

Acetylated histone H4 on the male X chromosome is associated with dosage compensation in *Drosophila*

James R. Bone,¹ Jayne Lavender,³ Ron Richman,^{1,2} Melanie J. Palmer,⁴ Bryan M. Turner,³ and Mitzi I. Kuroda^{1,2,5}

¹Department of Cell Biology and ²Howard Hughes Medical Institute, Baylor College of Medicine, Houston, Texas 77030 USA; ³Department of Anatomy, University of Birmingham, Birmingham B15 2TT, England; ⁴Department of Entomology, Oklahoma State University, Stillwater, Oklahoma, 74078 USA

Dosage compensation in *Drosophila* occurs by an increase in transcription of genes on the X chromosome in males. This elevated expression requires the function of at least four loci, known collectively as the *male-specific lethal (msl)* genes. The proteins encoded by two of these genes, *maleless (mle)* and *male-specific lethal-1 (msl-1)*, are found associated with the X chromosome in males, suggesting that they act as positive regulators of dosage compensation. A specific acetylated isoform of histone H4, H4Ac16, is also detected predominantly on the male X chromosome. We have found that MLE and MSL-1 bind to the X chromosome in an identical pattern and that the pattern of H4Ac16 on the X is largely coincident with that of MLE/MSL-1. We fail to detect H4Ac16 on the X chromosome in homozygous *msl* males, correlating with the lack of dosage compensation in these mutants. Conversely, in *Sxl* mutants, we detect H4Ac16 on the female X chromosomes, coincident with an inappropriate increase in X chromosome transcription. These data suggest that synthesis or localization of H4Ac16 is controlled by the dosage compensation regulatory hierarchy. Dosage compensation may involve H4Ac16 function, potentially through interaction with the products of the *msl* genes.

[Key Words: Histone acetylation; chromatin structure; transcription]

Received September 22, 1993; revised version accepted November 12, 1993.

Heterogametic organisms have evolved various dosage compensation mechanisms, by which the levels of sex-linked gene expression are equalized between females (XX) and males (XY). In *Drosophila*, dosage compensation is achieved via increased expression of genes on the male X chromosome relative to genes on the female X chromosomes. Analyses of X-linked genes transposed to autosomes suggest that this dosage compensation mechanism operates through many *cis*-acting sequences dispersed along the X chromosome (for review, see Lucchesi and Manning 1987; Henikoff and Meneely 1993; Kuroda et al. 1993).

The increased transcriptional activity of the male X chromosome correlates with its distinct morphology in polytene chromosome squashes. The single male X chromosome appears to be the same width as the paired X chromosomes in females or the autosomes of either sex, despite containing only half the amount of DNA (Offermann 1936; Aronson et al. 1954; Dobzhansky 1957). The resulting diffuse cytological appearance suggests that the male X chromosome has adopted a unique chromatin structure. However, the molecular basis of this altered

morphology and whether it is a cause or a result of increased transcription from X-linked genes is not known.

Four loci (*maleless* and *male-specific lethal-1*, -2, and -3) encode regulators of dosage compensation in males (for review, see Lucchesi and Manning 1987; Kuroda et al. 1993). Loss-of-function mutations in these genes (collectively referred to as the *msl* genes) result in the death of homozygous males as third-instar larvae or early pupae. Lethality in *msl* mutant males correlates with a failure to dosage compensate X-linked genes (Belote and Lucchesi 1980; Okuno et al. 1984; Breen and Lucchesi 1986).

The *maleless (mle)* gene encodes a product that shows sequence similarity to a family of putative ATP-dependent RNA and DNA helicases (Kuroda et al. 1991). The *male-specific lethal-1 (msl-1)* gene encodes a novel protein that contains amino-terminal acidic domains characteristic of a number of chromatin-associated proteins (Palmer et al. 1993). Polyclonal antisera raised against the products of these two genes detect a striking pattern of distribution on polytene chromosomes. Both MLE and MSL-1 are associated almost exclusively with hundreds of sites along the X chromosome in males, but not in females. The pattern of distribution for MLE and MSL-1

⁵Corresponding author.

suggests that they are direct regulators of X-linked transcription in males. In addition to the presence of MLE and MSL-1 on the male X, an isoform of histone H4, acetylated at lysine 16, is also predominantly associated with the male X chromosome (Turner et al. 1992). This suggests that increased expression of genes on the single male X chromosome may involve site-specific histone acetylation.

Positive correlations between histone acetylation and gene expression have been observed in many systems. Chromatin from transcriptionally active loci is often enriched in acetylated histones (for review, see Csordas 1990; Turner 1991). Conversely, hypoacetylation of histones has been associated with transcriptional repression in *Saccharomyces cerevisiae* (Braunstein et al. 1993). The recent finding that transcriptionally inactive regions of mammalian metaphase chromosomes, including the inactive X chromosome in females, are almost completely devoid of acetylated histone H4 is consistent with this pattern (Jeppesen and Turner 1993). If H4Ac16 contributes to the mechanism of dosage compensation in *Drosophila*, then it is likely that the abundance or localization of this modified histone would be controlled by the dosage compensation regulatory pathway. In addition, the pattern of H4Ac16 distribution on the X chromosome might be similar to that of the MLE and/or MSL-1 regulatory proteins.

To address this hypothesis, we have performed experiments to determine whether H4Ac16 is controlled by the *msl* genes. We observed that the presence of this H4 isoform on the X chromosome requires the wild-type function of all four of the *msl* genes. In addition, MLE and MSL-1 are associated with identical sites on the male X chromosome, and their pattern of distribution is largely coincident with that of H4Ac16. Our results support the hypothesis that the mechanism of dosage compensation involves H4Ac16 and that the dosage compensation regulatory proteins function together to regulate X-linked transcription in *Drosophila* males.

Results

Association of H4Ac16 with the male X chromosome requires wild-type function of all four msl genes

The predominant X chromosome localization of H4Ac16 suggests that it may play a role in dosage compensation (Turner et al. 1992). If this is the case, then the presence of H4Ac16 on the male X chromosome may be governed by genes of the dosage compensation regulatory hierarchy (Fig. 1). To examine the genetic requirements for detection of H4Ac16 on the single X chromosome in males, H4Ac16 immunolocalization experiments were carried out in all four *msl* mutants. *Drosophila* males that are homozygous for any of the four *msl* mutations die as late third-instar larvae or early pupae. These larvae yield poor polytene chromosome preparations, but dissected whole larval salivary glands can be successfully labeled with antisera specific for chromatin-associated proteins. Figure 2 demonstrates that H4Ac16 is not lo-

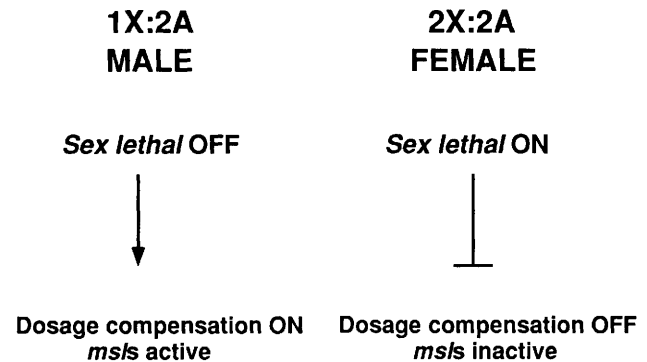


Figure 1. Genetic hierarchy of the dosage compensation pathway. A ratio of one X chromosome to two sets of autosomes (males) prevents expression of the *Sex-lethal* (*Sxl*) gene, allowing dosage compensation of the single X chromosome. A ratio of two X chromosomes to two sets of autosomes in females allows the *Sxl* gene to be expressed, which functions to prevent dosage compensation.

calized to the X chromosome in salivary gland nuclei from males mutant for *mle*, *msl-1*, *msl-2*, or *msl-3* (a–d). In contrast, wild-type H4Ac16 staining is observed in *msl* + sibling males (Fig. 2, e–h). A low level of staining is observed on all chromosomes in the mutants, similar to the anti-H4Ac16 staining observed in females (Turner et al. 1992). However, the high-level accumulation of H4Ac16 on the X chromosome in wild-type males is not observed in *msl* mutant males. In contrast, all chromosome arms can be labeled with antisera specific for RNA polymerase II in *msl* mutants, similar to wild type (e.g., *mle* vs. wild type, Fig. 3; data not shown). Therefore, the lack of wild-type levels of H4Ac16 on the X chromosome in *msl* mutant males is not attributable to a general loss of all chromatin-associated proteins. We cannot yet distinguish whether mutations in the *msl* genes interfere with synthesis, stability, or localization of H4Ac16 on the X chromosome.

X-chromosome association of H4Ac16 is inhibited in females by the action of the Sex-lethal gene

The *Sex-lethal* (*Sxl*) gene encodes an RNA-binding protein that acts as a genetic switch in *Drosophila* females to determine phenotypic sex and prevent dosage compensation (for reviews, see Baker and Belote 1983; Cline 1985; Baker 1989). *Sxl* functions, probably indirectly, to inhibit binding of MLE to the X chromosome (Gorman et al. 1993). As detection of H4Ac16 on the X chromosome in males is dependent on wild-type *mle* function, it is likely that it is also regulated by *Sxl*. In double-labeling experiments, we examined anti-H4Ac16 and anti-SXL staining in polytene chromosome preparations from *Sxl^{f1}/Sxl^{fhv1}* female larvae. Owing to a partial defect in activation of *Sxl* expression, these animals are mosaics of male and female cells, as judged by polytene chromosome immunolocalization of SXL and MLE (Gorman et al. 1993). Consistent with this observation, in all nuclei

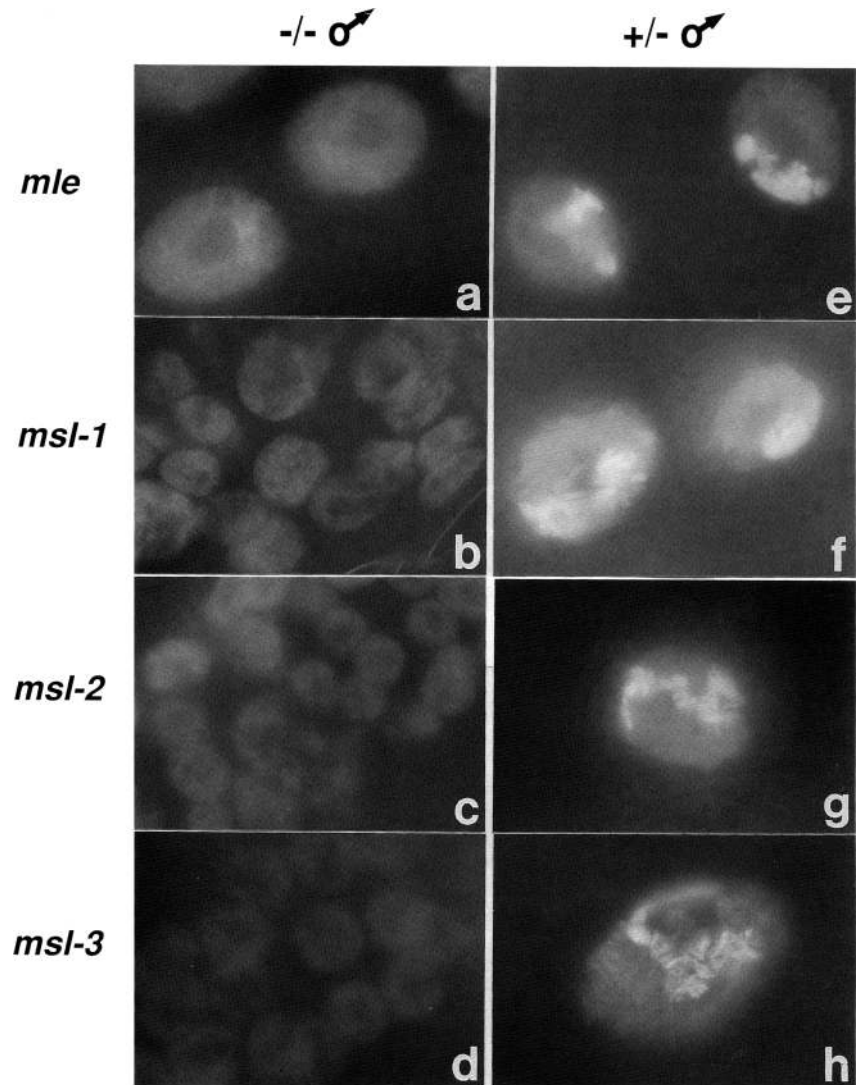


Figure 2. Immunolocalization of H4Ac16 in wild-type and *msl* mutant males. (Left) Nuclei from intact salivary glands from third-instar male larvae homozygous for mutations in *mle^{pm18}* (a), *msl-1^{γ216}/msl-1^{γ269}* (b), *msl-2/msl-2* (c), or *msl-3^P/msl-3^{MAK}* (d) stained with anti-H4Ac16 antibodies. (Right) Nuclei from intact salivary glands from heterozygous sibling males stained with anti-H4Ac16 antibodies. The *msl* mutants are much smaller than their heterozygous siblings, and this difference is reflected in the smaller size of the *msl* mutant polytene cells and nuclei.

from *Sxl^{f1}/Sxl^{fhv1}* females that follow a female pathway, and thus stain positively with SXL antibodies, elevated H4Ac16 staining is not detected on the paired X chromosomes (data not shown). Conversely, H4Ac16 is detected on the X chromosomes in all *Sxl^{f1}/Sxl^{fhv1}* nuclei that lack SXL protein and have followed a male developmental pathway (Fig. 4a). H4Ac16 is associated with multiple sites along the length of the paired X chromosomes from a *Sxl^{f1}/Sxl^{fhv1};msl-2⁻/+* female. Identical results are obtained from experiments with *Sxl^{f1}/Sxl^{fhv1}* females that are wild type for the *msl* genes (data not shown). Thus, the function of the *Sxl* gene prevents accumulation of H4Ac16 on the X chromosomes in females. These data are consistent with the finding that *Sxl* also regulates the presence of MLE and MSL-1 on the X chromosome (Gorman et al. 1993; M.J. Palmer and M.I. Kuroda, unpubl.).

Because of the poor health of *msl* mutant males, we also examined H4Ac16 staining in *Sxl^{f1}/Sxl^{fhv1};msl/msl* females to verify the results of our whole gland staining

experiments. These females yield high quality polytene chromosomes and can be used to assess the phenotype of *msl⁻* cells that follow the male dosage compensation pathway (Gorman et al. 1993). Figure 4b shows a SXL-negative polytene chromosome spread from a *Sxl^{f1}/Sxl^{fhv1};msl-2/msl-2* female labeled with antibodies against H4Ac16. No specific H4Ac16 staining is observed on the pair of X chromosomes, whereas nuclei from *msl-2⁻/+* sibling females clearly exhibit H4Ac16 on the X chromosomes (Fig. 4a). We have also examined H4Ac16 staining in *Sxl^{f1}/Sxl^{fhv1}* females that are homozygous for *mle*, *msl-1*, or *msl-3*. Without exception, no X chromosome staining of H4Ac16 above the level seen on all chromosome arms is observed in these animals (data not shown), confirming our previous results. Thus, all four of the *msl* genes are required for the synthesis, stability, or localization of H4Ac16 on the X chromosome.

Loss of *msl* gene function does not result in a general defect in histone H4 acetylation, as each of the

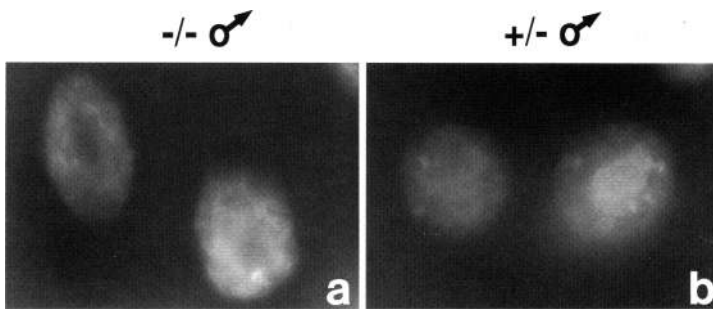


Figure 3. Salivary gland nuclei from *msl* mutant males stain positively for RNA polymerase II. Nuclei from an *mle^{pm18}/mle^{pm18}* mutant male (a), and an *mle^{pm18}/CyO* sibling male (b), stained with antibodies that recognize RNA polymerase II.

Sxl;msl mutants has a wild-type distribution of histone H4 acetylated at lysine 5 and/or 12 (e.g., Fig. 4; data not shown). All chromosome arms from the *Sxl^{f1}/Sxl^{thv1};msl-2⁻/+* nucleus (Fig. 4c) and the *Sxl^{f1}/Sxl^{thv1};msl-2/msl-2* nucleus (Fig. 4d) stain at multiple sites with anti-H4Ac5/12. These data indicate that *msl* mutations specifically result in the absence of H4Ac16 on the male X chromosome and do not noticeably alter histone H4 acetylation in general.

MLE and MSL-1 are associated with identical sites on the male X chromosome, which are largely coincident with H4Ac16

MLE and MSL-1 encode regulators of dosage compensation in males, and both exhibit predominant X chromosome localization in male polytene nuclei (Kuroda et al. 1991; Palmer et al. 1993). The male X chromosome-specific staining of H4Ac16 and its inverse dependence on *Sxl* gene function suggest that this modified histone may also play a role in dosage compensation. If these three proteins act in concert to regulate hyperexpression of genes on the single male X chromosome, then one might

expect their polytene chromosome distributions to be very similar or identical.

To examine potential interactions between MLE, MSL-1, and H4Ac16, we performed double-labeling immunolocalization experiments to determine whether the three proteins are associated with the same sites on polytene chromosomes. Polyclonal antisera raised in different species were used to detect MLE and MSL-1 on the polytene chromosomes of wild-type larvae. As seen in Figure 5, a and b, MLE (red) and MSL-1 (green) exhibit banded patterns of association along the length of the male X chromosome. In Figure 5c, a double exposure micrograph demonstrates that MLE and MSL-1 antibodies stain identical sites on the X chromosome. The generation of the yellow color is attributable to photographic superimposition of the Texas Red- and fluorescein-conjugated secondary antisera used to visualize the two specific primary antisera. In control experiments, we determined that these species-specific anti-IgG secondary antibodies do not cross-react (data not shown), so the overlap of the fluorochromes must be the result of the presence of MLE and MSL-1 antibodies at identical sites. The distribution patterns of MSL-1 (green) and H4Ac16 (red) are compared in Figure 5 d–f. The double

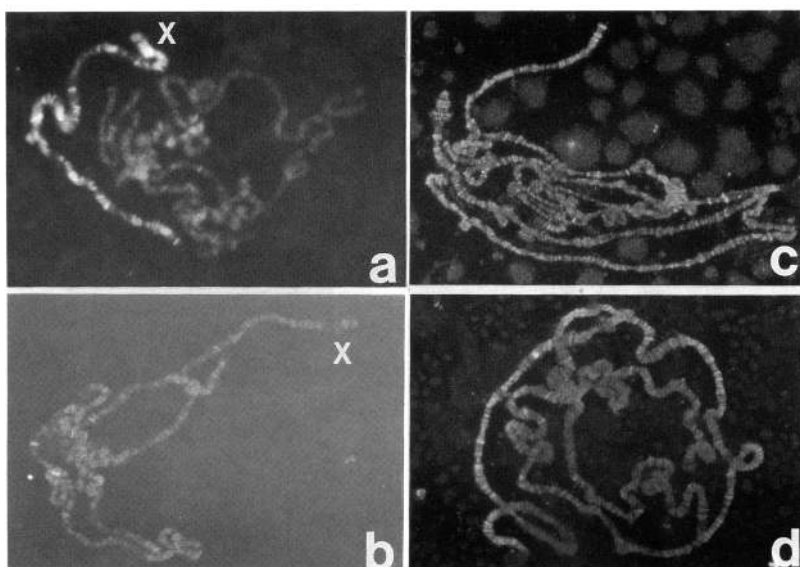


Figure 4. Immunolocalization of H4Ac16 or H4Ac5/12 in SXL-negative nuclei from *Sxl^{f1}/Sxl^{thv1}* females. (a,c) *Sxl^{f1}/Sxl^{thv1};msl-2/+* nuclei. (b,d) *Sxl^{f1}/Sxl^{thv1};msl-2/msl-2* nuclei. (a,b) Anti-H4Ac16 staining; (c,d) anti-H4Ac5/12 staining.

Bone et al.

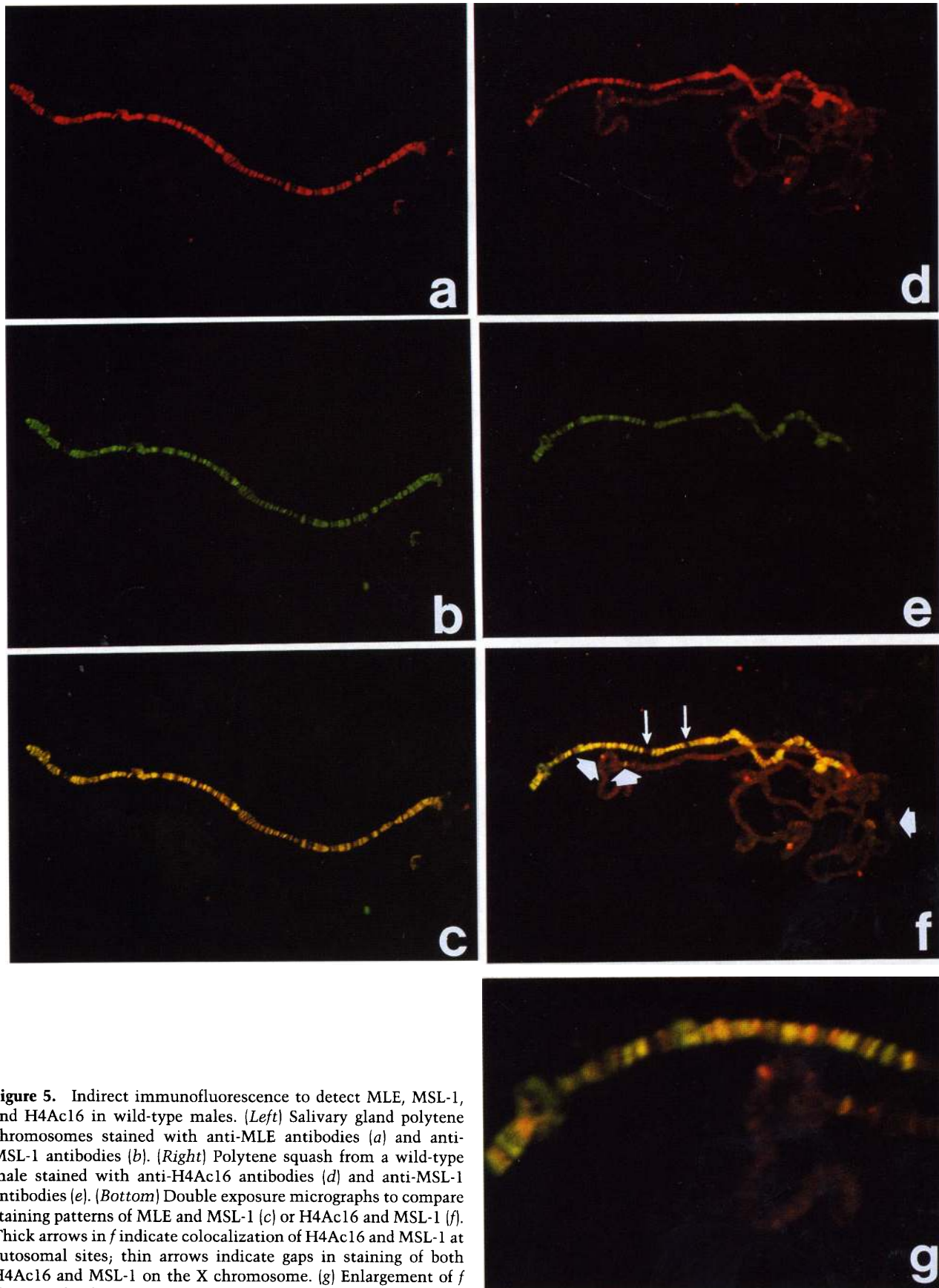


Figure 5. Indirect immunofluorescence to detect MLE, MSL-1, and H4Ac16 in wild-type males. (*Left*) Salivary gland polytene chromosomes stained with anti-MLE antibodies (*a*) and anti-MSL-1 antibodies (*b*). (*Right*) Polytene squash from a wild-type male stained with anti-H4Ac16 antibodies (*d*) and anti-MSL-1 antibodies (*e*). (*Bottom*) Double exposure micrographs to compare staining patterns of MLE and MSL-1 (*c*) or H4Ac16 and MSL-1 (*f*). Thick arrows in *f* indicate colocalization of H4Ac16 and MSL-1 at autosomal sites; thin arrows indicate gaps in staining of both H4Ac16 and MSL-1 on the X chromosome. (*g*) Enlargement of *f* showing the distal end of the X chromosome.

exposure micrograph in Figure 5f shows that the patterns of staining are overlapping and largely coincident. H4Ac16 is excluded from the same reproducible gaps observed in MLE/MSL-1 staining along the length of the X chromosome (Fig. 5f, thin arrows). Additionally, H4Ac16 is also found associated with all of the male-specific MSL-1 sites on the autosomes (e.g., 5f, thick arrows). Although it appears that MSL-1 and H4Ac16 are detected primarily at the same sites, the patterns exhibit several reproducible dissimilarities, evident as non-yellow regions in an enlargement from Figure 5f, depicting the distal third of the X chromosome (Fig. 5g).

Discussion

msl mutants lack H4Ac16 on the male X chromosome

In this study we present evidence that the presence of histone H4 acetylated at lysine 16 (H4Ac16) on the X chromosome in *Drosophila* is dependent on the function of the *msh* genes. We have not yet determined whether the *msh* genes act on H4Ac16 at the level of synthesis, stability, or localization. One possibility is that the *msh* gene products are required for the specific acetylation of histone H4 resident on the X chromosome or to prevent deacetylation of H4Ac16. Alternatively, the MSLs may target modified H4 to sites on the X chromosome or stabilize its chromosomal association. These alternatives could be distinguished by comparing the amounts of H4Ac16 in wild-type and *msh* mutant males. However, there is only a threefold difference in H4Ac16 levels between wild-type males and females (Turner et al. 1992). To date, our attempts at examining the relative levels of H4Ac16 in wild-type and mutant males have not been definitive, perhaps because of the difficulty in measuring small differences in protein levels in mutant animals. It is significant to note that mutations in the *msh* genes do not noticeably alter immunolocalization of H4 acetylated at lysine 5 and/or 12. Thus, general histone acetylation is apparently not perturbed by abolishment of *msh* gene function. One attractive possibility is that a hypothetical lysine 16-specific acetylase exists in both sexes but is more active and/or is only localized to the X chromosome in males.

Comparison of the staining patterns of MLE, MSL-1, and H4Ac16 reveals a correlation between dosage compensation and histone acetylation

In double-labeling experiments, we have observed that MLE and MSL-1 are associated in an identical pattern along the length of the X chromosome. This result is consistent with existing genetic evidence, suggesting that the products of the *msh* genes interact with each other to regulate dosage compensation in concert (Gorman et al. 1993). However, these cytological observations do not allow us to determine how closely these proteins might be distributed on a molecular level.

The observation that the H4Ac16 banding pattern is largely coincident with MLE/MSL-1 suggests a potential

link between the signals required for localizing these proteins to the male X chromosome. There are, however, several dissimilarities in their patterns of localization. Many of the noticeable differences between H4Ac16 and MSL-1 can be ascribed to an overabundance of one protein relative to the other in particular loci, but at a few sites along the X there is association of MSL-1 and an obvious absence of H4Ac16. For example, the ecdysone-induced puff at cytological position 2B on the X chromosome exhibits a marked lack of H4Ac16. It has been reported previously that acetylated histone H4 isoforms are underrepresented in highly puffed chromatin (Turner et al. 1990; Sommerville et al. 1993). It has not been determined whether this phenomenon represents the actual distribution of acetylated histone or is a consequence of the experimental procedure. Histones are very sensitive to the fixation conditions used for polytene chromosome spreads. If acetylated histones are present in the extended chromatin structure of a polytene puff, they may be more vulnerable to removal than nonacetylated histones, which can be detected in puffed regions (Sommerville et al. 1993). Despite the subtle differences in the staining patterns, the vast majority of the sites are common, suggesting that H4Ac16 may interact with MLE and MSL-1, or that these proteins are colocalized as a consequence of their function.

In addition to the male X chromosome-specific pattern of staining, H4Ac16 is detected at 30–40 loci on the autosomes in polytene chromosome preparations of both sexes (this paper; Turner et al. 1992). MLE protein is also found at many autosomal sites in polytene chromosomes, but it is not known whether these are identical to the H4Ac16 autosomal loci (Kuroda et al. 1991). MSL-1 exhibits association with <10 autosomal loci, but these are detectable only in males (Palmer et al. 1993). We have found that MLE and H4Ac16 are associated with all of the MSL-1 autosomal sites (e.g., Fig. 5; J.R. Bone and M.I. Kuroda, unpubl.), suggesting that unknown genes at these locations may be regulated by dosage compensation regulatory proteins. That MLE, MSL-1, and H4Ac16 colocalize to these sites on the autosomes, supports the hypothesis that the proteins are targeted to particular loci by similar, potentially interdependent mechanisms.

Site-specific H4 acetylation is implicated in transcriptional regulation

Several lines of evidence suggest that increased levels of histone acetylation are not merely a consequence of transcription but serve to delimit regions of active chromatin (for review, see Csordas 1990; Turner 1991, 1993). Genetic studies in *S. cerevisiae* provide compelling evidence that the amino terminus of histone H4, and lysine 16 in particular, plays an important role in the regulation of gene expression. Substitution of a neutral amino acid for lysine 16 results in the specific derepression of genes at the normally silent mating type loci, whereas placement of an arginine at position 16 has little effect on expression of these genes (Johnson et al. 1990; Megee et al. 1990; Park and Szostak 1990). It is possible that neu-

tralization of the positive charge on lysine 16 of histone H4, which occurs upon lysine ϵ -acetylation, stimulates gene expression from the normally silent mating type loci. This hypothesis is consistent with the finding that the amino-terminal region of H4 is required for activation of expression of the *GAL1* and *PHO5* genes (Durrin et al. 1991). Post-translational modification of histone octamers may activate gene expression by allowing the repositioning of key nucleosomes that cover *cis*-acting control elements (Roth et al. 1992), or by promoting interactions between nucleosomes and transcriptional activators (Lee et al. 1993). Histone H4 acetylated at lysine 16, present at multiple loci along the single X chromosome in *Drosophila* males, might facilitate dosage compensation by such mechanisms.

The relationship between the diffuse cytological appearance of the polytene X and its structure at the nucleosomal level is not known. However, both the accumulation of H4Ac16 on the male X chromosome and its distinct cytological appearance require the function of all four of the *msl* genes (this study; Belote and Lucchesi 1980; Okuno et al. 1984; Gorman et al. 1993). The biochemical properties of acetylated histone H4 are consistent with a general loosening of chromatin structure (Cary et al. 1982; Hong et al. 1993), but it is unlikely that a single modification of a nucleosome constituent is sufficient by itself to account for a distinct higher order chromatin structure (Csordas 1990; Kornberg and Lorch 1992). It is possible that acetylation of H4 at lysine 16 is required for the initiation of a series of molecular events that result in the establishment of the novel cytological appearance of the male X chromosome. In future studies, it will be interesting to discover what other molecules may contribute to the characteristic architecture associated with increased transcription of the male X chromosome in *Drosophila*.

Materials and methods

Drosophila stocks

Flies were raised on standard cornmeal/yeast/agar/molasses medium, containing propionic acid as an antifungal agent. The *msl-1^{r216}* and *msl-1^{r269}* alleles are described in Palmer et al. (1993). All stocks not specifically mentioned in the text are described in Lindsley and Zimm (1992).

Crosses to generate *msl* mutant males for whole gland staining were performed at 18°C as follows: (1) *y; mle^{pm18} cn bw/CyO* T(1;2)B80 *y*⁺ females \times *y/Y; mle^{pm18} cn bw/CyO* T(1;2)B80 *y*⁺ males; (2) *y; msl-1^{r269} cn bw/CyO* T(1;2)B80 *y*⁺ females \times *y/Y; msl-1^{r216} cn bw/CyO* T(1;2)B80 *y*⁺ males; (3) *y; msl-2 cn bw/CyO* T(1;2)B80 *y*⁺ females \times *y/Y; msl-2 b pr cn wxt bw/CyO* T(1;2)B80 *y*⁺ males; (4) *msl-3^P red/TM3* females \times *msl-3^{MAK} red e/TM3* males.

Homozygous *mle*, *msl-1*, and *msl-2* larvae were identified by the absence of the *y*⁺ marker, present on the *CyO* *y*⁺ balancer chromosome (J. Botas, pers. comm.). Homozygous *msl-3^P*/*msl-3^{MAK}* larvae were identified by their red Malpighian tubules, because of the presence of the *red* marker. The *msl-3^P* allele is the original *mle-3ⁱ* allele (Uchida et al. 1981). *msl-3^{MAK}* is a new allele, induced by X-ray mutagenesis (M. Keene and T. Hazelrigg, pers. comm.).

Crosses to generate *Sxl* heteroallelic females were performed at 25°C as follows: (1) *cm Sxl^{thv1} ct; pr mleⁱ females* \times *Sxl^{f1} oc ptg v/Y; pr mleⁱ/SM1* males; (2) *cm Sxl^{thv1} ct; msl-1^b cn bw* females \times *Sxl^{f1} oc ptg v/Y; msl-1^b cn bw/SM1* males; (3) *cm Sxl^{thv1} ct; msl-2 b pr cn wxt bw* females \times *Sxl^{f1} oc ptg v/Y; msl-2 cn bw/CyO* males; (4) *cm Sxl^{thv1} ct; msl-2 b pr cn wxt bw* females \times *Sxl^{f1} oc ptg v/Y; msl-2 cn bw/In (2LR) Gla Bc Elp* males; (5) *cm Sxl^{thv1} ct; msl-3^P red* females \times *Sxl^{f1} oc ptg v/Y; msl-3^P red/TM6B* males.

Homozygous *mle*, *msl-1*, and *msl-2* squashes were identified cytologically. In heterozygous *msl/Bal* squashes, the second chromosome is largely asynapsed. In experiments to determine H4Ac5/12 immunolocalization, homozygous *msl-2* mutants were identified by the absence of the dominant marker *Bc*, present on the *In (2LR) Gla Bc Elp* balancer chromosome. Homozygous *msl-3^P* larvae were identified by the *red* mutation and the absence of the *TM6B* dominant marker *Tb*.

Preparation of antisera

Affinity-purified anti-MLE antibodies were prepared essentially as described in Kuroda et al. (1991), with the exception that rabbits were immunized with a 225-amino-acid polypeptide from the amino terminus of MLE, encoded by the *mle12* cDNA (Kuroda et al. 1991; L. Richter and M.I. Kuroda, unpubl.). Preparation and characterization of antisera specific for acetylated histone H4 isoforms can be found in Turner and Fellows (1989) and Turner et al. (1989). Affinity-purified polyclonal antibodies specific for MSL-1 were raised in goat against a fragment of the MSL-1-coding region, encoded by plasmid pGmsl1-1, as described in Palmer et al. (1993), with the exception that the purified protein was mixed with an equal volume of Freund's adjuvant and used to immunize a single goat (Bethyl Laboratories). Anti-RNA polymerase II antibodies were a gift from Dr. Arno Greenleaf (Weeks et al. 1982). Anti-SXL ascites fluid was the gift of Drs. Daniel Bopp and Paul Schedl (Bopp et al. 1991).

Immunostaining of polytene chromosomes and salivary glands

Polytene chromosome preparation was performed as described previously (Pirrotta et al. 1988; Rastelli et al. 1993), with few modifications. Salivary glands from third-instar larvae or prepupae were dissected in 0.7% NaCl and fixed for 45 sec in phosphate-buffered saline (PBS) at pH 7.2, containing 3.7% formaldehyde and 0.1% Triton X-100. For visualization of histone H4, the fixation step was carried out for 5 min in PBS containing 3.7% formaldehyde, 0.1% Triton X-100, and 0.2% NP-40, according to Turner et al. (1990). Glands were then transferred to a 20- μ l drop of distilled water containing 50% acetic acid and 3.7% formaldehyde on a siliconized coverslip for 2 min. The glands were squashed onto a glass slide and frozen in liquid nitrogen. The coverslips were flipped off with a razor blade, and the slides were immersed in 95% ethanol for a minimum of 1 hr. The slides were then washed in PBS for 30 min, in PBT (PBS plus 0.2% Triton X-100) for 30 min, and blocked in PBT with 2% BSA for 30 min. Primary antibody incubation occurred overnight at 4°C in a humidified chamber. For double labeling, both primary antisera were applied simultaneously. Affinity-purified rabbit anti-MLE and goat anti-MSL-1 antibodies were both employed at a concentration of 1:20. Affinity-purified rabbit anti-H4Ac16 antibodies were used at a dilution of 1:80. Antisera that recognize H4 acetylated at either lysine 5 or lysine 12 (H4Ac5/12) were used at a dilution of 1:150. Goat anti-RNA polymerase II antibodies were used at a concentration of 1:200. Mouse anti-SXL antibodies obtained from ascites fluid were used at a

dilution of 1:2000. Donkey FITC- and Texas Red-conjugated secondary antibodies (Jackson ImmunoResearch) were used at a 1:200 dilution. Secondary antibody incubation took place for 2 hr at room temperature. For double labeling, both secondary antibodies were applied simultaneously. Counter staining of the chromosomes was done for 10 sec in 0.03 $\mu\text{g}/\text{ml}$ of bis-benzimide (Hoechst 33258, Boehringer), followed by three washes in PBT. Slides were mounted in 80% glycerol, 2% *n*-propyl gallate, and 10 mM Tris-HCl (pH 9.0). Chromosomes were viewed using epifluorescence optics with a Nikon Microphot-FXA microscope and photographed with either Kodak Tri-X pan or Ektachrome 400 film.

Immunostaining of polytene chromosomes in whole salivary glands was carried out using a modification of the imaginal disc staining procedure of Pattatucci and Kaufman (1991). Salivary glands from third-instar larvae were dissected in PBT and kept at 4°C. Glands were then extracted with agitation for 45 sec in 900 μl of PBS containing 1.1% formaldehyde and 55% heptane. Fixation was carried out by rocking the glands for 20 min in 800 μl of PBS containing 3% formaldehyde and 5% DMSO. The glands were washed for 20 min in PBS, followed by two 20-min washes in PBT plus 2% BSA. Primary and secondary antibodies were employed as stated previously. Glands were mounted intact on slides in 80% glycerol, 2% *n*-propyl gallate, and 10 mM Tris-HCl (pH 9.0). Nuclei in intact glands were viewed under epifluorescence optics using a Zeiss Axiophot microscope and photographed with Kodak Plus-X pan film.

Acknowledgments

We thank Daniel Bopp and Paul Schedl for providing the anti-SXL antibodies; Arno Greenleaf for the anti-RNA polymerase II antibodies; Juan Botas for the *CyO y⁺* balancer chromosome; Tulle Hazelrigg for the *mSl-3^{MAK}* allele; Monica Gorman and Bruce Baker for providing *Sxl*; *mSl* stocks; and Liz Richter for the expression plasmid used to prepare the anti-MLE antibodies. We are grateful to S. Elledge, R. Kelley, L. Rastelli, and L. Richter for critical reading of the manuscript. This work was supported by National Institutes of Health grant GM45744 and National Science Foundation grant DMB-9158176 to M.I.K., and grants from the Wellcome Trust and the U.K. Cancer Research Campaign to B.M.T. J.R.B. was supported by the Life and Health Insurance Medical Research Fund. M.I.K. is a Searle Scholar and an Assistant Investigator of the Howard Hughes Medical Institute.

The publication costs of this article were defrayed in part by payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 USC section 1734 solely to indicate this fact.

References

- Aronson, J.F., G.T. Rudkin, and J. Schultz. 1954. A comparison of giant X-chromosomes in male and female *Drosophila melanogaster* by cytophotometry in the ultraviolet. *J. Histochem. Cytochem.* **2**: 458–459.
- Baker, B.S. 1989. Sex in flies: The splice of life. *Nature* **340**: 521–524.
- Baker, B.S. and J.M. Belote. 1983. Sex determination and dosage compensation in *Drosophila melanogaster*. *Annu. Rev. Genet.* **17**: 345–393.
- Belote, J.M. and J.C. Lucchesi. 1980. Control of X chromosome transcription by the *maleless* gene in *Drosophila*. *Nature* **285**: 573–575.
- Bopp, D., L.R. Bell, T.W. Cline, and P. Schedl. 1991. Developmental distribution of female-specific *Sex-lethal* proteins in *Drosophila melanogaster*. *Genes & Dev.* **5**: 403–415.
- Braunstein, M., A.B. Rose, S.G. Holmes, C.D. Allis, and J.R. Broach. 1993. Transcriptional silencing in yeast is associated with reduced nucleosome acetylation. *Genes & Dev.* **7**: 592–604.
- Breen, T.R. and J.C. Lucchesi. 1986. Analysis of the dosage compensation of a specific transcript in *Drosophila melanogaster*. *Genetics* **112**: 483–491.
- Cary, P.D., C. Crane-Robinson, E.M. Bradbury, and G.H. Dixon. 1982. Effect of acetylation on the binding of N-terminal peptides of histone H4 to DNA. *Eur. J. Biochem.* **127**: 137–143.
- Cline, T.W. 1985. Primary events in the determination of sex in *Drosophila melanogaster*. In *The origin and evolution of sex* (ed. H.O. Halvorson and S. Monroy), pp. 301–327. Alan R. Liss, New York.
- Csordas, A. 1990. On the biological role of histone acetylation. *Biochem. J.* **265**: 23–38.
- Dobzhansky, T. 1957. The X-chromosome in the larval salivary glands of hybrids *Drosophila insularis* × *Drosophila tropicalis*. *Chromosoma* **8**: 691–698.
- Durrin, L.K., R.K. Mann, P.S. Kayne, and M. Grunstein. 1991. Yeast histone H4 N-terminal sequence is required for promoter activation *in vivo*. *Cell* **65**: 1023–1031.
- Gorman, M., M.I. Kuroda, and B.S. Baker. 1993. Regulation of the sex-specific binding of the maleless dosage compensation protein to the male X chromosome in *Drosophila*. *Cell* **72**: 39–50.
- Henikoff, S. and P.M. Meneely. 1993. Unwinding dosage compensation. *Cell* **72**: 1–2.
- Hong, L., G.P. Schroth, H.R. Matthews, P. Yau, and E.M. Bradbury. 1993. Studies of the DNA binding properties of histone H4 amino terminus. *J. Biol. Chem.* **268**: 305–314.
- Jeppesen, P. and B.M. Turner. 1993. The inactive X chromosome in female mammals is distinguished by a lack of histone H4 acetylation, a cytogenetic marker for gene expression. *Cell* **74**: 281–289.
- Johnson, L.M., P.S. Kayne, E.S. Kahn, and M. Grunstein. 1990. Genetic evidence for an interaction between SIR3 and histone H4 in the repression of the silent mating type loci in *Saccharomyces cerevisiae*. *Proc. Natl. Acad. Sci.* **87**: 6286–6290.
- Kornberg, R.D. and Y. Lorch. 1992. Chromatin structure and transcription. *Annu. Rev. Cell Biol.* **8**: 563–587.
- Kuroda, M.I., M.J. Kernan, R. Kreber, B. Ganetzky, and B.S. Baker. 1991. The maleless protein associates with the X chromosome to regulate dosage compensation in *Drosophila*. *Cell* **66**: 935–947.
- Kuroda, M.I., M.J. Palmer, and J.C. Lucchesi. 1993. X chromosome dosage compensation in *Drosophila*. *Sem. Dev. Biol.* **4**: 107–116.
- Lee, D.Y., J.L. Hayes, D. Pruss, and A.P. Wolffe. 1993. A positive role for histone acetylation in transcription factor access to nucleosomal DNA. *Cell* **72**: 73–84.
- Lindsley, D.L. and G.G. Zimm. 1992. *The genome of Drosophila melanogaster*. Academic Press, San Diego, CA.
- Lucchesi, J.C. and J.E. Manning. 1987. Gene dosage compensation in *Drosophila melanogaster*. *Adv. Genet.* **24**: 371–429.
- Megee, P.C., B.A. Morgan, B.A. Mittman, and M.M. Smith. 1990. Genetic analysis of histone H4: Essential role of lysines subject to reversible acetylation. *Science* **247**: 841–845.
- Offermann, C.A. 1936. Branched chromosomes as symmetrical duplications. *J. Genet.* **32**: 103–116.
- Okuno, T., T. Satou, and K. Oishi. 1984. Studies on the sex-specific lethals of *Drosophila melanogaster*. VII. Sex-specific lethals that do not affect dosage compensation. *Jpn. J. Genet.*

Bone et al.

59: 237–247.

- Palmer, M.J., V.A. Mergner, R. Richman, J.E. Manning, M.I. Kuroda, and J.C. Lucchesi. 1993. The *male-specific lethal-one (msl-1)* gene of *Drosophila melanogaster* encodes a novel protein that associates with the X chromosome. *Genetics* **134**: 545–557.
- Park, E.C. and J.W. Szostak. 1990. Point mutations in the yeast histone H4 gene prevent silencing of the silent mating locus HML. *Mol. Cell. Biol.* **10**: 4932–4934.
- Pattatucci, A.M. and T.C. Kaufman. 1991. The homeotic gene *Sex combs reduced* of *Drosophila melanogaster* is differentially regulated in the embryonic and imaginal stages of development. *Genetics* **129**: 443–461.
- Pirrotta, V., S. Bickel, and C. Mariani. 1988. Developmental expression of the *Drosophila zeste* gene and localization of *zeste* protein on polytene chromosomes. *Genes & Dev.* **2**: 1839–1850.
- Rastelli, L., C.S. Chan, and V. Pirrotta. 1993. Related chromosome binding sites for *zeste*, suppressors of *zeste* and *Polycomb* group proteins in *Drosophila* and their dependence on *Enhancer of zeste* function. *EMBO J.* **12**: 1513–1522.
- Roth, S.Y., M. Shimizu, L. Johnson, M. Grunstein, and R.T. Simpson. 1992. Stable nucleosome positioning and complete repression by the yeast $\alpha 2$ repressor are disrupted by amino-terminal mutations in histone H4. *Genes & Dev.* **6**: 411–425.
- Sommerville, J., J. Baird, and B.M. Turner. 1993. Histone H4 acetylation and transcription in amphibian chromatin. *J. Cell Biol.* **120**: 277–290.
- Turner, B.M. 1991. Histone acetylation and the control of gene expression. *J. Cell Sci.* **99**: 13–20.
- . 1993. Decoding the nucleosome. *Cell* **75**: 5–8.
- Turner, B.M. and G. Fellows. 1989. Specific antibodies reveal ordered and cell cycle-related use of histone H4 acetylation sites in mammalian cells. *Eur. J. Biochem.* **179**: 131–139.
- Turner, B.M., L.P. O'Neill, and I.M. Allan. 1989. Histone H4 acetylation in human cells. Frequency of acetylation at different sites defined by immunolabelling with specific antibodies. *FEBS Lett.* **253**: 141–145.
- Turner, B.M., L. Franchi, and H. Wallace. 1990. Islands of acetylated histone H4 in polytene chromosomes and their relationship to chromatin packaging and transcriptional activity. *J. Cell Sci.* **96**: 335–346.
- Turner, B.M., A.J. Birley, and J. Lavender. 1992. Histone H4 isoforms acetylated at specific lysine residues define individual chromosomes and chromatin domains in *Drosophila* polytene nuclei. *Cell* **69**: 375–374.
- Weeks, J.R., D.E. Coulter, and A.L. Greenleaf. 1982. Immunological studies of RNA polymerase II using antibodies to subunits of *Drosophila* and wheat germ enzyme. *J. Biol. Chem.* **257**: 5884–5891.



Acetylated histone H4 on the male X chromosome is associated with dosage compensation in *Drosophila*.

J R Bone, J Lavender, R Richman, et al.

Genes Dev. 1994, **8**:

Access the most recent version at doi:[10.1101/gad.8.1.96](https://doi.org/10.1101/gad.8.1.96)

References

This article cites 38 articles, 16 of which can be accessed free at:
<http://genesdev.cshlp.org/content/8/1/96.full.html#ref-list-1>

License

Email Alerting Service

Receive free email alerts when new articles cite this article - sign up in the box at the top right corner of the article or [click here](#).

