in accordance with previous work (Hiebert and Birchler 1992). Likewise, in TE89 the same transcripts are equally expressed in one-copy mlep/+ males and females, showing that copia fails to dosage compensate in either position at this developmental stage. Nevertheless, the abundance of the copia readthrough in mlep males in TE89 is significantly greater than that in the mlep/+ males (Figure 5B). This difference in readthrough transcripts in mlep males cannot be attributed to a generalized sexual dimorphism of copia expression, but is caused instead by the mutational consequences of mlep.

On Dp(1;3) w/+ Df(1;3) w/+ stock (duplicate for the 3A–3E region) used in immunostaining experiments described above was combined with Df(1) w1118, mlep8. We determined the effect of mlep on white transcripts emanating from the 3A–3E translocated segment. Four classes of segregating progeny were generated that carry a single copy of the X-chromosomal fragment but are heterozygous or homozygous for the mlep mutation. The level of white transcripts in mlep/+ males is nearly twofold greater than that in the one-copy mlep/+ females conforming to the expectations of complete dosage compensation in this case (Figure 5C). In contrast to other autosomal inserts, the amount of white transcripts accumulated in mlep8/mlep8 larvae is almost equal to mlep8/+ males (Figure 5C). These results indicate that mlep does not alter white expression in this autosomal construct. Thus, the mlep mutational effect is least effective on the regions of the genome exhibiting MSL association under normal circumstances.

Effect of the maleless mutation on chromosomal morphology: Squash preparations of polytene chromosomes from mlep/mlep male larvae show an altered morphology compared to wild-type chromosomes. We performed a quantitative measure of the X and autosomes using confocal microscopy (see materials and methods) to determine the average cross-sectional area of defined regions in wild-type, mlep/SM 6a, and mlep/mlep males and females.

The diffuse appearance of the normal male X chromosome has been postulated to be the cytological manifestation of the hyperactivation of X-linked genes (Mukherjee and Beermann 1965). The average cross-sectional area of the 1A–5A bands of the X chromosome in wild-type males is not significantly reduced compared to that of the X chromosomes in females (Figure 6). Likewise, the area of the autosomal segment (21A–23F) is similar between the sexes. The same areas of both the X and autosomal regions in mlep8/mlep8 males are significantly decreased compared with mlep8/mlep8 6a brothers, but remain greater than the asynapsed autosomal segment in the mlep8/SM 6a nuclei (Figure 6). The ratios of the respective measurements between mlep/mlep and wild-type males or mlep males and females shows that both the X and autosomal segments in mlep/mlep male nuclei are reduced by a similar magnitude relative to their controls. These results suggest that disruption of
Figure 5.—Effect of the maleless mutation on expression of larger autosomal insertions. (A) The genomic map and transcripts of \( w^a \). Selected restriction sites and position of the probe used in the Northern analysis are shown. (B) The effect of \( mle \) on two tightly linked promoters (white initiated and copia readthrough) were analyzed in \( w^a \) on the X and in TE89 on the third chromosome. The transcripts and their sizes are indicated on the left and right sides. In both locations, \( w^a \) is present in one copy in both males and females. (C) The Northern analysis of RNA from the X:A transposition Dp(1;3)w\(^{+67K} \) larvae with the \( mle^{m5} \) mutation. The transposition is present in one copy in both males and females. An asterisk denotes values that are significantly different from that of the \( mle^+/+ \) male control at the 95% confidence level as determined by triplicate measurements. The male values denoted with a circle are significantly different from that of the control female.
The MSL complex leads to a generalized reduction in chromosome size, providing no evidence for a differential change of either the X or the autosomes. The genome-wide changes in chromatin composition in the mle/mle males (see below) may account for this observation.

**MSLs in males compared to females:** To understand the differential response of the sexes to mle, salivary glands of normal males and females and selected msl mutants were mixed and their chromosomes were examined on the same slides for MSL binding and histone acetylation to allow a direct comparison. First, we examined the immunolocalization of MSL proteins on the polytene chromosomes in a mixture of normal male and female nuclei. The chromosomes were stained with either MLE or MSL-1 antibodies. In some cases, double staining for MSL-2, which is present only in males (Kelley et al. 1995; Zhou et al. 1995; Bashaw and Baker 1997), confirmed from which sex each nucleus was derived. In female nuclei, no trace of MSL-2 binding was found (data not shown), while both MLE and MSL-1 are associated with all chromosomes. In the male nuclei, all three proteins were associated with only the X (Figure 7A, a and b). In earlier work, Kuroda et al. (1991) also found a uniform MLE binding on all female chromosomes consistent with our findings. We cannot detect autosomal labeling in male nuclei (Figure 7, bottom). Therefore, the binding of the MSLs occurs on all chromosomes in females but appears to be limited to the X in males.

**MLE and MSL-1 binding is disrupted in msl mutant females:** Similar to X binding in males, a heteromic MSL protein complex is implicated for the MSL binding on the female chromosomes, although MSL-2 is absent in females and therefore is not included. Immunolocalization of MLE and MSL-1 was performed using normal, mle, msl-1, and msl-3 female nuclei each on separate slides and then the respective mutants in mixtures with normal nuclei. Chromosomal localization of MLE was found in all wild-type female nuclei but was disrupted in msl-1, msl-3, and mle females (data not shown). The lack of MSL-2 in females causes reduced levels of MSL-1 (Kelley et al. 1995). We therefore examined binding of MSL-1 in mle (Figure 7B, a and b), msl-1, and msl-3 females (data not shown). As with MLE, MSL-1 is clearly present in normals but fails to associate with msl mutant chromosomes, suggesting that a heteromeric complex presumably involving at least three proteins (MLE, MSL-1, and MSL-3) is required for the association with female chromosomes.

**Distribution of histone-4 acetylated lysine-16 isoform:** H4Ac16 is evenly associated with female chromosomes. Normal male and female nuclei were prepared together and examined in the same confocal microscopic field using antibodies against the lysine-16 acetylated form of histone-4 (Figure 8A, a and b). Preferential H4Ac16 accumulation is found on the male X chromosome. All female chromosomes show a low level of labeling relative to the male X but stronger than that exhibited by the male autosomes. Equal labeling of all chromosomes was clearly visible in female but not in adjacent male nuclei (Figure 8, bottom). Similar findings were observed in earlier experiments (Turner et al. 1992; Bone et al. 1994).

H4Ac16 is enriched on the autosomal insertion of an X segment that binds MSLs but not with TEs. To test whether H4Ac16 enrichment is correlated with MSL binding on the X segment in the autosomal sites, we examined the H4Ac16 presence on the chromosomes from the TE89, TE16, and Dp(1;3)w67K males. H4Ac16 is enriched on

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**Figure 6.—Measurement of chromosomal size in mle and normal genotypes.** The average area (±SE) was calculated from the mean of five width and length measurements in each of six different cross sections of each chromosomal region (1A–5F and 21A–23F) on the respective chromosomal arm in each nucleus using confocal microscopic images (×100). The values for the six cross sections were then averaged for each nucleus. The means of the assayed nuclei are reported. The values denoted with an asterisk are statistically different from those of the respective wild-type controls (males and females) at the 95% confidence level.
Figure 7.—MSL protein association with male and female chromosomes. (A) (a) Wild-type male and female nuclei are viewed in the same confocal microscopic field (stained with PI). (b) The same field probed for MLE protein. Only the X chromosome in male nuclei is labeled. (B) Effect of the mle mutation on MSL binding to female chromosomes. (a) A mixture of wild-type and mle female nuclei stained with PI in the same microscopic field. (b) MSL-1 staining of nuclei in the same field. MSL-1 proteins are only associated with wild-type female nuclei, but are not detectable in mle nuclei. (Bottom left) The magnified view of a male and a female nucleus stained with PI (boxes). (Bottom middle) MLE labeling of the same nucleus and (bottom right) merged images produced by superimposition of the DNA and antibody figures are shown. MLE proteins are associated with the X chromosome (greenish yellow) but fail to associate with the autosomes (red) in males. In the superimposed figure of female nuclei, all chromosomes are yellowish red indicating a low level of MLE binding. Bars, 10 μm.

the translocated 3A–3E region in the Dp(1;3)w^67k males but not detected at a high level on the X-chromosomal bands in the TEs (data not shown). The X chromosomes in the same nuclei in all cases show a high level of H4Ac16 enrichment. As a control, each preparation was stained in parallel with antibodies to histone 4 acetylated at lysine-8, which is not sex specific (Turner et al. 1992). No significant differences between preparations were found. These results suggest that the enrichment of H4Ac16 is correlated with the MSL binding on X transpositions.

H4Ac16 labeling in msl mutant males and females: An examination of mle or msl3 together with wild-type male nuclei in the same confocal microscopic field reveals that staining on the autosomes of msl mutants is more intense than on those of wild type but lower than the staining on the X chromosome in normal male nuclei. This low level of staining in msl mutant males is similar to the binding found with wild-type female chromosomes (Figure 8B, a and b). Thus, there is an equal accumulation of H4Ac16 residues on all chromosomes in mle and msl-3 mutants in lieu of an enriched H4Ac16 accumulation on the wild-type X. In contrast, no significant change in H4Ac16-binding intensity on the X and autosomes was found when a mixture of wild-type and either msl-1 or mle female nuclei were examined in the same microscopic field (data not shown). Thus, disruption of the MSL complex in mle females has no detectable effect on the H4Ac16 labeling.

MOF labeling in normal and msl mutants: The product of the mof gene is predicted to have histone acetylase activity (Hilfiker et al. 1997) and has been postulated...
to be responsible for the H4 acetylation that is correlated with the presence of the MSL complex. In mixtures of normal males and females, there is a preferential association of MOF with the X chromosome in males with a lower level on the male autosomes than on the female chromosomes present on the same slides (data not shown). When normal and mle male chromosomes are mixed and probed with anti-MOF, the distribution in the mle mutants is uniform on all chromosomes, but with a strong accumulation on a few specific bands (Figure 9). Thus, unlike other members of the MSL complex, MOF is still capable of associating with the chromosomes in msl mutant individuals. It appears to be able to dissociate from other members of the MSL complex under these circumstances and act independently.

**MSL-2 Initiates Sequestration of MSL Proteins to the X:** MSL proteins are sequestered to the X chromosome in males in the presence of MSL-2, suggesting that MSL-2 initiates the process. The expression of MSL-2 protein is inhibited in normal females by the action of Sex lethal (Kelley et al. 1995; Zhou et al. 1995; Bashaw and Baker 1997). We examined a stock (H83M2) in which MSL-2 is ectopically expressed in females. This P-element construct lacks the internal 52-nt intron and the putative Sxl-binding sites at the 5′- and 3′-untranslated terminal regions of msl-2 transcripts and is marked with a mini-white reporter gene (Kelley et al. 1995). Immunolocalization of MLE, MSL-1, and MSL-2 was carried out with the polytene nuclei of wild-type and H83M2 females prepared on the same slides. In all cases, MLE and MSL-1 proteins are associated with all female chromosomes in wild-type nuclei, but are associated only at a high level on the X chromosomes of the female nuclei in which MSL-2 is expressed (data not shown) in accordance with the results of Kelley and Kuroda (1995).

Double staining with H4Ac16 and MSL-2 antibodies reveals that H4Ac16 enrichment parallels MSL-2 localization in the H83M2/+ cells. This H4Ac16 enrichment on the female X chromosomes is accompanied by a
Figure 10.—H4Ac16 and MSL antibody binding in ectopically expressing MSL-2 females revealed by double immunolocalization using H4Ac16 and MSL-2 antibodies on the polytene chromosomes from wild-type and H83M2 females. (a) A mixture of PI-stained wild-type and H83M2 nuclei in the same confocal microscopic field. (b) MSL-2 antibody-stained nuclei in the same field showing that the X chromosomes in H83M2 nuclei are intensely stained. (c) Distribution of H4Ac16 residues of the same nuclei. H4Ac16 is strongly accumulated on the X’s in H83M2 females whereas in normal females a low level of staining is found on all chromosomes. Bars, 10 μm.

(Figure 11B). Ordinarily, the autosomally inserted X-derived transgene mini-white shows partial dosage compensation; i.e., females have significantly more than 50% of the male value (Qian and Pirrotta 1995; Roseman et al. 1995). Values in females of less than 50% indicate a reduction in expression of this autosomally inserted mini-white.

To analyze this effect further, we combined yellow transgenes used in the mle experiment above (one on the X and the other on the third chromosome) with the MSL-2-expressing transgene. The Northern profile of the segregating classes reveals that excess MSL-2 product in H83M2/+ males does not increase yellow transcripts in either location. However, in ectopically expressing MSL-2 females, the X insert is not affected but the autosomally inserted yellow gene is significantly reduced in expression (Figure 11C). These results suggest that H4Ac16 depletion on the autosomes reduces X-derived transgene expression under these circumstances.

**Gene expression in mof1 mutant individuals:** A survey
of selected X and autosomally encoded genes was tested in mof\(^1\) mutant individuals to examine the relative contribution of acetylation and dosage effects in males. This allele produces normal levels of MOF protein but has a single amino acid change that predicts a nonfunctional product (Hil\(\ddot{f}\)ker et al. 1997). Although the X association of other members of the MSL complex is destabilized to some degree in the mof\(^1\) mutants, the complex is still preferentially present on the X (Hil\(\ddot{f}\)ker et al. 1997). This experiment will determine the effect on gene expression of strongly reduced acetylation levels on the X.

Most X-linked genes are unaffected or increased in expression in mof\(^1\) mutants compared to normal brothers (Table 1; Figure 12). The only gene that exhibits a loss of dosage compensation is Salivary gland secretion protein 4 (Sgs4). The two genes, white and yellow, examined in other situations in this study, do not lose compensation. The white gene can only be examined between males and females, because the Basc balancer used in the cross carries a mutant allele, but compensation is maintained in this comparison. Of the autosomal genes examined, several show no difference or an increase in mutant males. Compared to the spectrum of effects seen with mle (Hiebert and Birchler 1994), Adh is not as strongly increased and \(\alpha\)Gpdh is not increased at all.

If acetylation were solely responsible for allowing the dosage effect of the X to be expressed, one would expect that the X-chromosomal genes would not exhibit dosage compensation compared to normal males and that the autosomal genes would be more or less equivalent in expression between mutant and normal. Sgs4 may represent such a case, but it appears to be exceptional. Clearly
compensation is still operative for most genes and autosomal increases are still present in some cases, but to a lesser degree than with mle. One potential explanation is that other components of the MSL complex, which is still intact in these individuals, render genes unresponsive to the histone acetylation level but permit a response to the X dosage. Another explanation, which is not mutually exclusive, is that some fraction of dosage-dependent regulators is normally expressed highly in the presence of H4 acetylation, but in its absence their expression falls, similarly to Sgs4, producing a new combination of effective modifying factors which generate an altered set of responses.

**DISCUSSION**

Three different approaches, namely chromosomal morphology, specific RNA quantitation, and binding of MSL proteins, were performed to investigate the role of the MSL complex on dosage compensation. Both chromosomal morphology and the measurement of specific transcripts reveal that the lack of MSL binding and H4 modification in the mle mutant males neither reduces X-chromosomal size specifically nor eliminates dosage compensation of most X-linked transcripts (Hiebert and Birchler 1994; present study). Two X-derived transgenes, white and yellow, which are normally partially compensated on the autosomes, are elevated in expression by the homozygous mle mutation. Thus, transgenes do not lose dosage compensation in the mle/ mle males; rather they become fully compensated on the autosomes in the absence of the MLE protein. In addition, the presence of MSL-2 in H83M2 females does not promote hypertranscription of the X-derived mini-white and yellow transgenes inserted into the autosomes. Rather the strong association of the MSL proteins and H4Ac16 enrichment with the two X chromosomes and concomitant reduced H4Ac16 residues on the autosomes is correlated with reduced autosomal transgene expression. Sequestration of chromatin proteins from one location in the genome to another as a means to affect gene expression has also been described in the case of Sir silencing proteins in yeast (Marcand et al. 1996). In contrast, changes of the acetylation level on the X chromosome appear to have minimal consequences.

**Evidence for nucleation sites for the MSL complex:**

MSL proteins do not associate with the X-derived transgenes or with a small X segment (>179 kb) in the

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**TABLE 1**

**Effect of the mof<sup>1</sup> mutation on X and autosomal gene expression**

<table>
<thead>
<tr>
<th>Locus</th>
<th>Chromosome</th>
<th>Ratio ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>mof&lt;sup&gt;1&lt;/sup&gt;/Y</td>
</tr>
<tr>
<td></td>
<td></td>
<td>+/Y</td>
</tr>
<tr>
<td>yellow</td>
<td>X</td>
<td>1.07 ± 0.06</td>
</tr>
<tr>
<td>white</td>
<td>X</td>
<td>1.79 ± 0.05&lt;sup&gt;a,b&lt;/sup&gt;</td>
</tr>
<tr>
<td>rudimentary</td>
<td>X</td>
<td>3.53 ± 0.11&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Sgs-4</td>
<td>X</td>
<td>0.55 ± 0.04&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>runt</td>
<td>X</td>
<td>0.92 ± 0.05</td>
</tr>
<tr>
<td>zeste</td>
<td>X</td>
<td>2.11 ± 0.08&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>αGpdh</td>
<td>2L</td>
<td>0.53 ± 0.04&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Adh</td>
<td>2L</td>
<td>1.87 ± 0.14&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>β Tub56D</td>
<td>2R</td>
<td>1.26 ± 0.06&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Sgs-3</td>
<td>3L</td>
<td>0.85 ± 0.10</td>
</tr>
<tr>
<td>scarlet</td>
<td>3L</td>
<td>4.17 ± 0.23&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Band densities were measured by Fuji (Stamford, CT) Bas 2000 phosphorimageter. The mof male vs. normal male and mof<sup>1</sup> female vs. normal female ratios are means of triplicate ratios obtained from phosphorimageter data from multiple Northern blots. The band density values were adjusted for rRNA levels which acts as a control for loading differences.

<sup>a</sup>Ratios significantly different from a value of 1.0 at the 95% confidence level.

<sup>b</sup>For white, mof male vs. mof<sup>1</sup> female ratios are used because of the presence of w<sup>1</sup>.

<sup>c</sup>Ratios significantly different between male and female at the 95% confidence level.
autosomes. This result indicates that MSL proteins do not initiate binding to every gene on the X chromosome because the lack of binding within the >179-kb segment suggests the absence of potential nucleation sites in that region, while the same cytological bands are associated with MSL proteins when residing on a larger segment. Apparently, the MSL proteins associate with nucleation sites on the X that allow initial recognition followed by polymerization.

It perhaps could be argued that binding to one or two X-derived genes inserted into the autosomes could not be detected by standard fluorescence or confocal microscopy. However, mini-white constructs of comparable length that carry a Polycomb Response Element (PRE) exhibit labeling with antibodies against Polycomb [MSL-2 is related to some Polycomb Group members (Bashaw and Baker 1996)] when similar techniques are used (Chan et al. 1994). Indeed, with the TEs the autosomal insertions are cytologically visible but exhibit no detectable MSL binding despite the fact that the same bands on the X in the same nuclei have recognizable signal. Taken together, these considerations argue that binding to single, double, and TE autosomal insertions could be detected, were it to occur.

**Gene expression studies with msl mutants:** It has been previously suggested that gene expression experiments on msl mutants are not informative (Kelley and Kuroda 1996) and that the presence of MSL proteins on the X is sufficient evidence to conclude they condition dosage compensation. However, we feel that measures of gene expression are necessary to understand the consequences of the msl mutations. Without such experiments, one can come to no conclusions about an involvement of the msls in any process.

We believe our data are an accurate reflection of the consequences of altered msl expression for several reasons. First, all of our results follow the same trends as previous reports of others (Belote and Lucchesi 1980; Okuno et al. 1984), in that levels of autosomal gene expression increase and dosage compensation is retained in msl mutants, although the treatment of the data and hence the interpretation differ. The cited studies involved chromosomal autoradiography. The number of grain counts over the X between normal and msl genotypes is quite similar, but that over the autosomes is increased in both sets of data. The grain counts were analyzed as X/A ratios and found to be significantly reduced between msl and normal males. Because the data lack a means to determine absolute expression, they could equally well represent a significant A/X increase. On the basis of our previous (Hiebert and Birchler 1994) and present results, we believe the latter to be the appropriate interpretation. The reduction of the levels of the X-linked Sgs4 gene product in mle males (Breen and Lucchesi 1986; Hiebert and Birchler 1994) is an exception (Hiebert and Birchler 1994). In a broader sense, we are not aware of any gene expression (or antibody-binding) data that differ among the laboratories reporting them. Further, our autosomal gene expression results are consistent with the changes in histone acetylation patterns that we observe in accordance with a current understanding of their effects (Wolfe and Pruss 1996; Grunstein 1997).

Moreover, the RNA measurements were performed on segregating progenies for direct comparisons of sibling genotypes. Within normals, where dosage compensation is expected between males and females, an approximately twofold difference was observed, illustrating by an internal standard the accuracy of the method. Previous work has demonstrated that Northern analysis of Adh and white can readily reveal twofold changes (Birchler et al. 1990; Hiebert and Birchler 1992). For yellow, a comparison of the normal male and female values between the mle and ectopic MSL-2 experiments illustrates the reproducibility of the method. The mean male/female ratio for the mle + genotype was 1.41 ± 0.06. The mean for the same male/female comparison in the ectopic MSL-2 experiment was 1.56 ± 0.07. A t-test of these two means indicates that they are not significantly different. Lastly, in the case of the ectopic expression of MSL-2, the w RNA measurement is a close match with the phenotypic expression of mini-white as determined by pigment assays.

**Trans-acting dosage effects and dosage compensation:** There are five levels of gene expression to be explained with regard to dosage compensation. The most commonly discussed is the twofold increase of the X in males compared to females. However, dosage compensation also occurs in females that have three X chromosomes (metafemales 3X;2A) (Stern 1960; Lucchesi et al. 1974; Birchler et al. 1989). In order for this to occur, the expression of each of the gene copies present must be reduced to two-thirds so that the total amount from the three X’s is equivalent to a normal female. Autosomal expression in these flies is also reduced to approximately two-thirds of normal (Birchler et al. 1989). Dosage compensation in males and metafemales is related as shown by the fact that several white alleles that fail to compensate in males also fail in metafemales (Birchler 1992).

Triploid females with three sets of all chromosomes have the same per gene expression as diploid females (Lucchesi and Rawls 1973a; Birchler et al. 1990). Reduction in the dosage of the X chromosome to two results in triploid intersexes (2X;3A), while further reduction to one copy is referred to as triploid metamales (X;3A). Both exhibit dosage compensation of their X chromosome(s) (Lucchesi and Rawls 1973b; Lucchesi et al. 1977; Mann et al. 1986). The increases in per gene expression for compensation in these cases are 1.5- and 3-fold, respectively.

The magnitude of the inverse dosage effect, commonly observed in aneuploids, can account for the different levels of gene expression. When the dosage of
large chromosomal segments is reduced from 2 to 1, the expression of unlinked genes goes from 1 to 2. When the dosage of the same segment is increased from 2 to 3, gene expression is reduced to 2/3. Genes present on the varied segment that are similarly affected become dosage compensated because the structural gene dosage effect is canceled by this inverse dosage effect simultaneously produced (Birchler 1979, 1981; Birchler et al. 1990; Guo and Birchler 1994). The magnitude observed in triploid situations follows closely an inverse correlation between the dosage and the effect on gene expression (Rabinow et al. 1991; Guo and Birchler 1994). Because the inverse effect is the most prevalent type of dosage response, it is likely to be the basis of the five levels of gene expression that occur with the different doses of the X. Indeed, when the white gene is present on the autosomes in constant copy number and a dosage series of the X is produced in the form of males (1X), females (2X), and metafemales (3X), an inverse effect upon w expression is found (Birchler 1992).

Invoking an inverse dosage effect alone as the basis of X-chromosomal dosage compensation is inadequate on two counts. First, although large aneuploids exhibit dosage compensation for the majority of linked genes (Devlin et al. 1982, 1988; Birchler et al. 1990; Guo and Birchler 1994), this fraction of compensated genes is not as great as that for the X chromosome. Second, this hypothesis predicts an increased autosomal gene expression in males compared to females concomitant with dosage compensation of the X. In general, this is not the case.

However, the results of the present study resolve these two issues. The increased acetylation of the X in normal males might intensify the response of some X-linked genes to the inverse dosage effect to increase the fraction of X-linked genes exhibiting full compensation. An example in the present study is Adh and from previous work, Salivary gland secretion protein 4 (Sgs4; Breen and Lucchesi 1986; Hebert and Birchler 1994), which both show lower expression on the X in mle mutant males. However, there is only a minority of X-linked genes whose expression is lowered in mle males, when the histone acetylation drops on the X to female levels. Indeed, in the absence of MOF activity, the majority of the genes resident in their normal locations on the X retain compensation. The failure of most X-linked genes to respond to lowered acetylation in mof1 mutants or, in the example of y+, to increased levels in H 83M 2/+ females, suggests that at least some component of the MSL complex nullifies a response of most X-linked genes to their acetylation levels. These results would also suggest that the presence of the MSL complex on the X in normal males prevents genes from responding to the high levels of H 4Ac16. The consequence of this action would be that the hyperactivation of these genes would remain at the twofold level because of the inverse dosage effect of the X, rather than a greater overexpression brought on by extremely high levels of acetylation.

The lowered acetylation of the autosomes in normal males appears to reduce the strength of the inverse dosage effect there. In general, histone acetylation is thought to result in a more open chromatin configuration and is associated with higher levels of gene expression (Brownell and Allis 1996; Wolffe and Pruss 1996; Grunstein 1997). In the mle mutant males, the acetylation is increased on the autosomes and the inverse dosage effect is intensified (Figure 13A). The reduced acetylation of the autosomes in normal males compared to females provides a basis for the nearly equal expression of the autosomes between the sexes despite the “monosomic” condition of nearly 20% of the genome, which would be expected to increase gene expression throughout all chromosomes under other circumstances. We suggest that autosomal genes inserted on the X become compensated (Spradling and Rubin 1983; Laurie-Ahlberg and Stam 1987), because they are moved from a weakly acetylated region to one where the dosage of the X can be effective upon them. This acquisition of dosage compensation of autosomal genes is consistent with our conclusion that the MSL complex spreads from nucleation sites and that individual genes on the X do not have MSL binding sites. On the other hand, the partial compensation of white on the autosomes (Qian and Pirrotta 1995; Roseman et al. 1995) as well as other genes (Krumm et al. 1985; McNabb and Beckendorf 1986; Gutierrez et al. 1989; Sass and Meselson 1991; Scott and Lucchesi 1991) can be explained because they are moved from a chromosome where the dosage of the X can be effective on them to one where it cannot. Thus, the MSL sequestration would maximize equality of expression between males and females for the whole genome.

In this article, we present evidence that chromosomal proteins are sequestered from the autosomes to the X in normal males. Therefore, when any of the msl loci are mutated, there is no sequestration. The X remains basically compensated and the autosomal expression is increased in general because MOF becomes uniformly distributed in the nucleus, resulting in a return of acetylation levels to those of females. The msl mutations have little effect in females because they are chromosomally balanced and therefore have no dosage effects operating. Also, there is a similar distribution of acetylation in mutant and normals. In contrast, in H 83M 2/+ females, the product of MSL-2 promotes sequestration of the acetylase to the X, which lowers the level of autosomal acetylation. As a result, the expression of transgenes inserted in the autosomes is reduced.

The recent finding that a gene on the murine X is doubled in expression compared to its evolutionary progenitor raises the possibility that genes on the mammalian X are also hyperactivated in males (Adler et al. 1997). This realization would explain the paradox that...
Figure 13.—Model for dosage compensation. (A) Relationship of MSL binding and the mle mutational effect on gene expression in X:A transpositions. In X-derived transgenes and TEs, the MSL complex sequesters acetylase (blue color intensity) from the autosomes. Thus, X-derived transgenes are partially compensated. In mle mutants, acetylase returns to the autosomes so transgene expression is increased significantly. In the large X:A transposition, lowering the acetylase on the autosomes does not reduce the expression of the included white gene, because the MSL complex binds to the X segment (dark blue color). In the mle mutant, the MSL complex is not associated with the X so the autosomes have increased acetylation. Therefore, gene expression is increased on the autosomes. (B) Model for the evolution of dosage compensation. Degeneration of a member of a homologous pair of chromosomes in males to form the Y chromosome leads to trans-acting dosage effects. As a result, most of the X-linked genes are dosage compensated. Simultaneously X-linked modifiers produce trans-acting inverse (predominantly) effects to increase autosomal gene expression. In males, the MSL complex sequesters histone acetylase (blue color intensity) from the autosomes (light blue color) to the X chromosome (dark blue color) deintensifying the inverse effect on autosomal genes in males (light blue color).
males with a single X are viable whereas individuals monosomic for chromosomal segments of comparable length abort early in development (Graves et al. 1998). This finding also raises the interesting possibility that an inverse response of X-linked genes in males is a common feature of X/Y heteromorphic chromosomal systems and that the MSL sequestration in Drosophila and X inactivation in mammals are reactionary modifications of the dosage effects of the sex chromosomes.

Evolution of the Drosophila sex chromosomes: There is much to be learned before a full comprehension of the evolution of this situation can be formulated. However, one speculation might be that the degeneration of one member of a homologous pair of chromosomes to begin the formation of the Y (Charlesworth 1991; Marin et al. 1996; Orr and Kim 1998) would leave a small segment of the opposing homologue in the haplo state. A selective advantage would be afforded to individuals carrying any change, such as elevated acetylation, that would result in increased expression of haplo-insufficient genes in this region. Although we have focused on the situation in which acetylation or lack thereof modifies the effect of sex-chromosomal dosage, it is also the case, as exemplified by the ectopic expression of MSL-2, that changes in acetylation will modify gene expression without involving a dosage effect. As the haplo region expands, dosage-dependent regulatory genes would be altered in dosage. It is interesting to note that dosage-dependent regulatory genes are more likely to have an effect in the haplo condition than genes encoding metabolic functions. Their acetylation might be selected to eliminate their global effects on target genes throughout the genome, but some fraction must remain dosage dependent in normal males to produce compensation and the observed autosomal effects.

Because an inverse effect is more common than a positive dosage response, there would be a tendency to increase gene expression throughout the genome, including rendering the haplo regions at least partially dosage compensated. To date there are over twenty genes identified that produce a trans-acting dosage effect upon white (see Introduction and unpublished results). In analogy with modifiers of position-effect variegation, many of which have been identified as transcriptional regulators, there are scores of dosage-dependent modifiers of a single process (Henikoff 1996; Bhadra et al. 1998). These genes appear to operate in a dosage-dependent cascade such that any one or several produce much the same effect on the target locus (Bhadra et al. 1998). Thus, statistically speaking, any region involving only a few percent of the genome will potentially carry a dosage-dependent modifier of any one target gene.

Despite the large number of such modifiers, varying multiples does not in general generate cumulative effects beyond the inverse limits. This conclusion is derived from two different types of observations. First, combinations of mutations do not exceed this limit (Bhadra et al. 1998; Pal-Bhadra et al. 1998) and second, large aneuploids produce trans-acting dosage effects that remain within this range (Birchler 1979; Birchler and Newton 1981; Devlin et al. 1982, 1988; Guo and Birchler 1994). Thus, the combination of acetylation and the inverse dosage effect could provide for a synergistic expansion of the haplo regions. This combination would produce dosage compensation whether the affected target genes are haplo insufficient or not. The inverse dosage effect provides a numerical explanation of how the process of dosage compensation doubles the expression of many genes transcribed at widely differing rates, although the mechanism remains to be elucidated. The sequestration of acetylase to the X in increasingly larger quantities would lower the autosome acetylation and thus mute the increased expression of the autosomes that would be expected to result from a lowered dosage of the evolving X. On the X, some component of the MSL complex or associated proteins would eventually render most genes unresponsive to the acetylation level. Indeed, there may be a selective advantage to maintaining transcriptional regulators as dosage dependent to maintain compensation.

Such a situation might evolve if continued increases in acetylation caused a tendency for extensive overexpression of the X. Inactivation of a response to acetylation would hold the hyperactivation at the twofold level because of the effect of the dosage of the X. Moreover, there is a strong skewing in favor of TATA-less promoters on the X chromosome (Arkhipova 1994). Therefore, it is also possible that many individual X-linked genes have evolved features that render them more sensitive to the X dosage effects or less sensitive to the acetylation level.

Consequently, with the available information, we envision that as the heteromorphic sex chromosome situation evolved in Drosophila by degeneration of one member of a homologous pair of chromosomes to form the Y, dosage effects would come into play and produce compensation of most X-linked genes together with a tendency for the doubling of the expression of the autosomes in males (Figure 13B). The sequestration of a histone acetylase to the X chromosome would mute the effect on the autosomes. The MSL complex on the X would inactivate the response to H4Lys16 acetylation for most genes, to maintain the hyperactivation at the twofold level. Thus, our model proposes a single mechanism that explains the five levels of transcription involved with the various cases of dosage compensation, the MSL binding on the X in males, and the gene expression pattern in the msl mutants.

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