Histone Acetylation and Gene Expression Analysis of *Sex lethal* Mutants in Drosophila

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ABSTRACT

The evolution of sex determination mechanisms is often accompanied by reduction in dosage of genes on a whole chromosome. Under these circumstances, negatively acting regulatory genes would tend to double the expression of the genome, which produces compensation of the single-sex chromosome and increases autosomal gene expression. Previous work has suggested that to reduce the autosomal expression to the female level, these dosage effects are modified by a chromatin complex specific to males, which sequesters a histone acetylase to the X. The reduced autosomal histone 4 lysine 16 (H4Lys16) acetylation results in lowered autosomal expression, while the higher acetylation on the X is mitigated by the male-specific lethal complex, preventing overexpression. In this report, we examine how mutations in the principal sex determination gene, *Sex lethal* (*Sxl*), impact the H4 acetylation and gene expression on both the X and autosomes. When *Sxl* expression is missing in females, we find that the sequestration occurs concordantly with reductions in autosomal H4Lys16 acetylation and gene expression on the whole. When *Sxl* is ectopically expressed in Sxl^M mutant males, the sequestration is disrupted, leading to an increase in autosomal H4Lys16 acetylation and overall gene expression. In both cases we find relatively little effect upon X chromosomal gene expression.

THE evolution of sex determination mechanisms is often accompanied by changes in chromosomal structure (Charlesworth 1996). These occur to hold the sex determination components together on one chromosome in the face of meiotic recombination or to capitalize upon changes in gene dosage as part of the mechanism. A secondary consequence is that the dosage of many genes unrelated to sex determination is also altered. Included are regulatory genes that have the potential to have effects on whole groups of target genes throughout the genome. Many transcription factors and chromatin proteins are dosage sensitive in diploid organisms (Birchler 1979; Henikoff 1979, 1996; Bhadra *et al.* 1998). For the X-linked *white* eye color gene, there are at least 40 dosage-dependent autosomal modifiers (*e.g.*, Rabinow *et al.* 1991; Bhadra *et al.* 1997; Frolov *et al.* 1998; J. Birchler, U. Bhadra, M. Pal-Bhadra, M. Frolov and E. Benevolenskaya, unpublished data). The number on the X is more difficult to define because *white* is also there. In other words, regulatory genes have evolved to be expressed at a level and to operate mechanistically in such a way that they are rate limiting in the diploid state for phenotypic characteristics. The genetic behavior of many quantitative and developmental traits is likely to be a reflection of this fact (Guo and Birchler 1994; Bhadra *et al.* 1998). Indeed, many sex determination mechanisms have utilized dosage-dependent regulators as part of their system (Charlesworth 1996).

The generation of heteromorphic sex chromosomes, such as the X/Y situation in Drosophila and mammals, results in changes in the dosage of hundreds or thousands of genes. Typically, similar changes experimentally produced as aneuploids result in rather detrimental effects on vigor and, in extreme cases, lethality. These effects, which are thought to be a consequence of the dosage-sensitive regulatory genes (Birchler 1979; Birchler and Newton 1981; Guo and Birchler 1994; Bhadra *et al.* 1998), must be counteracted during the evolution of the sex chromosomes.

The most common dosage effect is one in which there is an inverse correlation between the regulatory gene dosage and the expression of the target loci (Birchler 1979; Devlin *et al.* 1988; Guo and Birchler 1994; Bhadra *et al.* 1998). Even though there are numerous modifiers of any one gene, those varied together generally are not cumulative beyond the inverse limits of their dosage (Devlin *et al.* 1988; Bhadra *et al.* 1998). Thus, reductions in dosage of a sex chromosome from two to one by degeneration of one member of a homologous pair would cause a generalized doubling of gene expression throughout the genome in the absence of any modification. Indeed, a dosage series of the X chromosome as a whole produces an inverse dosage effect on the X-derived *white* eye color gene held at one copy on the autosomes (Birchler 1992). Consequently, the expres-

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sion of genes on the chromosome present in one dose are dosage compensated because this twofold regulatory effect counteracts the halfing of expression of the target "housekeeping" genes. In the two cases where the situation has been carefully analyzed, Drosophila and mouse, the expression of the single male X has been doubled (Adler *et al.* 1997; Arkhipova *et al.* 1997).

X inactivation in mammals and the male-specific lethal (MSL) complex in Drosophila have been considered as dosage compensation mechanisms. In fact, they appear to be reactionary modifications to the *trans*-acting dosage effects that are generated by the evolution of heteromorphic sex chromosomes (Adler *et al.* 1997; Bhadra *et al.* 1999). We have shown previously that the MSL complex in Drosophila sequesters a histone acetylase from the autosomes to the X (Bhadra *et al.* 1999). As a consequence, the level of histone 4 lysine 16 acetylation (H4Ac16) is lower on the autosomes and higher on the X in males (Turner *et al.* 1992) compared to females. This chromatin change mitigates the doubling of autosomal gene expression in males, while on the X, the MSL complex appears to prevent genes from overexpressing due to the greatly increased histone acetylation (Bhadra *et al.* 1999), although some exceptional genes on the X are affected. The twofold effect due to the reduced dosage of the X provides the proper level of sex chromosome dosage compensation without an extensive increase caused by high levels of H4 acetylation, but at the same time the inverse effect on the autosomes is diminished. In other words, the single X in males tends to double expression throughout the genome, the sequestration of acetylase from the autosomes reduces this effect there, and the MSL complex on the X prevents overexpression that might otherwise result from the highly acetylated histone 4 levels. In this study, we examine the role of *Sex lethal* (*Sxl*), a principal sex determination gene (Cline 1984), in this process.

SEX LETHAL is a female-specific RNA-binding protein that controls alternative splicing in the sex determination cascade (Cline 1984; Bell *et al.* 1991). In females, the precursor mRNA of the *Sxl* gene is normally spliced to produce a functional SXL protein, while in males defective splicing inserts an abortive translational stop codon that generates a truncated protein. The sex-specific expression of *Sxl* is initiated by dosage-dependent regulators on the X *vs.* the autosomes, which constitute the X/A balance determinant of sex (Cline 1988; Erickson and Cline 1993). SXL also represses the expression of *msl-2*, the critical member of the MSL complex needed for X chromosome localization (Bashaw and Baker 1997; Kelley *et al.* 1997), by coupling to multiple sites in the untranslated region of the *msl-2* transcripts. In the absence of functional SXL protein in males, the *msl-2* gene is normally expressed. The MSL proteins [MSL-1, MSL-2, MSL-3, and MLE (*maleless*)] together with the *rox1* and *rox2* RNAs form a complex that is strongly associated with the X chromosome, including MOF (*males absent on the first*), a putative histone acetylase (Kuroda *et al.* 1991; Baker *et al.* 1994; Kelley and Kuroda 1995; Amrein and Axel 1997; Hilfiker *et al.* 1997; Meller *et al.* 1997; Franke and Baker 1999). The JIL-1 kinase, which phosphorylates H3 histone, might also be a member of this group (Jin *et al.* 1999). In females, the complex, minus MSL-2, is uniformly distributed on all chromosomes (Bhadra *et al.* 1999).

In this article, we examine the effect of *Sxl* mutations on MSL protein sequestration and histone acetylation as well as on X and autosomal gene expression. Previously published models of *Sxl* action have suggested that loss of function in females results in increased expression of the X chromosomes and that ectopic expression of SXL in males eliminates X-chromosome dosage compensation. Our data indicate that the predominant role of *Sxl* can be understood within the context of modifying the sequestration of the MSL complex, which alters the impact of the dosage of the X chromosome.

MATERIALS AND METHODS

Crosses: The following crosses were performed to generate the segregating classes for the Northern and Western blots as well as immunostaining: (1) $mle^{pml\,8}/mle^{pml\,8}$ females × $mle^{pml\,8}$/*T(2;3)CyO Tb* males. This cross produces males and females that are homozygous for *mle* and that can be distinguished by their normal appearance at the third larval instar. Control males and females are also produced that are heterozygous for *mle* and that have a Tubby phenotype. (2) *y* $Sxl^{M\#4}$/*Basc* females × *y*/Y males. This cross produces brothers that are *y* $Sxl^{M\#4}$ or normal *Basc* (y^+) as well as females heterozygous for $Sxl^{M\#4}$ with a yellow phenotype and those heterozygous for the *Basc* (y^+) balancer. (3) *y* $Sxl^{fhv\#1}$/*y* $Sxl^{fhv\#1}$ females × $Sxl^{f\#1}$/Y males. The *Sxl* heteroallelic daughters produced from this cross have low viability in contrast to the normal $Sxl^{fhv\#1}$ males. (4) *cm* $Sxl^{fhv\#1}$ *ct*/*cm* $Sxl^{fhv\#1}$ *ct;* $mle^{pml\,8}/mle^{pml\,8}$ females × $Sxl^{f\#1}$ *oc ptg v*/Y*;* $mle^{pml\,8}$/*In(2LR) Gla Bc Elp* males. This cross produces heteroallelic females that are either homozygous for *mle* or heterozygous. The latter exhibit the *Black cell* (*Bc*) phenotype. The $Sxl^{fhv\#1}/Y$ males are also segregating for *mle*.

Northern blots: RNA preparations, electrophoresis of total mRNA, blotting to nylon membranes, hybridization, and antisense RNA preparation were performed as described (Hiebert and Birchler 1994; Bhadra *et al.* 1999).

Because the major portion of total RNA is rRNA, it is possible to address the question of whether equivalent amounts of total RNA from different genotypes is contributed from equal amounts of DNA. This determination allows one to test whether each *Sxl* mutation affects the cellular expression of rRNA. Normalization of the values of each gene in the survey to rRNA provides a means to make a "per cell" expression comparison among *Sxl* genotypes. Analysis of total DNA/rRNA ratios for all the *Sxl*, *mle*, and *Sxl*; *mle* genotypes was performed as described previously (Hiebert and Birchler 1994). For this experiment, we isolated total nucleic acid of each genotype. Quadruplicate isolations of each genotype were separated electrophoretically on 1.0% agarose gels (as shown in Figure 1). Separate DNase I and RNase A digestions confirmed which bands on the ethidium-stained gels corresponded to DNA and RNA (not shown). A dilution series of these nucleic acid preparations was used to establish that differences in quantity could be detected under these conditions. However,

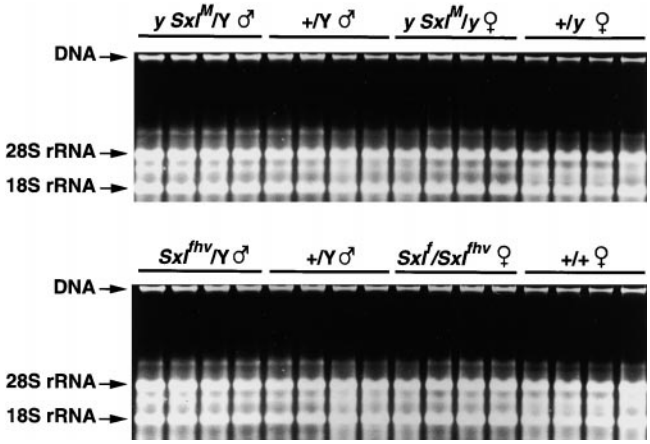

Figure 1.—Comparison of rRNA/DNA ratios in Sxl^M and Sxl^f with the respective normal genotypes. The ratio of rRNA/DNA in the critical *Sxl* genotypes does not differ from the respective controls, as described in the text. This result indicates that using rRNA as a gel-loading control allows direct "per cell" comparisons among genotypes.

the results show that there were no significant differences between DNA/28S rRNA ratios or DNA/18S rRNA ratios among these classes of larvae. The data for the most critical genotypes ($Sxl^{M\#4}$/Y *vs. Basc*/Y as well as $Sxl^{fhv\#1}/Sxl^{f\#1}$ *vs.* $Sxl^{fhv\#1}$/Y and Canton-*S* females) are shown in Figure 1.

Western blots: Crude extracts were prepared from third instar larvae using Laemmli loading buffer (10 μl/larvae) containing proteinase inhibitors as described (Kelley *et al.* 1995). The extracts were boiled for 5–10 min and then centrifuged for 1 min. Each sample was loaded in a 4% stacking–6% PAGE-SDS gel. Proteins were transferred to a nylon membrane using an electrocell electroblot apparatus for 3–4 hr at 500 mA. Blots were incubated overnight with rabbit anti-MSL-2 and MLE antibodies and detected using an alkaline phosphatase-conjugated goat antirabbit detection system.

Immunostaining: Polytene chromosomes from the third instar larvae were prepared as described (Kuroda *et al.* 1991; Bhadra *et al.* 1999). The slides were incubated overnight with affinity-purified rabbit anti-MSL-1, MSL-2, MLE, or MOF antibodies with appropriate dilution. Slides were processed as described (Kuroda *et al.* 1991; Bhadra *et al.* 1999).

For confocal microscopy, Cy-5-conjugated goat secondary antibodies were used. The slides were mounted with Vectashield mounting media and propidium iodide mixture. The slides were examined with a Bio-Rad 600 confocal microscope (Bio-Rad, Richmond, CA) using a ×100 oil lens.

RESULTS

The rationale for these experiments was to examine how loss- and gain-of-function alleles of *Sxl* affect the genomic distribution of MOF and hence histone 4 acetylation, which has not been determined previously. In examining the distribution of various MSL proteins, MOF, and histone acetylation, we used mixtures of salivary gland polytene chromosomes of the mutant genotypes with the appropriate male or female control so that any changes in either the X or the autosomes could be detected under the same conditions of preparation and analysis. In parallel, a survey of X and autosomal gene expression was conducted and correlated with the respective acetylation levels to establish the role of *Sxl* in modifying the *trans*-acting dosage effects of the X chromosome.

Binding of MSL proteins: Loss of function of *Sxl* results in female lethality. However, certain alleles of the *Sxl* gene partially complement to give viable heteroallelic individuals. One combination is $Sxl^{f\#1}/Sxl^{fhv\#1}$, in which a few percent of the female progeny can survive to the third instar larval stage. In each individual, ~50% of the cells produce functional SXL protein (Gorman *et al.* 1993). We used the salivary glands of heteroallelic larvae for double staining with MSL-2 and H4Ac16 antibodies (Figure 2A). The MSL-2 protein is only associated with the Xs in cells lacking SXL, as described previously by others (Gorman *et al.* 1993; Bashaw and Baker 1995; Kelley *et al.* 1995). In the MSL-2-expressing nuclei, H4Ac16 and the other MSL proteins assayed (MSL1 and MOF) showed a male level association with the Xs, instead of the genomewide binding typically present in normal females (Figure 2B). We confirmed that, under our experimental conditions, an intermediate level of MSL-2 is present in these larvae, as detected by Western analysis (Figure 3).

We next examined the X chromosomal association of MSL proteins in larvae carrying the gain-of-function allele $Sxl^{M\#4}$. Individuals with this mutation have a constitutive expression of SXL protein in males that accumulates during development more slowly than it occurs in normal females (Bernstein *et al.* 1995). The anti-MLE antibody was associated with the X chromosome in the Sxl^M larvae. However, MSL-2 was not obviously detected on a particular chromosome using standard microscopy (confocal microscopy detects a very low level of MSL2 on the X, as shown below), while binding of the MSL-1 antibody on the X was very weak (Figure 4A). This nonstoichiometric MSL binding is likely the case because complex formation was normal early in development, when SXL levels were extremely low, but there was a subsequent depletion of MSL-2, concordant with the rise in SXL concentration.

To determine whether the low level of MSL-2 binding is due to a reduced total amount in the Sxl^M males, we performed Western blot analysis using MSL-2 and MLE probes. MLE acts as a loading control because it was found in all genotypes tested (except the *Sxl*; *mle* females). The amount of MSL-2 protein is reduced in the Sxl^M male larvae relative to their controls (Figure 3), confirming that expression of the female-specific form of SXL in Sxl^M reduces the MSL-2 levels.

Given the residual binding of the MSL complex in these larvae, the question arises as to the basis of their male lethality. One possibility is that the MOF acetylase is incompletely sequestered to the X chromosome. To determine whether the reduced level of MSL-2 protein causes failure to sequester acetylase to the male X, a mixture of Sxl^M and normal male nuclei were examined

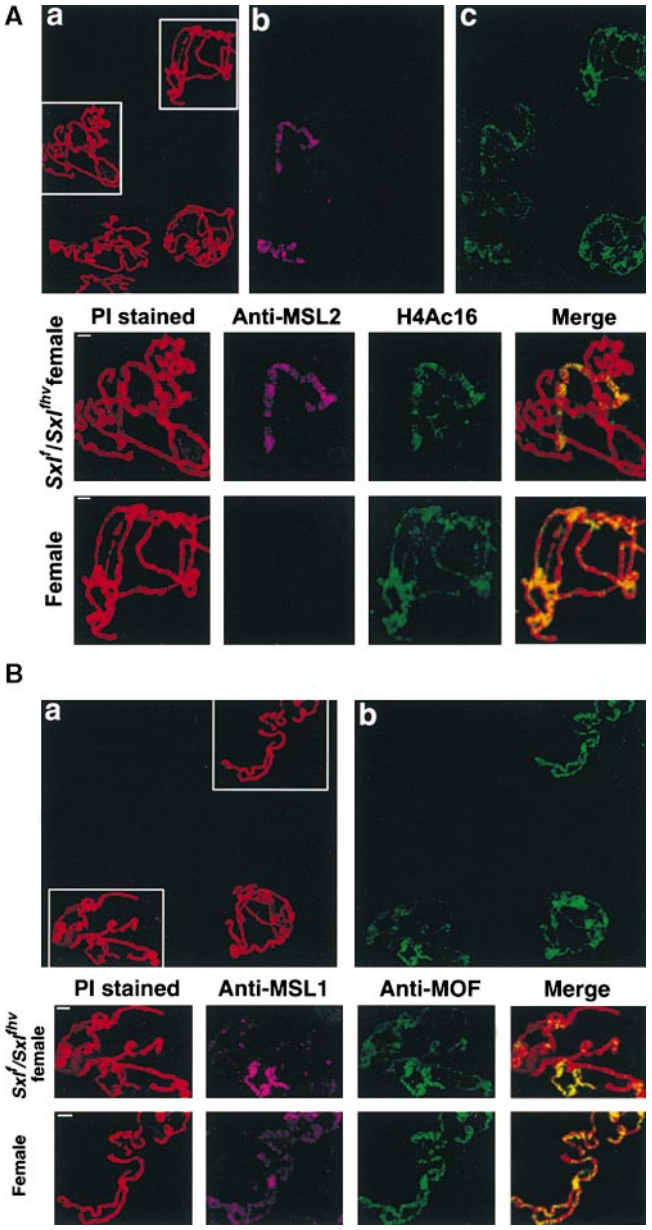

Figure 2.—MSL protein binding in Sxl^f nuclei. (A, a) Mixture of $Sxl^{f\#1}/Sxl^{fhv\#1}$ and wild-type female nuclei in the same confocal microscopic field stained with propidium iodide (PI; red). (b) Same field probed with anti-MSL-2 (purple) and (c) with H4Ac16 antibodies (green). Below, magnified images of a $Sxl^{f\#1}/Sxl^{fhv\#1}$ and a normal female nucleus (boxed in a) showing anti-MSL2 and anti-H4Ac16 labeling with the merged PI and H4Ac16 images (yellow) at the right. (B) Mixture of $Sxl^{f\#1}/Sxl^{fhv\#1}$ and wild-type females stained with PI (a) and anti-MOF (b). Below are magnified views of boxed nuclei in a also showing staining with anti-MSL-1. The merged PI and anti-MOF images are at the right. Bar, 10 μm.

in the same microscopic field using antibodies against MOF. A preferential labeling of the X was found in the wild-type nuclei, while the acetylase levels show a uniform genomewide distribution in the Sxl^M larvae (Figure 4B), as previously found in *mle* males and wild-type females (Bhadra *et al.* 1999).

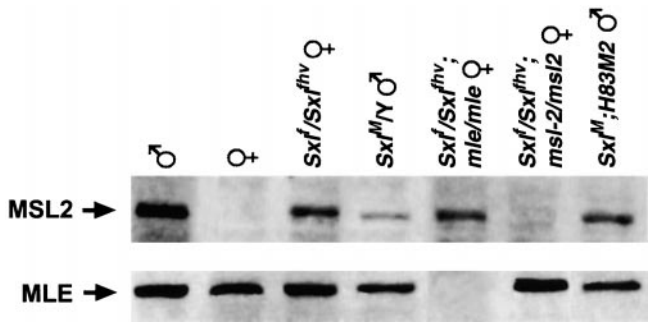

Figure 3.—Western analysis of MSL-2 and MLE in *Sxl* mutants. The relative amount of MSL-2 protein in *Sxl* and *Sxl; msl* larvae was estimated by Western blotting. The genotype is noted at the top. Detection of MLE from a duplicate blot (shown below) acts as a control.

To confirm that the reduced level of MSL-2 is responsible for the female type of MOF distribution in the Sxl^M larvae, we used a transgenic stock, *H83M2*, which ectopically expresses the MSL-2 protein even in the presence of SXL protein (Kelley *et al.* 1995) to reintroduce MSL2 into the Sxl^M mutants. Double staining with MSL-1 and H4Ac16 antibodies was performed on a mixed preparation of polytene nuclei from Sxl^M and Sxl^M*; H83M2* males. MSL-1 is associated weakly with the X in Sxl^M nuclei, but is present at a high level on the X in the MSL-2-expressing cells (Figure 5A). The uniform H4Ac16 distribution on the Sxl^M mutant chromosomes is intermediate between that of the autosomes and X in Sxl^M*; H83M2* males (Figure 5A). An additional staining for MOF and MSL-2, which is much more strongly represented on the X in Sxl^M, *H83M2* males, confirmed the identity of the nuclei in the mixed spreading (Figure 5B). Therefore, the constitutive synthesis of MSL-2 protein in the Sxl^M males restores preferential binding of the MSL complex and H4Ac16 enrichment on the X chromosome.

The double-mutant *Sxl; msl* flies are phenotypically intersex and contain a mixture of female and male cells. They survive at a high percentage relative to each single mutant. By removing a member of the MSL complex using an *msl* mutation, it should be possible to eliminate the sequestration of the MSL complex on the Xs, which otherwise occurs in the *Sxl* mutant. Therefore, we double stained a mixture of Sxl^f/Sxl^{fhv} and Sxl^f/Sxl^{fhv}*;* $mle^{pml\,8}/mle^{pml\,8}$ chromosomes with anti-MSL-1 and H4Ac16 antibodies and in separate preparations with anti-MLE and anti-MOF. In the former genotype, MSL-1 and MLE are either present only on the X chromosome (in the non-SXL-expressing cells) or uniformly distributed, as normally occurs in females. In the double-mutant genotype, there is no labeling with MLE in any of the cells. It has been noted previously for the X chromosome by Lyman *et al.* (1997) that in this genotype MSL-1 and MSL-2 associate with discrete sites at a low level. We find that this is also the case with autosomal labeling. This distri-

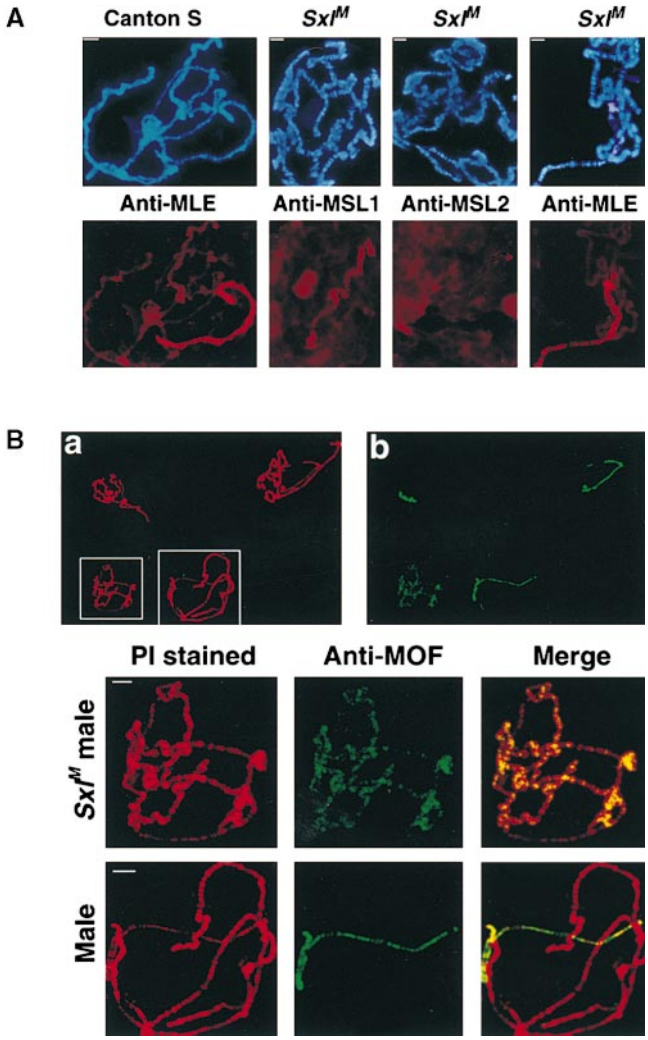

Figure 4.—Comparison of MSL protein association with chromosomes in Sxl^M and normal male larvae. (A, top) Polytene chromosomes of Canton-S and Sxl^M larvae stained with 4′,6-diamidino-2-phenylindole. (A, bottom) Each nucleus stained with different anti-MSL antibodies as noted above the panel. (B, top) Mixture of Sxl^M and normal males stained with PI (a) and anti-MOF (b). (B, bottom) Enlarged images of boxed nuclei in a and their merged images. Bar, 10 μm.

bution is in contrast to the *mle* mutant alone, which does not support the respective type of binding in either males or females of any of the other members of the MSL complex. It was of interest to determine the distribution of MOF and H4 acetylation under these circumstances. In the *Sxl; mle* nuclei there is binding of anti-H4Ac16 and anti-MOF on all chromosomes but with a stronger discrete association with many X and autosomal sites than is typically observed with normal or *mle* females (Bhadra *et al.* 1999; Figure 6, A and B). This result suggests that the discrete binding of MSL-1 and MSL-2 sequesters MOF to these sites, but also that these sites are saturated for MOF, resulting in the remainder being uniformly distributed in the genome. Therefore this pattern differs from the situation in males and females, where *msl* mutants exhibit a more uniform genomewide distribution of MOF and H4 acetylation (Bhadra *et al.* 1999). This result also suggests that *Sxl* affects the expression of a gene product that influences the ability for and distribution of MSL-1 / MSL-2 binding in the absence of MLE.

Gene expression: Gene expression studies are important to define the effect of changes in histone acetylation on both the X and the autosomes. To determine the relationship between the mutational effect on gene expression and the sex-specific sequestration of MSL proteins and acetylase, we performed Northern analyses with total RNA on the same classes of mutant larvae that were studied in the binding assays. Steady-state mRNA levels were measured with randomly selected probes for 9 X-linked and 11 autosomal loci. Ribosomal RNA served as a gel-loading control. Individual genes react differently to the changes in histone acetylation, but general trends were evident with each type of mutant. In $Sxl^{M\#4}$ and $mle^{pml\,8}$ males, most of the X-linked transcripts remain unaffected, relative to normal males, represented by *Basc / Y* and *mle / +*, respectively. However, the *rudimentary* and *vermilion* mRNA were significantly increased, while *sis-b* and *Sgs-4* were reduced in both mutants (Figures 7 and 8). In addition, the majority of the 11 tested autosomal loci were increased in their expression. Overall, these results suggest that the basis of the male lethality either by $mle^{pml\,8}$ or $Sxl^{M\#4}$ results from a similar trend of effects on X and autosomal gene expression. It appears that the effect of *mle* and Sxl^M on X chromosomal RNA levels is negligible, whereas a general elevation in expression is found for autosomal loci. The latter trend correlates with an autosomal increase of H4Lys16 acetylation found in the antibody-labeling experiments described above.

For the X-linked transcripts in the $Sxl^{f\#1}$ / $Sxl^{fhv\#1}$ heteroallelic females, the expression of the majority of transcripts was unchanged (Figures 7 and 8). However, transcript levels of all 11 tested autosomal loci were altered significantly. The *scarlet* and *Gpdh* RNAs showed an elevation, whereas the majority of loci were reduced in expression relative to the Sxl^{fhv} males in the same genetic background (Figures 7 and 8). Because these females are mosaics of normal and SXL^- cells (Gorman *et al.* 1993; see also Figures 3 and 8), the gene expression data reflect only partially the response occurring in the latter cell type. Relative to normal females, the autosomal H4 acetylation is reduced, which correlates with the gene expression trend.

RNA analysis was also performed on the double-mutant *Sxl; mle* larvae. In this case, the homozygous *mle* mutation would hinder the sequestration of MOF to the X that occurs otherwise in the SXL^- cells. To evaluate the fraction of cells expressing the different forms of *Sxl* in the *Sxl* and *mle* genotypes and to compare this value to the Western blots of Sxl^f alone (described above), a Northern analysis of *Sxl* RNA was performed.

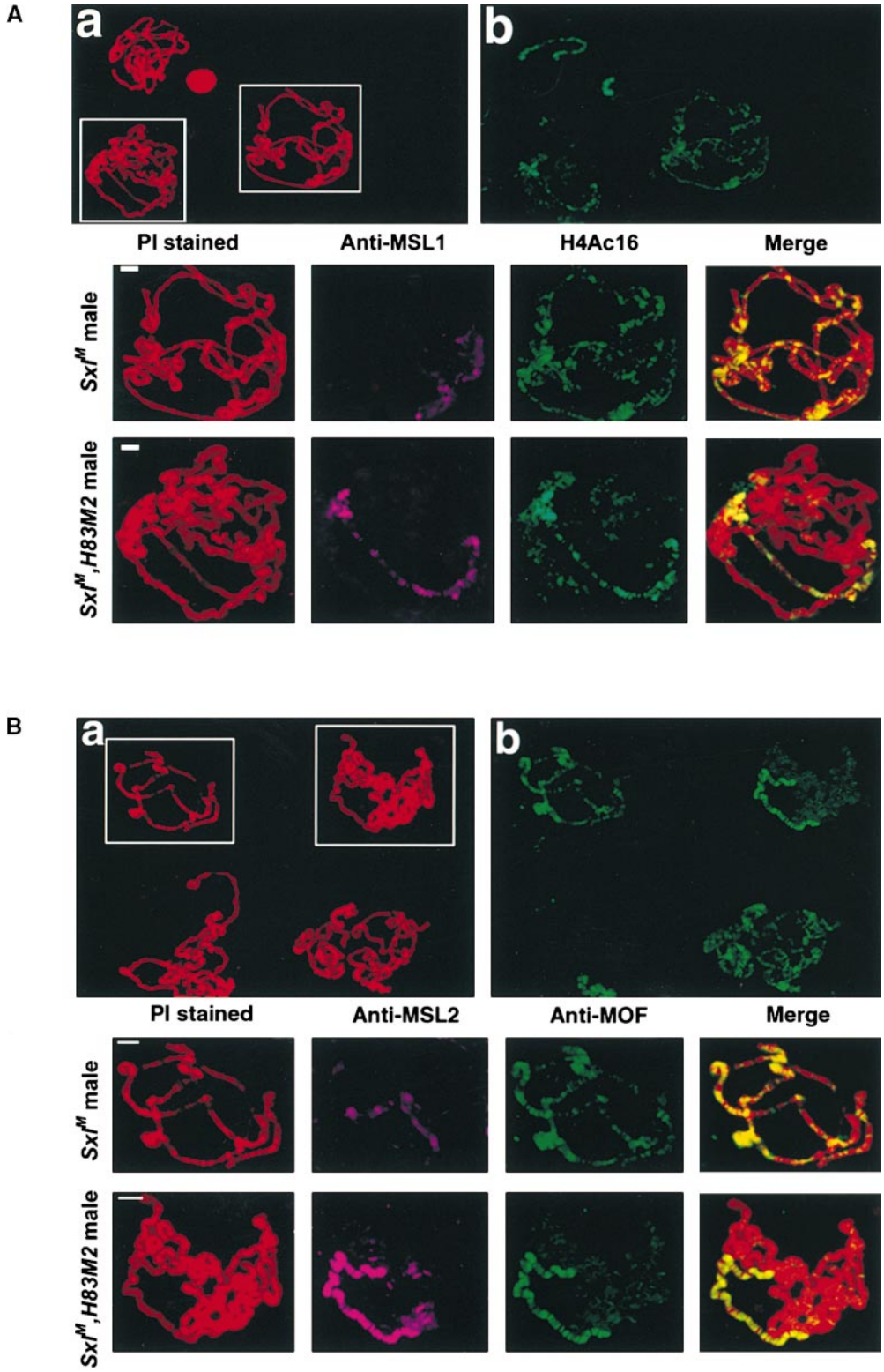

Figure 5.—Comparison of MSL protein association with chromosomes in Sxl^M and Sxl^M/*H83M2* larvae. (A, a) Mixture of $Sxl^{M\#4}$ and $Sxl^{M\#4}$; *H83M2* (constitutive source for MSL-2) nuclei stained with PI (red). (b) H4Ac16 staining of the same field. (A, bottom) Magnified image of a Sxl^M male and a Sxl^M; *H83M2* nucleus (boxes in a) with PI-stained (red), anti-MSL-1 antibody (purple), H4Ac16 antibody (green), and the merged DNA and H4Ac16 images (yellow). (B) Same type of mixture stained with PI (a) and anti-MOF (b). (B, bottom) Enlarged images boxed in a also showing anti-MSL2 labeling. The merged images of PI and anti-MOF are at the right. Bar, 10 μm.

The size of the *Sxl* transcript differs depending on the sex. Using this size difference as a marker, we were able to determine the percentage of male- and female-specific cells present in the mosaic larvae using a quantitative Northern blot, assuming minimal effect of *Sxl* on its own total RNA expression. Indeed, the total RNA from *Sxl* is not obviously different between the mutant and normal females and the intermediate level of the female form matches the level of protein found in the Western analysis noted above. These results indicate that *Sxl* has little impact on the level of its own total RNA (although due to the splicing function, the level of

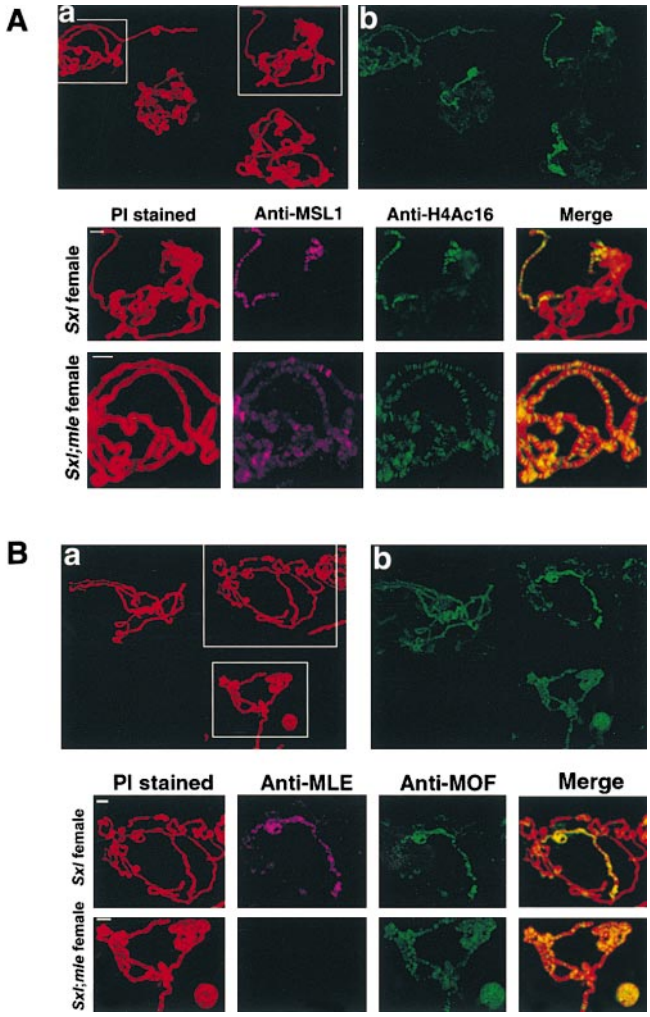

Figure 6.—H4Ac16, MOF, and MSL binding in *Sxl; mle* females. (A, a) Microscopic field containing a mixture of PI-stained *Sxl* and *Sxl; mle* female nuclei. Chromosomes were stained with H4Ac16 antibodies (b). Enlarged views of a *Sxl* and a *Sxl; mle* nucleus (boxed in a) probed with anti-MSL-1 and H4Ac16. The merged images of PI and anti-H4Ac16 are shown at the right. (B) Same type of mixture stained with PI (a) and anti-MOF (b). (B, bottom) Enlarged views of boxed nuclei in a also showing anti-MLE labeling. The merged PI and anti-MOF images are shown at the right. Bar, 10 μm.

SXL protein is altered). Both male- and female-specific transcripts are present in the *Sxl; mle* genotype. Triplicate measurements relative to the rRNA control revealed that 54% of total *Sxl* mRNA is male specific (Figures 7 and 8). Because this value is quite similar to the percentage found in Sxl^f alone, there is a similar number of male and female cells in the two genotypes, which allows a direct comparison of the effects on gene expression.

For the X chromosome, the level of the majority of transcripts is not significantly different from those of the *Sxl/Y; mle/+* males produced in the same cross (Figures 7 and 8). Of the autosomal loci, the majority of the transcripts in such females was quite similar in level to that of control males. Thus, the blockage of the MOF sequestration by the *mle* mutation prevents the reduced autosomal expression that otherwise occurs in Sxl^f females. Differences in gene expression between *Sxl; mle* females and *mle* females (Hiebert and Birchler 1994; Bhadra *et al.* 1999) alone might be attributed to the more discrete *vs.* uniform genomic distribution of MOF and H4Ac16 in these two genotypes, as noted above. Nevertheless, the autosomal reductions seen with Sxl^f itself are greatly reduced in the *Sxl; mle* double mutants.

DISCUSSION

In this study, the effect of *Sxl* mutations was examined to define the relationship of the sex determination mechanism to the sequestration process. In Sxl^M males, the slow accumulation of the SXL protein during development eventually prevents significant MSL-2 expression and hence reduces the MSL complex association with the X chromosome. This results in X and autosomal gene expression quite similar to that found in the *mle* mutant, *i.e.*, little response of the X-linked genes, but an overall increase in autosomal expression (Figure 9). Similarly, the association of the MSL proteins in ~50% of the cells of heteroallelic *Sxl* females causes sequestration of MOF to the two X chromosomes. This sequestration reduces the H4Ac16 on the autosomal loci, resulting in a lowered expression. There is a concomitant increase of acetylation on the X chromosome, but little overall response of the X-linked genes (Figure 9).

In the Sxl^M and Sxl^f*; mle* genotypes a low level of MSL-1/MSL-2 shows chromosomal binding to some degree, although present in distinct patterns in the two cases. This low level of binding, however, is insufficient to sequester all the available MOF present in the cell. Previous and present data suggest that in the absence of a functional MSL complex, MOF still associates with the chromosomes and is active in modifying H4. The reduced amount of MSL-1/MSL-2 appears saturated with MOF, allowing the remainder to be uniformly distributed across the genome, which modulates gene expression.

The general trends of X and autosomal gene expression in the Sxl^M and Sxl^f mutants match the autoradiographic data of Lucchesi and Skripsky (1981) when the latter is considered as absolute levels rather than relative X to autosomal ratios. In the cited study, autoradiographic grain counts over the X chromosome were changed very little in Sxl^M larvae compared to normal, but the counts over the autosomes were increased. Conversely, in Sxl^f females, the autosomal counts were lower than in normal females with little change over the X. Because the data reported here are anchored to rRNA levels, which in turn do not vary per unit of DNA, the "per cell" expression trends can be determined on an absolute rather than a relative comparison and indicate greater changes of the autosomes compared to the X chromosome.

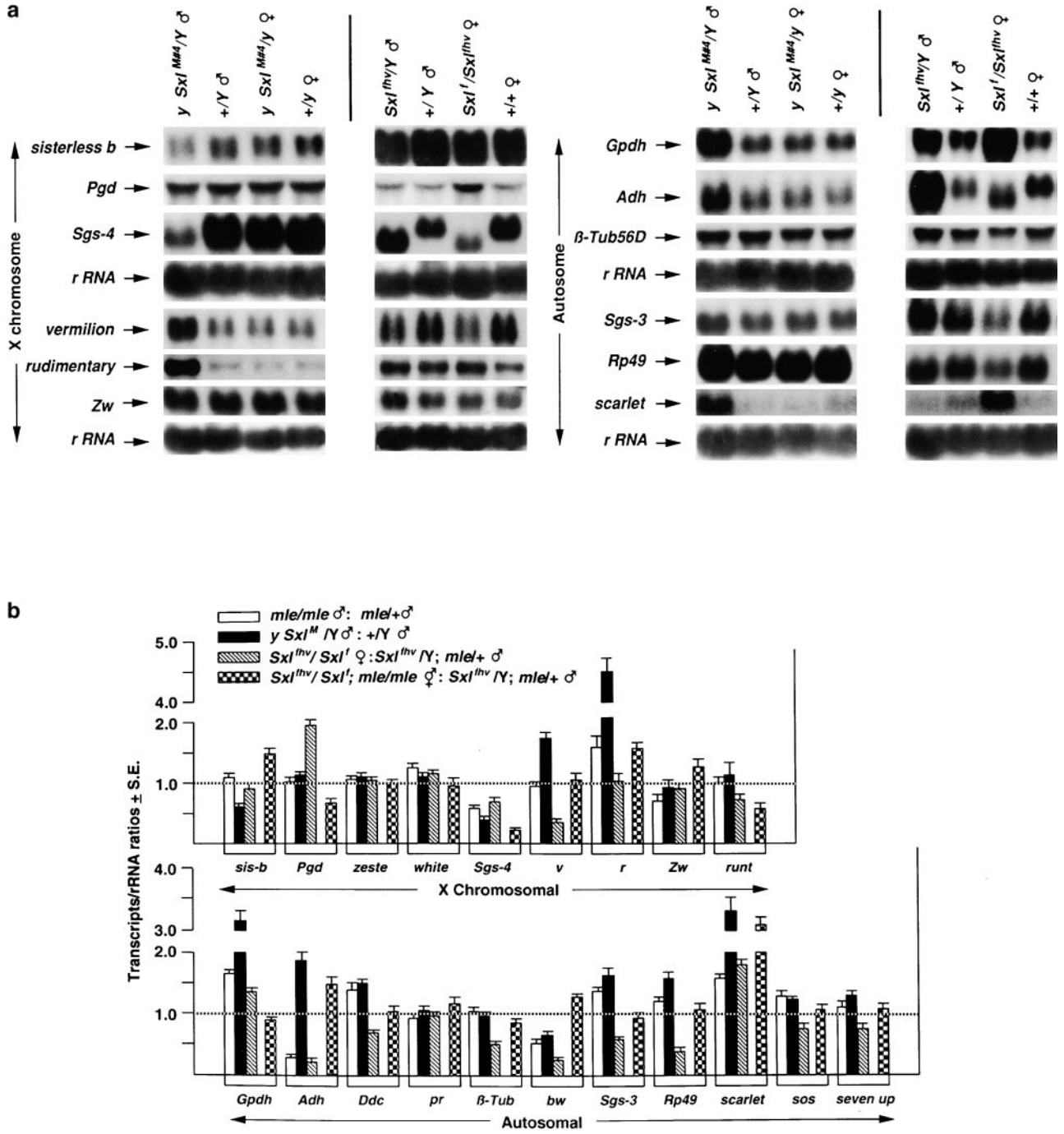

Figure 7.—(a) Effect of $Sxl^{M\#4}$ and $Sxl^{f\#1}/Sxl^{fhv\#1}$ mutations on selected X and autosomal genes. Transcript levels of each gene were determined via Northern analysis of the four genotypes, as noted at the top. Ribosomal RNA acts as a gel-loading control. (b) The quantitative value of 20 transcripts in *mle* and *Sxl* mutants relative to a normal respective control, noted in the key, is represented by the bar diagram. The dotted line represents the control level arbitrarily set at 1.0. Each ratio is determined by triplicate measurements. The 95% confidence interval is shown for each comparison.

A previous study has shown that the loss of individual components of the MSL complex in the *msl* mutant males releases the MOF acetylase from the X and a uniform H4 acetylation distribution results (Bhadra *et al.* 1999). Accordingly, autosomal gene expression is generally increased, reflecting an inverse effect of the X on the autosomes, because the normally sequestered acetylase is now dispersed, which results in higher acetylation levels on the autosomes. An increase or decrease of acetylation level on the X is not reflected in major changes in gene expression, suggesting that some member of the MSL complex insulates genes on the X from responding to the much increased acetylation level (Bhadra *et al.* 1999).

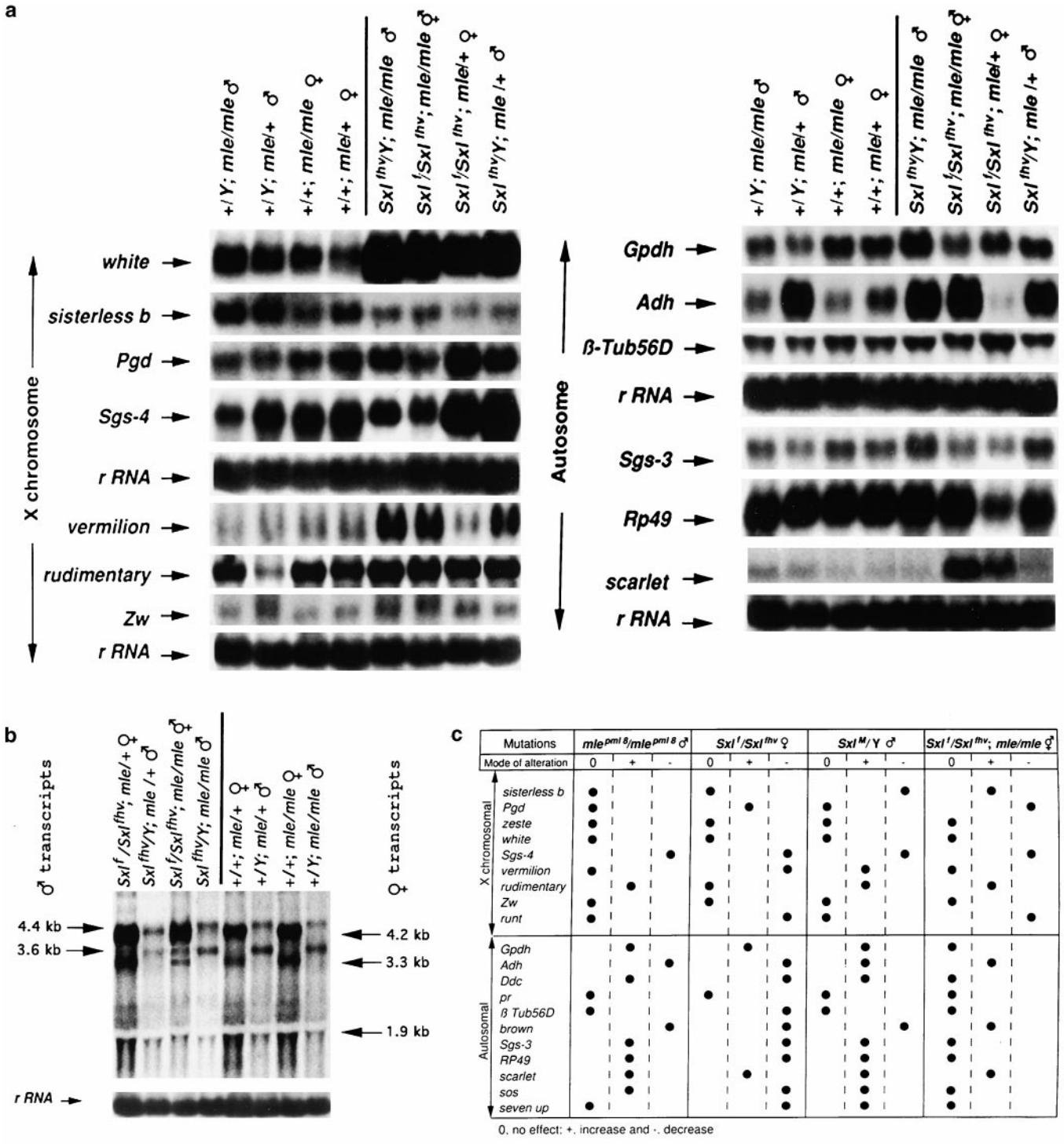

Figure 8.—(a) Expression of selected X and autosomal genes in $mle^{pml\ 8}$ and Sxl^{f}/Sxl^{fhv}; $mle^{pml\ 8}/mle^{pml\ 8}$ mutants relative to normal. Each gene was tested by triplicate Northern hybridizations of the genotype noted at the top. Antisense rRNA served as a gel-loading control. The relative ratio of each genotype compared to the controls is represented by the bar diagrams in Figure 7. (b) An autoradiogram of a Northern gel on the same genotypes probed with both male- and female-specific *Sxl* transcripts. The size of the male- and female-specific transcripts is noted. (c) The effect of various sex-specific mutations on selected X and autosomal genes is summarized to illustrate the generalized effect on the X and the autosomes. The transcript levels that are significantly greater or less than the control at the 95% confidence level are noted in the + or − columns, respectively.

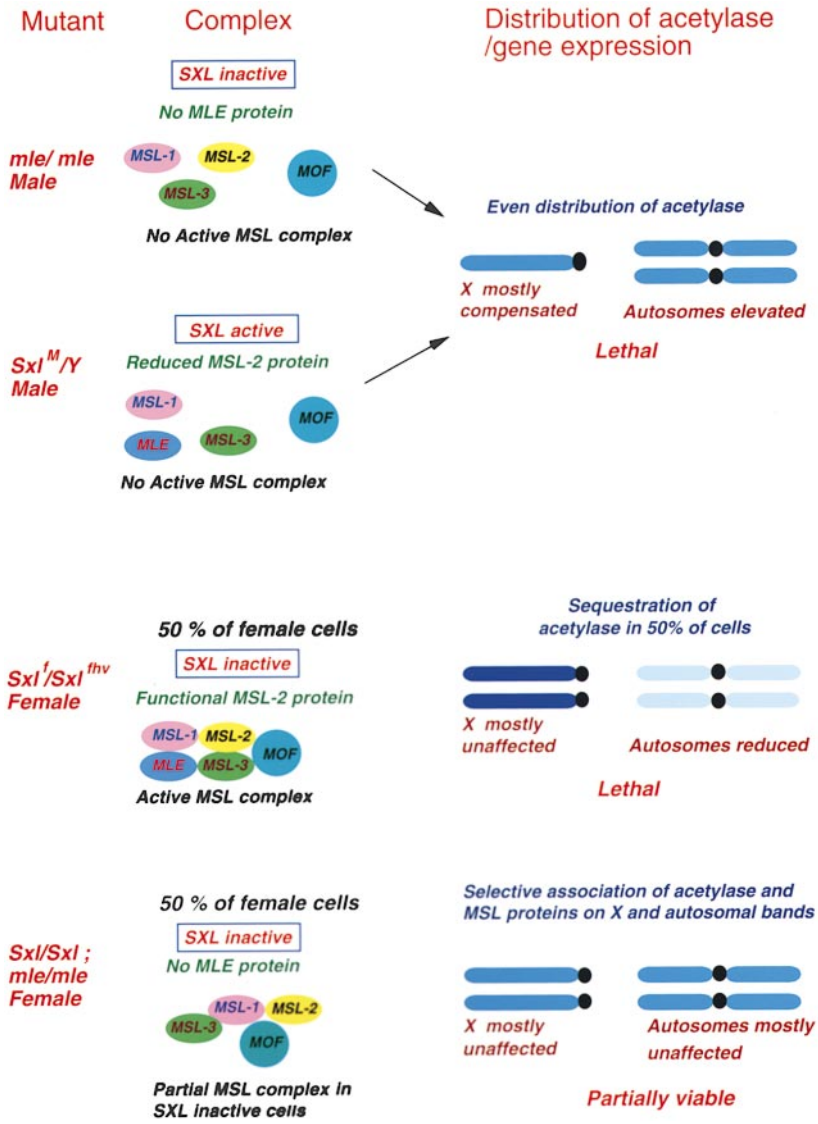

Figure 9.—MSL complex in different sex-specific mutant combinations. The schematic diagram illustrates the distribution of acetylase and the X and autosomal gene expression in each combination.

The collective data indicate that models that posit an association of the MSL complex with a gene for dosage compensation to occur are not supported. First of all, genetic destruction of the complex does not eliminate dosage compensation of most X-linked genes (Hiebert and Birchler 1994; Bhadra *et al.* 1999; present study). However, one could perhaps argue that this action eliminates compensation of the regulatory genes on the X, which, because they are now dosage dependent, will compensate most of the "housekeeping" genes that were assayed. We do not favor this alternative for three reasons. First, ectopic expression of MSL2 in females as a transgene (Bhadra *et al.* 1999) or in the *Sxl*^{f} mutants (present study) have the MSL complex present on their Xs but gene expression in general is not increased as predicted if the MSL complex alone conditions hyperactivation. Second, dosage compensation also occurs in metafemales (3X chromosomes with diploid autosomes), where there is no complete complex and this compensation is related to that occurring in males (Birchler 1992). Last, autosomal insertions of many X-derived genes still exhibit some degree of compensation despite the fact that these genes have no association with the MSL complex (Bhadra *et al.* 1999; Kelley *et al.* 1999). Thus, there are several circumstances known in which compensation occurs without the MSL complex. The function of the MSL complex on the X chromosome appears to be to inhibit the response of most X-linked genes to high levels of histone acetylation.

When these data are taken together along with previous studies on *mle* (Hiebert and Birchler 1994; Birchler 1996; Bhadra *et al.* 1999), a consistent model (Figure 9) is supported, indicating that the effect of the *Sex lethal* gene is mediated through its control of the presence or absence of MSL-2. When the MSL-2 protein is expressed, the sequestration of the MSL complex occurs with a resultant increase of H4Lys16 acetylation on the X at the expense of acetylation on the autosomes. In the absence of MSL-2, there is a uniform genomewide distribution of MOF and H4Lys16 acetylation. In gen-

eral, gene expression on the autosomes responds positively to the level of acetylation, but the X is refractory to it in the presence of the MSL complex. In this way, the twofold inverse-dosage effect of the X is used to achieve a proper level of dosage compensation, but the effect on the autosomes is diminished. Thus, as the heteromorphic sex chromosomes have evolved, both the X and the autosomes have maintained nearly equal expression between the sexes.

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