Recruitment of the Male-specific Lethal (MSL) Dosage Compensation Complex to an Autosomally Integrated \( \text{roX} \) Chromatin Entry Site Correlates with an Increased Expression of an Adjacent Reporter Gene in Male \text{Drosophila}\n
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Drosophila dosage compensate (equalize X-linked gene products) by doubling the transcription of most X-linked genes in males. The MSL (male-specific lethal) ribonucleoprotein complex consisting of at least five proteins and two non-coding RNAs (\( \text{roX}1 \) and \( \text{roX}2 \)) is essential for this transcription response. Recently it has been shown that the X-linked \( \text{roX}1 \) and \( \text{roX}2 \) genes each contain at least one chromatin entry site for the MSL complex. In this study we show that insertion of either \( \text{roX}1 \) or \( \text{roX}2 \) DNA sequences, upstream of an insulated lacZ reporter gene controlled with the constitutive armadillo promoter (arm-lacZ), results in a significant elevation of expression of lacZ in males. However, full compensation, that is a precise doubling of lacZ expression in males relative to females, was only observed in some lines carrying autosomal insertions of either \( \text{roX}1 \)-arm-lacZ or \( \text{roX}2 \)-arm-lacZ transgenes. Furthermore, we found that a 419-base pair fragment of \( \text{roX}1 \) that contains an MSL binding site was sufficient to cause a modest elevation of expression of lacZ in males, but this response was significantly less than obtained with a full-length \( \text{roX}1 \) cDNA. This is the first direct demonstration that insertion of an MSL chromatin entry site on an autosome results in elevated expression in males of genes near the entry site.

In the vinegar fly Drosophila melanogaster, males have one X chromosome and females have two. Males dosage compensate by doubling the transcription of most X-linked genes (1). The MSL complex is required for this male-specific hypertranscription (2, 3). The complex is comprised of at least five proteins, MSL1, MSL2, MSL3, MLE, and MOF and two non-coding RNAs, \( \text{roX}1 \) and \( \text{roX}2 \). All components of the complex co-localize to hundreds of sites along the male X chromosome (4–6). Two of the proteins have enzymatic activity; MLE is an RNA helicase (7) and MOF is a histone acetylase (8). In addition, a kinase, JIL-1, preferentially associates with the male X chromosome (9), but it is not known if this protein is essential for dosage compensation. The complex only assembles in males as one of the components, MSL2, is not present in females (10–12).

The complex is initially targeted to the male X chromosome through binding to 30–40 “high affinity” or “chromatin entry” sites (13). The complex is then thought to spread from these sites to other sites on the X chromosome (2). Two of these sites are the X-linked \( \text{roX}1 \) and \( \text{roX}2 \) genes (14). That is, the same genes that encode the RNA components of the complex also appear to contain DNA sequences that are recognized by the complex.

It has recently been shown that a 217-bp DNA fragment of \( \text{roX}1 \) is sufficient to produce an ectopic chromatin entry site when inserted on an autosome (15).

Previously, we developed an insulated reporter gene system to search for \( \text{cis} \)-acting X-linked DNA sequences that are required for dosage compensation (16). The system consists of the constitutive armadillo promoter driving expression of the lacZ reporter gene and flanked by SCS and SCS’ insulator elements. Seven X-linked DNA fragments totaling 63 kb were tested with the system, but none were found to contain DNA sequences that caused elevated expression of the reporter in males. Here we report that insertion of either \( \text{roX}1 \) or \( \text{roX}2 \) DNA sequences upstream of the armadillo promoter results in elevated expression of the lacZ reporter gene in male Drosophila.

EXPERIMENTAL PROCEDURES

Recombinant DNA—All recombinant DNA manipulations were carried out using standard procedures (17) unless otherwise specified. The insulated reporter P transformation vector pHF11 contains a unique EcoRI site between the SCS’ element and the armadillo promoter (16). pHF11 also contains a unique NotI site immediately upstream of SCS’. A derivative of pHF11, pRH07, which contains a unique NotI site between SCS’ and the armadillo promoter, was constructed by first deleting the NotI site of pHF11 then inserting a linker that contains a NotI site into the EcoRI site. lacZ DNA fragments were inserted into either the EcoRI or NotI sites of pHF11 or pRH07. The fragments were a 4.9-kb EcoRI genomic \( \text{roX}1 \) (14), a 3.7-kb NotI fragment containing \( \text{roX}1 \) cDNA (18), a 1.1-kb NotI/PspOMI fragment containing \( \text{roX}2 \) cDNA (18), a 2.2-kb NotI/PspOMI fragment containing the hs83 promoter (19) and \( \text{roX}2 \) cDNA, a 4.0-kb NotI genomic fragment containing the \( \text{roX}2 \) gene (6), and 419- and 246-bp fragments of \( \text{roX}1 \) generated by polymerase chain reaction. The primers used to obtain the 419-bp fragment called \( \text{roX}1 \) BS were 5’-GTGCAAATTCATGCGATTCGACTGCTTCTG-3’, the 246-bp fragment called \( \text{roX}1 \) SIM were 5’-GTCGAATTCGAAAAACACATTTACTACAAATAAA-C3’ and 5’-GTCGAAATTCGAAAAACACATTTACTACAAATAAA-C3’.

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digested with EcoRI then ligated with EcoRI-cut pHF11. Based on the numbering of the roX1 gene (GenBank® accession number U97114), roX1 B5 is from nucleotides 1152 to 1570, and roX1 SIM is from 3556 to 3801. All roX fragments were inserted in the same 5’→3’ direction as lacZ except roX1 4.9 genomic, roX2 4.0 genomic, and roX2 cDNA, which are in the 3’→5’ orientation. The construct containing both roX1 and roX2 cDNAs was made by insertion of the 3.7-kb roX2 plasmid DNA using standard procedures (21). Microinjections were performed using standard procedures (20). Flies were raised on standard cornmeal-yeast-sugar-agar medium with methyl paraben. Crosses were performed at 25 °C unless otherwise indicated. All stocks not specifically mentioned are described in Lindsley and Zimm (20).

Germ-line Transformation—Manually dechorionated y w embryos were injected with a mixture of F transformation vector and helper plasmid DNA using standard procedures (21). Microinjections were performed using an Eppendorf transjector and Femtotips. Single G0 adults were mated with y, w, and offspring of these crosses were examined for non-white eye color. Single G1 transformants were backcrossed with y w. Homozygotes were selected in subsequent generations on the basis of a darker eye color. Linkage of P[w+1] was determined by following w− segregation in the appropriate crosses. For some lines the sequence flanking the integrated transgene was determined by inverse polymerase chain reaction according to the method of J. Rehm (www.fruitfly.org/methods). The chromosomal site of integration could then be inferred by comparison of the flanking sequence with the sequence of the Drosophila genome.

β-Galactosidase Assays—β-Galactosidase assays were performed as described previously (16). For each transgenic line β-galactosidase activity was standardized by both wet weight measurement and total protein microassays (Bio-Rad). Initial statistical analysis (standard error, 95% confidence limits) was determined by using Microsoft Excel 98. To make comparisons of lines carrying different constructs we performed an analysis of variance, using the line-means as data. The means were weighted by using 1/S.E.2 = Number of observations contributed to Mean/Variance of observations contributing to mean to allow for the fact that some means are more precise estimates because they are based on more observations, or on observations which are less variable, than others. The analysis was performed using SAS Proc Mixed. In most cases only two Treatments (e.g. two lines of the same dose) are being compared, and so the significance level of the Treatment effect is the significance level of the difference. When comparing the four combinations of Dose and line, Tukey’s honestly significant difference was used to compare the means for the different combinations. When analyzing ratios, there are good theoretical reasons for analyzing the log of the ratio, rather than the ratio itself; the mean of the ratio of A:B is not the ratio of the mean of A:mean of B, whereas the mean of log(A:B) is approximately log(mean of A:mean of B). However, in our analyses both the raw ratios and the log(ratios) were analyzed and produced similar results.

RESULTS

roX1 and roX2 DNA Sequences Are Sufficient to Cause Elevated Expression of Autosomally Integrated arm-lacZ Reporter in Males—We have previously developed an insulated reporter gene system to search for X chromosome-linked DNA sequences that are required for dosage compensation (16). The gene system is shown schematically in Fig. 1. The constitutive armadillo promoter controls expression of the lacZ reporter gene, which encodes β-galactosidase. The arm-lacZ construct is bracketed by the SCS and SCS’ insulator elements to protect against possible repressive effects of an autosomal environment. The insulated reporter is expressed equally in males and females when inserted on an autosome and fully dosage compensated when on the X chromosome (16). X-linked DNA fragments are generally inserted between the SCS’ element and the arm promoter (position B in Fig. 1). If the fragment contains a DNA sequence necessary for dosage compensation, then transgenic males carrying an autosomal insert of the construct should produce twice the β-galactosidase activity of females.

We made a series of constructs where either roX1 or roX2 genomic or cDNA fragments were inserted into site B of the insulated reporter vector. Because both roX1 and roX2 contain binding sites for the MSL complex, we anticipated that DNA sequences from either gene should cause elevated expression of arm-lacZ in males if recruitment of the MSLs is sufficient to cause hypertranscription. We assayed three lines carrying autosomal inserts of a 4.9-kb roX1 genomic-arm-lacZ construct (Table I). This construct would be expected to express roX1 RNA. We assayed males and females carrying either one or two copies of the transgene. For all lines, the male to female (M/F) ratio of β-galactosidase activity was significantly greater than one. However, the ratios were also significantly less than the 2-fold increase in activity in males expected if the arm-lacZ reporter was fully compensated. We assayed four lines carrying autosomal inserts of a 3.7-kb roX1 cDNA-arm-lacZ construct only two of which could be made homozygous (Table I). This construct would not be expected to make roX1 RNA unless by chance the transgene has become inserted adjacent to a promoter. As for the roX1 genomic construct, all lines showed significantly elevated expression of arm-lacZ in males. Furthermore, both lines 1 and 2 showed full male-specific hyperactivation, that is an M/F ratio of close to 2, when homozygous for the transgene. Inspection of the data suggested that lines, which were homozygous for the roX1 cDNA construct, gave higher male/female ratios than homozygous lines carrying the roX1 genomic construct. To determine if this difference was statistically significant, we performed an analysis of variance (ANOVA) using weighted means of the lines as data. We found that the homozygous lines carrying the roX1 cDNA did give a significantly higher M/F ratio than the homozygous roX1 genomic lines (protein ratio, p = 0.0009; log(protein ratio), p = 0.0006; weight ratio, p = 0.016; log(weight ratio), p = 0.024). Further analysis showed that the one dose roX1 cDNA lines also gave significantly higher M/F ratios than the two dose roX1 genomic lines (p < 0.05 for both protein and weight ratios).

Lines carrying autosomal insertions of either a 4.0-kb roX2 genomic fragment-arm-lacZ or roX2 1.1-kb cDNA-arm-lacZ construct were assayed for β-galactosidase activity (Table II). All lines gave M/F ratios of β-galactosidase activity that were significantly greater than one. However, there was significant variation between the roX2 1.1-kb cDNA-arm-lacZ lines. Line 1...
showed only a small increase in expression in males whereas both one dose and two dose line 3 flies had M/F ratios close to 2, indicating near full compensation.

Several arm-lacZ lines carrying either roX1 or roX2 DNA sequences do not appear to be fully compensated, that is the M/F ratios are less than two. Although there are several possible explanations for these results (see “Discussion”), one possibility we considered was that a single roX gene integrated in an autosomal environment may not recruit sufficient copies of the MSL complex to achieve full hyperactivation of the reporter. Thus we made a construct that carried both roX1 and roX2 DNA sequences (in that order) upstream of arm-lacZ. Two lines carrying autosomal insertions of this construct were assayed for β-galactosidase activity (Table III). Both lines showed a significant elevation of arm-lacZ expression in males, but the M/F ratios were also less than 2. Thus, insertion of both roX1 and roX2 did not lead to a greater male-specific hyperactivation of arm-lacZ than either alone. In addition to the autosomal lines, we obtained one line (number 3) with the construct integrated onto the X chromosome. In this line lacZ was fully compensated with one dose males having twice the β-galactosidase activity of one dose females (Table III). This was the expected result, because we had previously found that the insulated arm-lacZ reporter was fully compensated when inserted onto the X chromosome (15).

### A Fragment of roX1 Is Sufficient to Cause a Small Increase in arm-lacZ Expression in Males—Two regions of roX1 have been indicated as potentially being important for function. A 217-bp fragment near the 5′-end has been shown to contain a binding site for the MSL complex (15) (Fig. 2). At the 3′-end of roX1 there is a region of 30 bp that shows high similarity to a sequence in roX2 (5). We tested whether either of these regions are sufficient to cause elevated expression of lacZ in males. A 419-bp fragment containing the MSL binding site (roX1 BS) (Fig. 2) and a 246-bp fragment that contains the region of similarity (roX1 SIM) (Fig. 2) were inserted into site B of the insulated arm-lacZ reporter (Fig. 1). Three lines carrying autosomal insertions of each construct were assayed for β-galactosidase activity in males and females from n number of independent experiments. If males and females have equal copy numbers of the arm-lacZ construct, a M/F ratio of 2.0 corresponds to full dosage compensation.

### TABLE I
**roX1 DNA sequences cause an elevated expression in males of an autosomally integrated insulated lacZ reporter gene**

| roX1 construct | Line | Doses | Standardization method | M/F ratio | n | S.E. | 95% confidence limit |
|----------------|------|-------|------------------------|-----------|---|------|-------------------|
| 4.9 genomic    | 1    | 1     | Protein                | 1.41      | 4 | 0.03 | 0.09              |
|                |      | 2     | Protein                | 1.39      | 5 | 0.05 | 0.14              |
| 4.9 genomic    | 2    | 1     | Protein                | 1.42      | 3 | 0.08 | 0.32              |
|                |      | 2     | Protein                | 1.21      | 5 | 0.05 | 0.14              |
| 4.9 genomic    | 3    | 1     | Protein                | 1.47      | 6 | 0.05 | 0.12              |
|                |      | 2     | Protein                | 1.46      | 6 | 0.03 | 0.09              |
| 3.7 cDNA       | 1    | 1     | Protein                | 1.54      | 6 | 0.03 | 0.07              |
|                |      | 2     | Protein                | 1.81      | 5 | 0.11 | 0.29              |
| 3.7 cDNA       | 2    | 1     | Protein                | 1.57      | 6 | 0.07 | 0.18              |
|                |      | 2     | Protein                | 1.58      | 6 | 0.03 | 0.07              |
| 3.7 cDNA       | 3    | 1     | Protein                | 1.51      | 4 | 0.02 | 0.07              |
|                |      | 4     | Protein                | 1.67      | 4 | 0.08 | 0.24              |

* M/F ratio is the mean ratio of β-galactosidase activity in males and females from n number of independent experiments. If males and females have equal copy numbers of the arm-lacZ construct, a M/F ratio of 2.0 corresponds to full dosage compensation.

### TABLE II
**roX2 DNA sequences cause an elevated expression in males of an autosomally integrated insulated lacZ reporter gene**

| roX2 construct | Line | Doses | Standardization method | M/F ratio | n | S.E. | 95% confidence limit |
|----------------|------|-------|------------------------|-----------|---|------|-------------------|
| 4.0 genomic    | 1    | 1     | Protein                | 1.41      | 5 | 0.03 | 0.07              |
| 1.1 cDNA       | 1    | 1     | Protein                | 1.25      | 5 | 0.05 | 0.14              |
|                |      | 2     | Protein                | 1.34      | 5 | 0.04 | 0.11              |
| 1.1 cDNA       | 2    | 1     | Protein                | 1.60      | 6 | 0.09 | 0.24              |
| 1.1 cDNA       | 3    | 1     | Protein                | 1.74      | 7 | 0.07 | 0.17              |
|                |      | 2     | Protein                | 1.97      | 4 | 0.09 | 0.28              |
controls less than was obtained with the full-length cDNA construct. However, in all of the lines carrying genomic fragment, and some of the lines carrying the full-length cDNA had significantly higher M/F ratios of β-galactosidase activity than lines carrying the roX1 BS construct (protein ratio, p = 0.0002; weight ratio, p = 0.0018).

We considered several explanations for the difference in M/F ratios of the roX1 BS and roX1 SIM fragments. The roX1 gene is shown in a 5′–3′ orientation with the boxes representing the two exons. The roX1 BS fragment contains a known binding site for the MSL complex, whereas the roX1 SIM fragment contains the 30-bp region of similarity between roX1 and roX2.

FIG. 2. roX1 gene showing relative location of the roX1 BS and roX1 SIM fragments. The roX1 gene is shown in a 5′–3′ orientation with the boxes representing the two exons. The roX1 BS fragment contains a known binding site for the MSL complex, whereas the roX1 SIM fragment contains the 30-bp region of similarity between roX1 and roX2.

We have previously found that the insulated arm-lacZ reporter is fully compensated when inserted on the male X chromosome (16). Thus the insulator elements did not appear to be able to block the transcription elevation due to the MSL complex. Because the roX genes contain MSL binding sites, we decided to test this more directly by inserting roX sequences either upstream or downstream of the SCS′ insulator element (Fig. 1). We used a hsp83.roX2 1.1 cDNA construct, which should express a roX2 RNA from the constitutive hsp83 promoter (19). The construct was inserted either upstream of the SCS′ element (position A in Fig. 1) or between the SCS′ element and the arm promoter (position B in Fig. 1). We found that all lines gave a significant elevation of expression of the arm-lacZ reporter in males (Table V). Furthermore, there was no significant difference between the lines that had the hsp83.roX2 construct inserted upstream of SCS′ (i.e. position A) compared with those lines where the construct was inserted downstream of SCS′ (position B). Thus the SCS′ insulator element appears to be unable to block hyperactivation of the arm-lacZ due to insertion of an MSL chromatin entry site.

### DISCUSSION

There are several lines of evidence that have shown that the MSL complex binds to hundreds of sites along the male X chromosome and causes a 2-fold increase in expression of most X-linked genes in male Drosophila (1, 2). The most direct evidence for the latter is that males that are homozygous for mutant alleles of msl1, msl2, or mle have significantly reduced levels of X-linked but not autosomal enzymes and mle males have a lower overall rate of X-chromosome transcription (22). In this study we have shown that binding of the MSL complex to either roX1 or roX2 DNA sequences integrated at autosomal sites correlates with an elevated expression of an adjacent lacZ reporter gene. Indeed, in some of the lines that had either roX1 or roX2 cDNA inserted upstream of lacZ we observed full compensation, that is a doubling of expression in males compared with females carrying the same number of copies of the construct. However, in all of the lines carrying roX1 genomic fragments, a line with a roX2 genomic fragment, and some of the lines carrying roX1 cDNA upstream of lacZ we observed partial compensation. That is the male/female ratios were significantly greater than one but less than the 2-fold expected if recruitment of the MSL complex leads to a precise doubling of transcription as occurs on the X chromosome. We considered that this partial compensation might be because the MSL complex is only binding to the autosomal roX sequence in a fraction of the cells in a tissue. However, we found that the proportion of nuclei that showed binding to the roX1 BS construct, which...
A fragment of roX1 that contains and MSL binding site is sufficient to cause a modest elevation of lacZ expression in males

| roX fragment (Fig. 2) | Line | Doses | Standardization method | M/F ratio | n  | S.E. | 95% confidence limit |
|----------------------|------|-------|------------------------|-----------|----|------|---------------------|
| roX BS               | 1    | 1     | Protein                | 1.18      | 5  | 0.03 | 0.08                |
|                      | 2    | 2     | Protein                | 1.28      | 5  | 0.03 | 0.09                |
|                      | 3    | 1     | Protein                | 1.25      | 4  | 0.01 | 0.03                |
|                      | 4    | 1     | Protein                | 1.34      | 4  | 0.05 | 0.17                |
| roX BS               | 2    | 1     | Protein                | 1.16      | 5  | 0.04 | 0.12                |
| roX BS               | 3    | 1     | Protein                | 1.25      | 5  | 0.03 | 0.08                |
| roX BS               | 4    | 1     | Protein                | 1.33      | 5  | 0.03 | 0.07                |
| roX SIM              | 1    | 1     | Protein                | 1.04      | 4  | 0.02 | 0.06                |
| roX SIM              | 2    | 1     | Protein                | 1.11      | 4  | 0.03 | 0.10                |
| roX SIM              | 3    | 1     | Protein                | 1.04      | 5  | 0.03 | 0.09                |
| roX SIM              | 4    | 1     | Protein                | 1.13      | 5  | 0.03 | 0.08                |

MSL complex binds to autosomal roX1 transgenes. MSL3 localization in male nuclei determined by immunostaining with anti-MSL3 antibodies (green) and DAPI (blue), which binds to all chromosomes. A, roX1 3.7 cDNA line 1 (chromosome 3, 85B). B, roX BS line 3 (chromosome 3, 79C). C, roX SIM line 2 (chromosome 3, 93B). In all nuclei strong binding is seen to many sites on the male X chromosome. In A and B, MSL3 binding is also seen at one autosomal site (arrowhead).

The SCS/H11032 insulator element does not block male-specific hyperactivation of lacZ

| roX construct | Insertion site (Fig. 1) | Line | Doses | Standardization method | M/F ratio | n  | S.E. | 95% confidence limit |
|---------------|------------------------|------|-------|------------------------|-----------|----|------|---------------------|
| hsp83.roX2    | A                      | 1    | 1     | Protein                | 1.34      | 4  | 0.04 | 0.13                |
|               |                        | 2    | 1     | Protein                | 1.60      | 4  | 0.03 | 0.09                |
|               |                        | 2    | 2     | Protein                | 1.44      | 5  | 0.03 | 0.10                |
|               |                        | 3    | 1     | Protein                | 1.65      | 5  | 0.03 | 0.08                |
|               |                        | 2    | 1     | Protein                | 1.82      | 5  | 0.10 | 0.27                |
|               |                        | 2    | 2     | Protein                | 1.60      | 5  | 0.04 | 0.11                |
|               |                        | 3    | 1     | Protein                | 1.68      | 5  | 0.07 | 0.19                |
|               |                        | 2    | 1     | Protein                | 1.50      | 6  | 0.08 | 0.2                 |
|               |                        | 3    | 1     | Protein                | 1.56      | 5  | 0.08 | 0.21                |
|               |                        | 2    | 2     | Protein                | 1.21      | 5  | 0.05 | 0.13                |
|               |                        | 3    | 1     | Protein                | 1.39      | 5  | 0.09 | 0.25                |

shows partial compensation, was the same as compared with a line that showed full compensation (3.7 cDNA line 2). Thus it appears that partial compensation cannot be explained by variability in MSL binding between cells within a tissue. However, we have only analyzed cells from one tissue, third instar larvae salivary glands. We also considered the possibility that one roX sequence might not be sufficient in some autosomal locations to recruit enough MSL complexes to achieve full compensation. If so, a construct that had both roX1 and roX2 cDNAs inserted upstream of lacZ may be more effective at recruiting the complex and would thus show full compensation in most lines. However, the lines tested both showed partial compensation. Thus there must be some other explanation for why some lines show only partial compensation. We think the most likely ex-
planation is that the local chromatin environment at the auto-
somal integration site influences the level of hyperactivation of
the lacZ reporter by the MSL complex. It’s known that some
autosomal sites are much more permissive to spreading of
the complex than others, indicating that the local autosomal chro-
matin environment can affect at least one function of the MSL
complex.

We found that lines that were homozygous for the roX1 4.9
genomic-arm-lacZ construct gave significantly lower male/female
ratios of β-galactosidase activity than lines that were either heterozygous or homozygous for the roX1 3.7
cDNA-arm-lacZ construct. Because both constructs contain a
binding site for the MSL complex, it’s not obvious why the roX1
genomic construct should give lower hyperactivation of lacZ in
males. One possibility is that this is simply because by chance
in all the roX1 genomic lines the transgene integrated into a
negative chromosomal environment that was not permissive to
full hyperactivation of lacZ in males. However, this would seem
unlikely, because all three lines gave similar male/female ra-
tios of β-galactosidase. The genomic construct contains addi-
tional DNA sequences from the roX1 gene region compared with
the cDNA construct. It’s possible that these additional
sequences may somehow inhibit hyperactivation of lacZ by the
MSL complex. The genomic construct would be expected to
produce roX1 RNA, whereas the promoter-less cDNA construct
would not. The function of the roX1 RNA in the complex is not
what. If the RNA has an inhibitory role, then a localized
excess of synthesis of roX1 RNA might result in assembly of an
MSL complex that is less effective at elevating expression of
the adjacent reporter gene. It has been suggested that in vivo
there must be some mechanism for dampening the transcrip-
tion elevation due to the MOF histone acetylase, because in vitro recombinant MOF is able to increase expression from a
nucleosomal template far more than 2-fold (23). Clearly, fur-
ther experiments with additional constructs are required to
determine the biological significance (if any) of the difference in
lacZ hyperactivation between roX1 genomic and cDNA
constructs.

The level of elevation of β-galactosidase activity in males
carrying the 419-bp fragment of roX1 that contains the MSL
binding site was significantly less than obtained with 3.7-kb
roX1 cDNA. It’s possible that by chance all three roX1 BS lines
are inserted into negative chromatin environments that inhibit
the MSL complex. We think this is unlikely, because all three
genes gave very similar male/female ratios of β-galactosidase
activity. Rather it is more likely that binding of the MSL
complex to the site in roX1 BS is not sufficient to achieve full
compensation. This suggests that other sequences in roX1 3.7
cDNA in addition to the MSL binding site in roX1 BS are
required for full roX1 function.

In Drosophila, the SCS and SCS’ insulator elements are able
to protect a gene from position effects and block a transcription
enhancer from acting on a promoter (24, 25). It has been pre-
viously shown that the SCS and SCS’ elements do not block
genes from being dosage compensated when inserted onto the X
chromosome (16, 24). This suggests that these insulators can-
not prevent hypertranscription in males due to the MSL com-
plex. In this study we have tested this directly by placing an
SCS’ insulator between a roX2 sequence, which contains an
MSL binding site, and the lacZ reporter. The SCS’ insulator
was unable to block elevation of expression of the reporter in
males. Because this insulator can block transcription enhan-
ers, this suggests that the mechanism by which the MSL com-
plex affects transcription may be different from how an en-
hancer acts on a promoter.

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