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The Drosophila *roX1* RNA Gene Can Overcome Silent Chromatin by Recruiting the Male-Specific Lethal Dosage Compensation Complex

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ABSTRACT

The Drosophila MSL complex consists of at least six proteins and two noncoding *roX* RNAs that mediate dosage compensation. It acts to remodel the male's X chromatin by covalently modifying the amino terminal tails of histones. The *roX1* and *roX2* genes are thought to be nucleation sites for assembly and spreading of MSL complexes into surrounding chromatin where they roughly double the rates of transcription. We generated many transgenic stocks in which the *roX1* gene was moved from its normal location on the X to new autosomal sites. Approximately 10% of such lines displayed unusual sexually dimorphic expression patterns of the transgene's mini-*white* eye-color marker. Males often displayed striking mosaic pigmentation patterns similar to those seen in position-effect variegation and yet most inserts were in euchromatic locations. In many of these stocks, female mini-*white* expression was very low or absent. The male-specific activation of mini-*white* depended upon the MSL complex. We propose that these transgenes are inserted in several different types of repressive chromatin environments that inhibit mini-*white* expression. Males are able to overcome this silencing through the action of the MSL complex spreading from the *roX1* gene and remodeling the local chromatin to allow transcription. The potency with which an ectopic MSL complex overcomes silent chromatin suggests that its normal action on the X must be under strict regulation.

IN Drosophila, males hypertranscribe most genes along their single X chromosome to match the output of females with two X chromosomes (Cline and Meyer 1996). This hypertranscription is mediated by a large RNA-protein complex distributed at hundreds of sites along the male X chromosome (Meller 2000). Mutations in the genes encoding five of the six known protein components display a distinctive *m*ale-specific *l*ethal (MSL) phenotype, and so the products are collectively referred to as the MSL proteins. The two known RNA components of the MSL complex are *roX1* and *roX2* (*R*NA *o*n the *X*; Amrein and Axel 1997; Meller *et al.* 1997). The genes producing these two noncoding RNAs are located on the X, and their products are thought to spread *in cis* from the sites of synthesis when complexed with MSL proteins (Kelley *et al.* 1999; Park *et al.* 2002). The two *roX* RNAs are very dissimilar in size and sequence, and yet share a poorly understood function in dosage compensation. The MSL complex can bind the male X with either *roX1* RNA or *roX2* RNA alone, but only weakly if both RNAs are absent, resulting in male lethality (Franke and Baker 1999; Meller and Rattner 2002).

When either *roX* gene is moved from the X to a random autosomal site, the MSL complex will bind to the *roX* DNA sequence anywhere in the genome (Kelley *et al.* 1999; Kageyama *et al.* 2001). The MSL complex also spreads into autosomal chromatin flanking such *roX* transgenes. If the MSL complex normally spreads *in cis* from the endogenous *roX* genes on the X, this could help explain how dosage compensation is targeted to the correct chromosome.

One prediction of the spreading model is that when the MSL complex is redirected to autosomal *roX1* transgenes, the surrounding chromatin should be remodeled to resemble the male X, resulting in inappropriate hypertranscription. We previously reported that the affected segment of autosome becomes hyperacetylated at lysine 16 on histone H4 (Kelley *et al.* 1999). Here we report that placing a *roX1* gene next to a mini-*white* reporter can allow male-specific activation of eye color when the transgene is embedded in a variety of repressive autosomal chromatin environments. This reversal of silencing provides evidence that when the MSL complex is redirected to an autosome, it can remodel nearby chromatin to allow elevated levels of transcription. Thus, isolated *roX* transgenes inserted on autosomes can serve as a model to study MSL targeting, spreading, and chromatin remodeling.

MATERIALS AND METHODS

Fly genetics: Mosaic stocks were recovered in a screen where a second chromosome GMroX1 transposon was mobilized

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with *P* transposase (Robertson *et al.* 1988). *y w*/Y; *P*{*w*$^+$ GMroX1}57E/CyO; *Ki* {*Js* Δ*2-3*}99B/+ males were crossed to *y w* virgins. CyO; +/+ sons with pigmented eyes, which must have lost the original insert on the second chromosome, were recovered. Consequently, most inserts were on the third chromosome with a few recovered on CyO and the fourth chromosome. The new inserts were balanced and made homozygous if viable. The inserts were mapped on polytene chromosomes by anti-MSL1 antibody staining, which binds *roX* genes (Kelley *et al.* 1999), and DNA *in situ* hybridization using a digoxygenin labeled *roX1* probe. Due to the difficult cytology of CyO, the two mosaic lines recovered on this balancer were mapped only by sequencing the DNA flanking one end of the transposons by inverse PCR. The GMroX1-64A transgene was remobilized to recover hops with solid eye color in both sexes. The GMroX1-69C and GMroX1-64A.2 lines were recovered during this screen because of their distinctive eye patterns. The latter is at the original location, but suffered a deletion of sequences flanking the mini-*white* end of the transposon. To generate XXY daughters and XO sons, transgenic females were crossed to attached XY males: *YS.X, In(1)EN, v ptg oc sn*^{5} *w y, YL oc*^{8} *y*$^+$ *oc ptg*.

Sequencing insertion sites: Genomic DNA was cut with either *Hpa*II or *Hha*I, ligated into circles, and then recut with *Hin*dIII. DNA flanking the transposon was amplified with primers 5′-TGAGAGGAAAGGTTGTGTGC-3′ and 5′-TATCGACGGGACCACCTTAT-3′, gel purified, and sequenced. The sequence was placed on Drosophila genome sequence (FlyBase release 3) using BLAST, Gadfly (http://www.fruitfly.org/annot/), and Flyenhancer (http://flyenhancer.org/Main).

Expressing MSL complex in female eyes: *P*{*y*$^+$ *YEM2*} (***y****ellow* ***e****yeless* ***msl2***) carries a 3.6-kb eye-specific enhancer from the first intron of the *eyeless* gene (Halder *et al.* 1998) driving transcription of the *msl2* coding sequence, minus the regulatory SXL-binding sites in the 5′ and 3′ untranslated regions (UTRs; Kelley *et al.* 1995). This was introduced into flies in the *P*{Carnegie 4-yellow} vector, a derivative of YES that lacks Su(Hw)-binding sites, so that eye pigmentation caused by GMroX1 could be assayed (Patton *et al.* 1992; Sigrist and Pirrotta 1997).

Photography of fly heads: Adults were aged for 3 weeks to maximize pigmentation and submerged in mineral oil. Brother and sister pairs were photographed side by side with Ektachrome 160T film using a Leica MZ12 microscope, digitized, and processed using Adobe Photoshop.

Scanning electron microscopy (SEM) of eyes: Adults were dehydrated through an ethanol series, soaked in hexamethyldisilazane, and vacuum dried. After mounting on adhesive blocks, the flies were coated under vacuum using a Bal-Tec MED 020 high-resolution sputtering device (Technotrade International, Manchester, NH) with a platinum alloy target for ∼400 sec. Samples were examined in a JSM-5900 scanning electron microscope (JEOL, Peabody, MA) at an accelerating voltage of 5 kV.

RESULTS

Unusual male-specific mosaic eye pigmentation in *roX1* transgenes: The *roX1* transgene used in this study was marked with the mini-*white* eye pigment gene (Pirrotta 1988). In the course of experiments to insert the *P*{*w*$^+$ *GMroX1*} transgene (hereafter referred to as GMroX1-location) in many autosomal sites, most new transgenic inserts showed the solid dark-orange (male) and solid light-orange (female) coloration typical of the CaSpeR vector. However, ∼10% of our *roX1* transgenic stocks displayed unusual, sex-specific eye pigmentation patterns (Figure 1). In many cases, mini-*white* expression was very low or silent in females. In these same lines, males expressed mini-*white*, but frequently in mosaic sectors. All males from any single stock showed a similar mix of pigmented *vs.* white sectors, but there were large differences in the patterns between independent transgene insertion sites. For instance, GMroX1-39DE males had almost solid red eyes, but GMroX1-84E males had only a few small red sectors. In both lines, females had solid white eyes. Some lines, such as 64A and 80C, had a few large sectors, indicating that the decision to activate or to silence the transgene was made shortly after the eye disc formed during embryogenesis (Figure 1).

The sectored eyes superficially resembled those reported in cases of position-effect variegation (PEV; Wakimoto 1998). One striking difference was that the mosaic patterns seen with *roX* transgenes were exclusively male specific. A second important difference was that PEV most often occurs when a normally euchromatic gene is placed near blocks of hererochromatin, but most *roX1* transgenes under study were located in unremarkable euchromatic regions of polytene chromosomes (Figure 1, Table 1). The exceptions were GMroX1-80C at the heterochromatic base of 3L and GMroX1-102C in the banded portion of the heterochromatic fourth chromosome. Genes subject to PEV are often sensitive to the presence of the heterochromatic Y chromosome. We examined the mosaic *roX1* transgenes in XO males and XXY females. In no case did the addition of a Y chromosome overcome silencing in females (Figure 1). In general, XO males displayed more severe transgene silencing compared to XY males. Not surprisingly, the strongest response was for the heterochromatic insertion at 80C, but some euchromatic inserts like 99C also had much less pigmentation (Figure 1). In several lines, such as GMroX1-64A and GMroX1-75C, the presence or absence of the Y had little or no effect on male pigmentation.

Numerous genes encoding proteins necessary for packaging silent chromatin have been identified as modifiers of PEV (Wallrath 1998; Grewal and Elgin 2002). We tested mutations in *Su(var)2-5* and *Su(var)3-7* (encoding HP1; Eissenberg *et al.* 1990) and a C2H2 Zn finger protein (Cleard and Spierer 2001), respectively, for dominant effects on GMroX1 eye pigmentation. We also tested *Su(z)12*, which encodes a C2H2 Zn finger protein that interacts with *Polycomb* group genes and was later found to affect variegation of *w*^{m4} (Birve *et al.* 2001). None affected female eye color of any transgenic line (Figure 1, bottom, and data not shown). The same modifiers also had variable effects on male eye color that depended strongly on insertion site. The centric heterochromatin insert GMroX1-80C and one on the heterochromatic fourth chromosome, GMroX1-102C, responded to *Su(var)3-7* and *Su(z)12*^{4} (Figure 1, bottom). In most cases, however, the suppressors of PEV

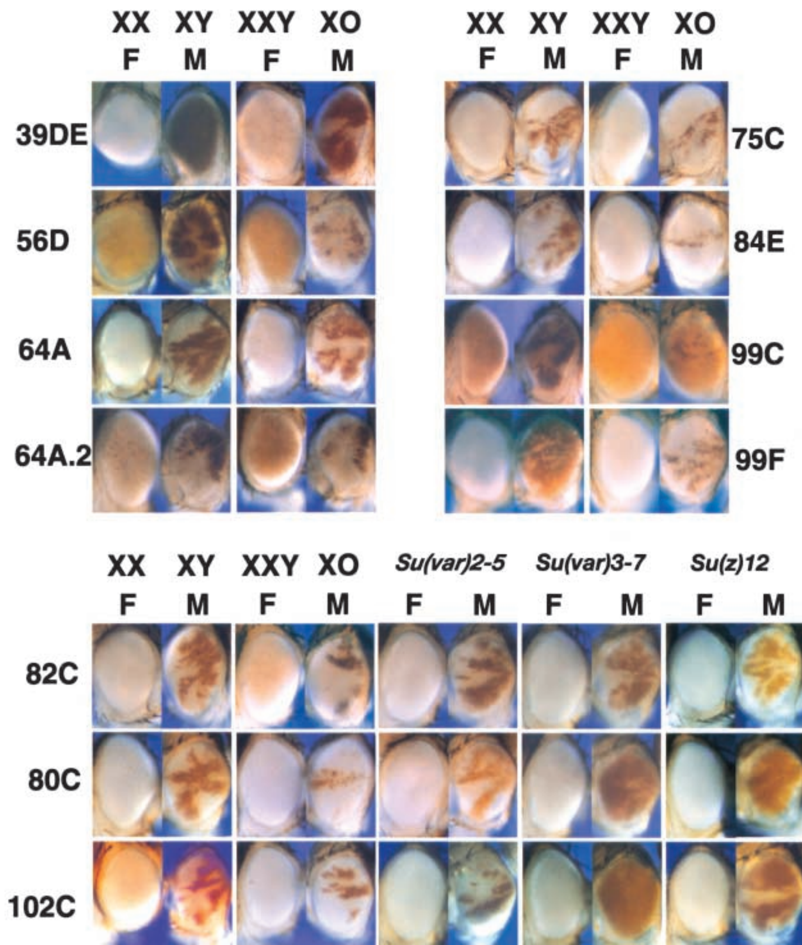

FIGURE 1.—Mosaic mini-*white* expression in GMroX1 transgenic flies. Female (left) and male (right) pairs of transgenic flies carrying hemizygous GMroX1 insertions at the indicated cytological locations are shown. For lines 35DE–99F (top) the order is XX females, XY males, XXY females, and XO males. For lines 82C, 80C, and 102C (bottom) the order is XX females, XY males, XXY females, and XO males, female and male *Su(var)2-5*^{05}/+, female and male *Su(var)3-7*/+, female and male *Su(z)12*^{4}/+. None of the modifiers increases mini-*white* expression in females. The inserts at 80C and 102C are located near heterochromatin and responded strongly to *Su(var)3-7*, moderately to *Su(z)12*, and not at all to *Su(var)2-5*. GMroX1-82C had the strongest response to *Su(var)* mutations of any euchromatic insertion. In each line, the males displayed a range of patterns with a slightly different percentage of the eye pigmented. Typical patterns are presented in each case.

had little effect on the male mosaic pattern (data not shown). We conclude that the *roX1* inserts displaying male-specific mosaicism represent a new phenomenon distinct from PEV.

Most mosaic insertions are in gene-rich regions: We favor a model in which the mosaic transgenes are inserted in locations unfavorable for mini-*white* expression. The male-specific MSL complex might assemble on nascent *roX1* transcripts and then remodel the surrounding chromatin into a conformation permissive for mini-*white* expression. This idea is consistent with the observation that when the strongly variegated GMroX1-64A line was mobilized with *P* transposase, almost all new hops gave brothers and sisters with solid orange eyes (data not shown). This indicates that the silencing element was most likely in flanking DNA around 64A rather than within the transposon.

Examining the nearby genes or DNA sequence surrounding the mosaic transgenes might provide clues to the nature of the repressive chromatin. The exact locations of most transgenes were determined by sequencing the flanking DNA generated by inverse PCR (Table 1). The heterochromatic insertion at 80C landed within a degraded *hoppel* mobile element, and the GMroX1-75C transgene landed in a *yoyo* element. GMroX1-84E inserted into a low-copy repeat. The others landed in single-copy sequences, often densely packed with genes. In such a small sample size, it is surprising to recover strongly mosaic insertions at the 5′ ends of both *zfh1* (99F) and *zfh2* (102C), the genes encoding large transcription factors containing both Zn fingers and a homeobox. The 64A insertion is near the 5′ end of the *scrt* gene. These sites are expected to be packaged in active chromatin in at least some tissues and developmental times. It is possible that such regions are silenced in tissues where the resident genes are not needed. By contrast, GMroX1-82C landed in a region devoid of predicted genes for >25 kb on either side.

The insertion at 39DE illustrates the activating potential of the *roX1* transgene. GMroX1-39DE landed in a copy of the *histone H3* gene and mini-*white* was fully silent in females but active in males (Figure 1). The 5-kb histone gene clusters occur in tandem arrays of ~100–200 copies. Others have reported that mini-*white* expression is repressed when embedded within tandem repeats (Dorer and Henikoff 1994). The only *P* elements that we are aware of in this large interval were recovered using the *yellow* (*y*) body color marker, not mini-*white* (FlyBase, R. Levis, personal communication).

The endogenous genes surrounding the mosaic

TABLE 1

Sequences of GMroX1 insertion sites

Insert line	Flanking sequence	Nearby genes
GMroX1-35E	*<AATAGAAGCATTTATGGATAAGAAACCCACCACACGGGTGCT CTCTCTCTCTCTCTCC	1.9-kb 3′ *BicC* 6.1-kb 5′ *beat*
GMroX1-39E	*TTCGCTTTTCGCTCGACAAATGAAATGGCCTCTGTTTTTCTCTC TCTCTCT	176-bp 5′ *histone H3* ATG codon
GMroX1-64A	*<GTTTTGTTTTCCAAAAACCCGAGGAGAAAATTGCCAGCCCAAA	∼700-bp 5′ *scrt*
GMroX1-69C	*TGGCCTGAATGAAAGGCCAACGCAAACAAACAAACACAC	Second intron of *ara*
GMroX1-75C	*<AGTGCAACTTGCGTTAACTGGCGCCC	YOYO mobile element
GMroX1-77A	*<CGGGACCACCTTATGTTATTTCATCATGGTACC	6-kb 5′ CG13812 2.2-kb 3′ CG7306
GMroX1-80C	*<CTTTGTGCGTTGAAAAGAGCTGTTCGCTGTAGC	Hoppel mobile element
GMroX1-82C	*GTATATAGTAATACATCAACAACGTGTAAAAGCGTTGA	27-kb 3′ CG12586 24.5-kb 5′ CG12587
GMroX1-84E	*<ACAGTACAGCAGGCATCTCTAGAAAATTGATTCCAAAGTGTA	Low-copy repeat between CG31146 and CG2616
GMroX1-93E	*<CTGTTCGGTCGTTTTCGAACTCTGTCTTTTGACCGCTTACAG AACTCTGTTAGTGCCG	2.1-kb 3′ *E2f*
GMroX1-95E	*<CATTTTAAAAGCTAAGCGTGGAAAA	Second intron of CG10716
GMroX1-98B	*GGGGGGAGTTTACGAACGACCCGCACAATGTA	6.5-kb 5′ CG5017
GMroX1-99C	*<ATTTTTGTGGTAGCAAATTCTAAGAATGCCTCTCAAATACCGA	5.9-kb 3′ CG1973 7.4-kb 5′ CG15507
GMroX1-99F	*GGCGAGGGCGATACGGCGGGGTTCTACGTTTCCACGGAGAGCG	200-bp 5′ of *zfh1*
GMroX1-102C	*GTACACCGTTTTATCACATCATCTCCCGTCTCGCTCTAACGTAA TTACCA	12.4-kb 5′ CG11533 19.9-kb 5′ of *zfh2*

Inverse PCR was performed on the *white* end of GMroX1 inserts and sequenced. The first column is the cytological location of each transgene line. The second column is a short sequence adjacent to the *P* element. "*" denotes the insertion site. "<" indicates that the sequence run did not extend across the junction with the *P* element and the precise junction may be up to ∼200 bp away from the sequence given. The third column indicates the approximate distances between the transposon and the predicted flanking genes. GMroX1-35E and 39DE inserted on the CyO balancer chromosome. No PCR product was recovered from the *white* end of GMroX1-64A.2, but primers from the *roX1* end did yield a product whose sequence was immediately adjacent to the parental GMroX1-64A. Sequences were not recovered for lines 21C or 56D.

GMroX1 inserts might be silent in the developing eye, but active elsewhere. Histone H4 methylation at lysine 20 is a candidate for such an epigenetic silencing factor. This modification is mutually exclusive with histone H4 acetylation at lysine 16 produced by the MOF protein within the MSL complex (Nishioka *et al.* 2002). The *pr-set7* gene is responsible for this methylation, which is found not only in centric heterochromatin, but also over the euchromatic arms. Because *pr-set7* is an essential gene, we could assay only adults with lowered PR-SET7 levels. We tested seven mosaic GMroX1 lines in *Df(3R) red*^{P93}/+ males, which should produce only 50% of the amount of PR-SET7 methylase. In each case the hemizygous *pr-set7* males showed mosaic eye patterns indistinguishable from their +/+ brothers (data not shown).

Pairing-sensitive repression: When most mini-*white* transgenes (without *roX1* sequences) are made homozygous, the eye pigmentation is darker than that in hemizygotes because of increased gene dose. Several viable mosaic inserts in this study showed significantly less pigmentation when homozygous compared to hemizygous (Figure 2). The most extreme case was GMroX1-84E in which the transgene inserted in a 4.5-kb low-copy repeat element present twice near the CG2616 gene with additional copies at 38D and 41F (FlyBase). Most GMroX1-84E homozygous males had solid white eyes with only ∼20% showing one or two small red sectors. In other lines, hemizygous animals had even pigmentation over the entire eye, but homozygous animals had decreased mini-*white* expression in both sexes. Males overcame this repression in a mosaic pattern (Figure 2, 21C, 95E, and 98B). Pairing-dependent silencing of the *white* gene has been reported in numerous other situations and is often due to the action of the PcG proteins (Kassis *et al.* 1991; Chan *et al.* 1994; Americo *et al.* 2002). We tested whether the most dramatic pairing-sensitive insert, GMroX1-98B, was affected by reduced PcG in *Scm* and *Pc*^{3} mutant backgrounds, but found no effect (data not shown).

A striking exception to this trend was the GMroX1-102C insert on the small, heterochromatic fourth chromosome. The transgene is silent in females whether

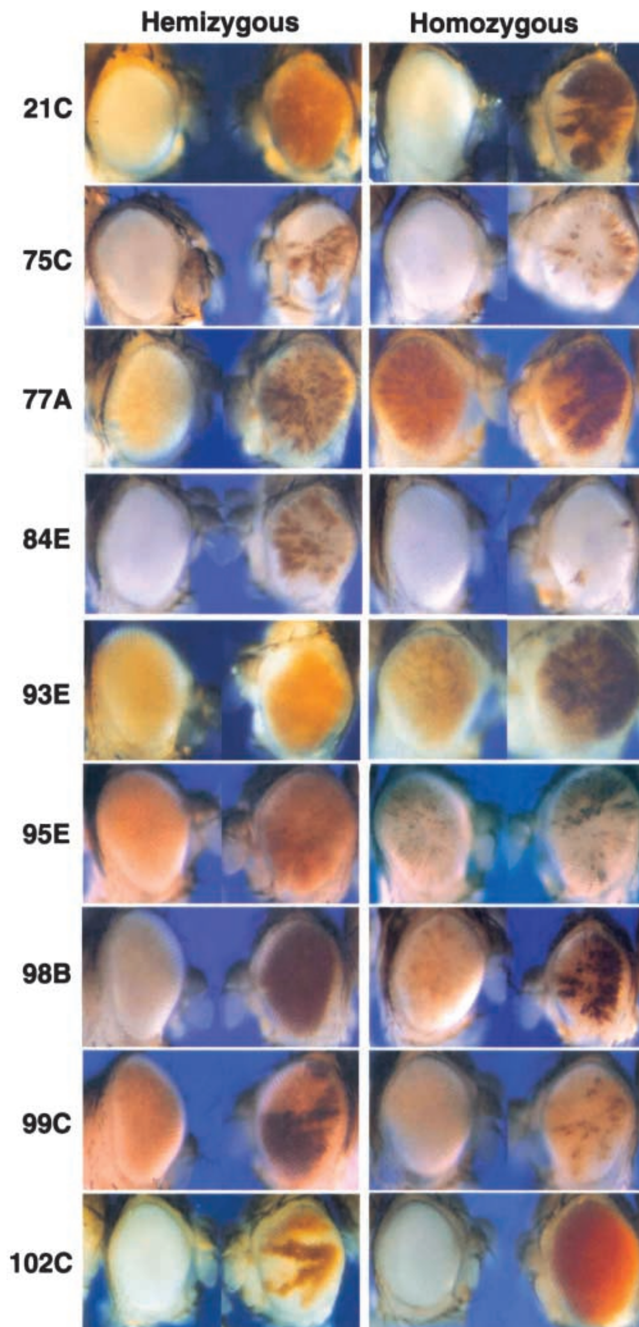

FIGURE 2.—Transgene pairing affects mini-*white* expression. Insertions are shown as hemizygous (left pair) or homozygous (right pair). For each pair, females are on the left and males are on the right. The numbers at left indicate insertion sites of transgenes. Most insertions have lower mini-*white* expression when the transgene is paired except the fourth chromosome insert, 102C, where homozygous males have solid red eyes. Some pairing-sensitive lines (77A, 93E, 95E, and 98B) show a slight salt-and-pepper pattern in females, which is greatly exacerbated in males. The following lines carried lethal mutations on the same chromosome and could not be tested as homozygotes: 35E, 39DE, 56D, 64A, 64A.2, 80C, 82C, and 99F.

hemizygous or homozygous, but pairing dramatically increases pigmentation in males so that they have solid red eyes (Figure 2). This insert lies only a few hundred nucleotides from the M371.R insertion site of *P*{*hsp26-pt hsp70-w*$^+$} (SUN *et al.* 2000), and yet M371.R is a lethal insert in the 5′ end of *zfh2* whereas GMroX1-102C is homozygous viable. The *hsp70-w*$^+$ marker is strongly expressed in both sexes to give dark red eyes. It is not clear how much the dramatically different eye phenotypes between the two transposons are determined by the different promoters driving *white* or the slightly separated insertion sites.

***iroquois* insertion produces dorsoventral pattern:** The *GMroX1-69C* insertion landed in the second intron of *ara* and had a distinctive pattern of eye pigmentation. The dorsal 30% of the eye was pigmented in hemizygotes of both sexes, but the ventral 70% was completely white in females and sectored in males (Figure 3, A and B). Others have recovered transposon inserts with dorsal red/ventral white patterns (Figure 3F) and they all map near 69D, the location of the *iroquois-mirror* cluster of homeotic genes, which includes *ara* (SUN *et al.* 1995; GOMEZ-SKARMETA *et al.* 1996; NETTER *et al.* 1998). These genes are expressed in many tissues, but in the developing eye disc they are expressed only in the dorsal half (MCNEILL *et al.* 1997; KEHL *et al.* 1998). The ventral silencing of transgenes in the *iroquois* complex is due to action of the Polycomb complex and the PcG proteins are found at 69CD in salivary gland polytene chromosomes (RASTELLI *et al.* 1993; NETTER *et al.* 1998). When the *Pc*^{3} mutation was introduced into GMroX1-69C flies, weak derepression of the mini-*white* marker was evident by light pigmentation in the ventral region of both male and female eyes, suggesting that this transgene is also silenced by the PcG proteins (Figure 3C).

In contrast to pairing-dependent repression seen in most other lines, homozygous GMroX1-69C males have nearly solid red eyes with a lighter equator (Figure 3E). This is the converse pattern seen in the nearby Eq1 mini-*white* transgene lacking any *roX1* sequences (NETTER *et al.* 1998). Pairing has little effect in females except that ~10% of homozygous females have a small pigmented sector in the ventral half of the eye (Figure 3D).

Ectopic dosage compensation in females activates *white* expression: Formally, several sex-specific regulatory proteins might mediate silencing in females (SXL, TRA, DSX^f) or activation in males (DSX^m, FRU). However, given the well-documented interaction between the MSL complex and the *roX* genes, we tested whether ectopic MSL expression in females could overcome repressive chromatin around the GMroX1. Females normally make all the MSL proteins except MSL2 (BASHAW and BAKER 1995; KELLEY *et al.* 1995; ZHOU *et al.* 1995). The *P*{*y*$^+$ *YEM2*} transgene (Figure 4A) expressed MSL2 protein in the developing eye disc in both sexes under the control of the *eyeless* enhancer. This caused a low-penetrance female-specific eye defect (data not shown). The rough eye phenotype was most likely due to ectopic dosage compensation because it was strongly enhanced by simultaneous overexpression of MSL1 (Figure 4, C–E)

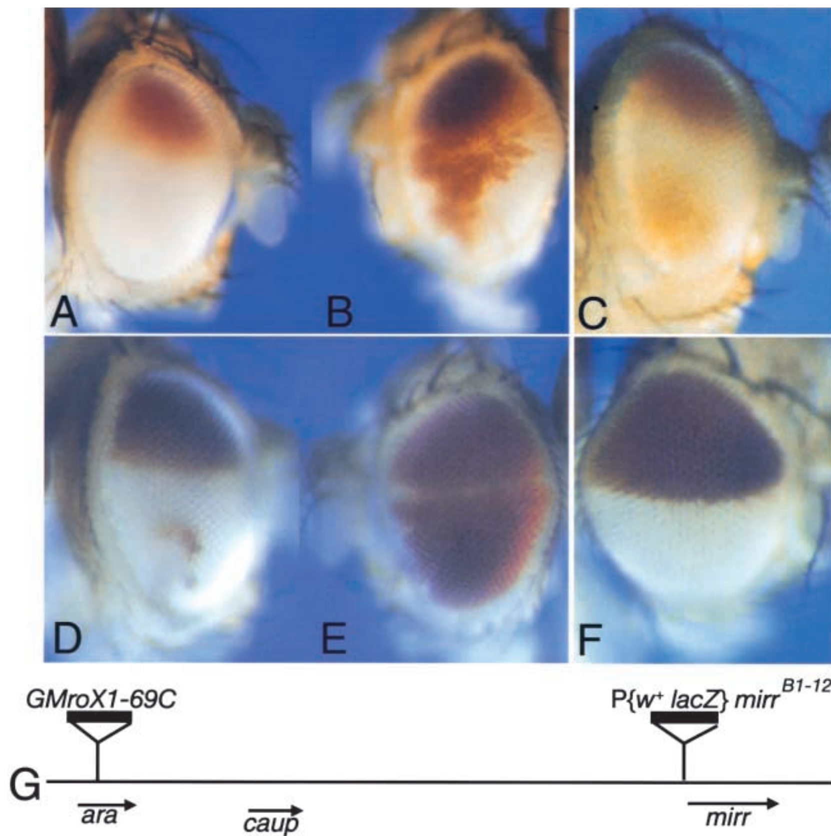

FIGURE 3.—Insertion into the *iroquois* cluster. GMroX1-69C is located in the second intron of the *ara* gene within the ~140-kb *iroquois* cluster of homeotic genes located at 69CD (G). The dorsal ~30% of female eyes are pigmented (A), but the ventral region is completely silenced as is typical for many other mini-*white* marked transgenes in this region like $P\{w^+\ lacZ\}\ mirr^{B1\text{-}12}$ (F). GMroX1-69C males have the same pattern as females except red sectors are present ventrally (B). The ventral silencing is mediated by the Pc group of proteins. *y w;* GMroX1-69C +/+ Pc^3 females show weak derepression of mini-*white* in the ventral half (C). Pairing of the transgene causes ventral derepression in the ventral half of the eye in small sectors in ~20% of homozygous GMroX1-69C females (D) or almost total derepression in most homozygous males (E). Such males often have a nonpigmented equator.

but had no effect in males (Figure 4B). When $P\{y^+$ *YEM2*} was crossed into the GMroX1-102C stock, females' eyes changed from completely silent to having red sectors (Figure 4, F *vs.* G). This demonstrates that the MSL complex is the key factor responsible for the male-specific activation of mini-*white* observed in these mosaic GMroX1 lines.

DISCUSSION

Expression of the *white* gene is exquisitely sensitive to its chromatin environment. The mini-*white* derivative used here is further debilitated by the loss of its eye enhancer. Several large screens have shown that expression of visible marker genes such as *white* and *yellow* variegate when placed near blocks of heterochromatin located around the centromeres (Roseman *et al.* 1995; Wallrath and Elgin 1995; Cryderman *et al.* 1998; Yan *et al.* 2002), the fourth chromosome (Sun *et al.* 2000), and at telomeres (Cryderman *et al.* 1999). A few examples of *white* silencing/variegation in euchromatin regions have been reported, for example, when multicopy arrays of *white* transposons are generated (Dorer and Henikoff 1994). In a screen using transposons carrying both *white* and a green fluorescent protein (GFP) reporter driven by PAX6-binding sites, up to 20% of the Drosophila euchromatic insertions expressed GFP, but not mini-*white* (Horn *et al.* 2000). The nature of these repressive regions is not understood, but they show that a large fraction of the euchromatin will silence mini-*white* expression. It is likely that all *P*-element screens generate many inserts at such sites that go unnoticed because the transgenic flies fail to express the marker. We postulate that we recovered inserts in a subset of these unfavorable sites because males could overcome silencing.

A model for male-specific mosaic eyes: We are unaware of other mini-*white* marked transgenes with the characteristics of those described here and infer that the *roX1* gene is responsible for this unusual behavior. We propose that male-specific pigmented sectors reported here are a visible manifestation of ectopic dosage compensation occurring around autosomal GMroX1 transgenes, which landed in repressive chromatin environments (Figure 5). The MSL complex is active by mid-embryogenesis and stays on throughout development (Rastelli *et al.* 1995; Franke *et al.* 1996). The mosaic eye patterns seen here suggest that the MSL complex spreads a more "open" chromatin architecture during embryonic development when the primordial eye disc has a small number of cells. This chromatin packaging competes with uncharacterized silencing factors and can be inherited through many mitotic divisions so that large clones of cells in the adult eye share the same on/off state. These results are similar to those reported for a w^+ transgene lacking *roX1* inserted at the heterochromatic base of the X (Wallrath *et al.* 1996). Such females suffered PEV but males had solid red eyes presum-

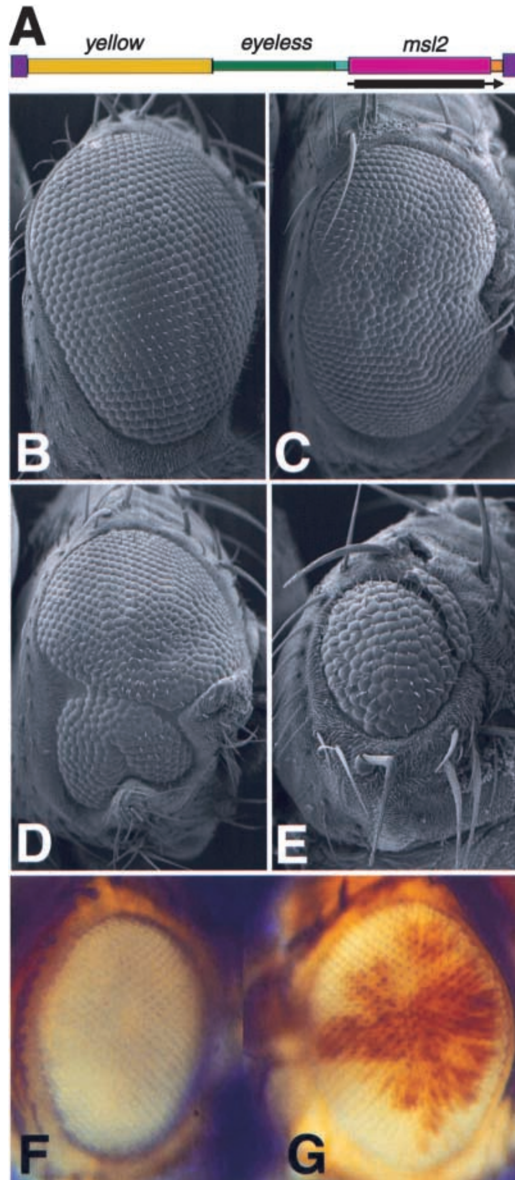

Figure 4.—The MSL complex overcomes mini-*white* silencing. *P*{y^+ *YEM2*} (A) was used to express the MSL complex in eyes using the eye-specific enhancer from the second intron of *eyeless* (dark green) to drive expression of the *msl2* coding sequence (violet) from a minimal *Hsp70* promoter (light green). The *tra2* 3′ UTR and polyadenylation site (orange) was placed downstream of *msl2*. When *P*{y^+ *YEM2*} was present alone, the MSL2 protein in the eye was sufficient to cause only a very mild rough eye defect in ∼2–5% of females due to ectopic dosage compensation (not shown). However, this phenotype is dramatically enhanced by overexpression of MSL1 from *P*{w^+ *H83M1*} (Chang and Kuroda 1998). (B) *y w/Y*; *P*{y^+ *YEM2*}9/*P*{y^+ *YEM2*}9 *P*{w^+ *H83M1*} males have wild-type eyes. (C–E) Approximately 80% of similar females show sectors of cell death due to ectopic dosage compensation. (F) The mini-*white* gene in *y w*; GMroX1-102C females is silent. (G) The mini-*white* gene is derepressed when MSL2 is expressed in the eyes of *y w*; *P*{y^+ *YEM2*}9; GMroX1-102C females.

ably due to the MSL complex spreading from flanking chromatin.

This model is supported by the finding that ectopic expression of MSL2 in females is sufficient to overcome silencing. Thus the MSL complex is responsible for the activation, but must be targeted to the transgene. This could happen either by MSL proteins assembling on nascent *roX1* transcripts (Park *et al.* 2002) or by mature MSL complex being recruited by DNA sequences within the *roX1* gene (Kageyama *et al.* 2001).

An alternative interpretation of dosage compensation in Drosophila, known as the inverse model, postulates that the MSL proteins normally have two key functions in wild-type males. First, they sequester the MOF histone H4 acetyltransferase away from the autosomes by targeting it to the X chromosome. Second, the MSLs block overexpression of X-linked genes that might otherwise result from MOF-mediated nucleosome acetylation (Bhadra *et al.* 1999). In this model, histone acetylation by MOF has little effect on gene transcription in the wild-type male X chromosome, but a significant toxic effect in *mle* mutant males where MOF escapes from the X and hyperacetylates the entire genome. In contrast to expectations of the inverse model, we find that histone H4 acetylation caused by MOF within complete MSL complexes is a potent activator in wild-type males.

The spreading model of MSL recognition predicts that the expression of autosomal genes in the vicinity of an ectopic *roX1* gene is elevated about twofold in nuclei where spreading occurs. The results reported here are consistent with the idea that the histone aectylation produced by the MSL complex near autosomal *roX* transgenes elevates expression of genes. In this situation we sometimes observed more than the expected twofold effect not because mini-*white* expression was elevated too much in males—the pigmented sectors were usually orange, suggesting modest expression—but rather the unusually low basal expression in the female eyes and silent male sectors was responsible for the large difference between "high" and "low" states. In a few lines such as 77A, 93E, 99C, and 98B, females did have detectable mini-*white* expression and males had sectors that appear about twofold darker than this basal expression. These results are consistent with a recent report that autosomal *roX1* transgenes could modestly elevate the expression of a flanking *lacZ* reporter in males (Henry *et al.* 2001).

Placing Polycomb response elements (PREs) near mini-*white* can cause mosaic silencing by recruiting the Polycomb complex (Fauvarque and Dura 1993; Chan *et al.* 1994; Sigrist and Pirrotta 1997; Americo *et al.* 2002). This prompted us to consider whether the *roX1* gene itself, rather than flanking chromatin, somehow caused repression of mini-*white* in females. The strongest argument against this idea is that the 69C and 80C inserts are clearly silenced by flanking sequences. Also, in many lines the males also have silent sectors, but it is difficult to imagine how *roX1* would act to repress genes in males. The fact that females from the large majority of GMroX1 transgenic lines have solid eye pig-

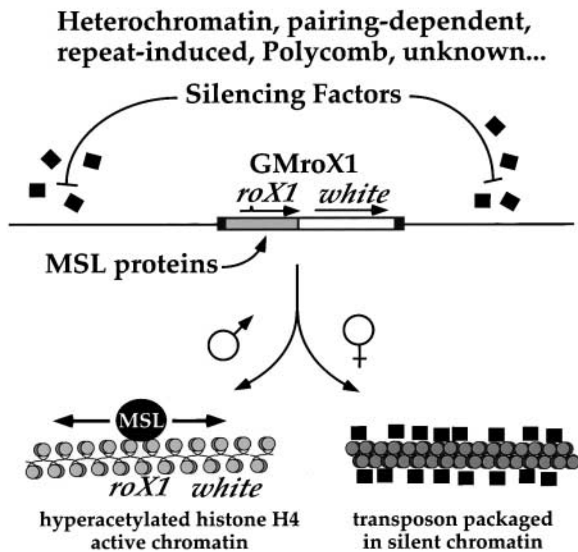

Figure 5.—Model for mosaic eyes. In ~10% of all cases, GMroX1 inserts into a chromatin domain that is subject to silencing due to the action of heterochromatin, the Polycomb complex, repeat-induced silencing, pairing-induced silencing, or other unknown factors. In a female, these factors spread so that the transgene is packaged in the same repressive chromatin (right). The MSL complex present in males recognizes the *roX1* gene and spreads into flanking chromatin as it is postulated to do on the X. The resulting histone modifications overcome silencing, allowing the mini-*white* reporter to be transcribed (left). The fraction of cells in which the MSL complex successfully remodels the local chromatin determines the eye pigmentation pattern.

mentation also suggests that the *roX1* sequence does not act as a silencer. The mosaic lines presented here account for only ~10% of all inserts. However, we cannot exclude the possibility that *roX1* sequences mediate female silencing in combination with unknown factors in some lines.

In some circumstances PRE-mediated silencing is pairing dependent (Chan *et al.* 1994; Americo *et al.* 2002). It is not clear whether *roX1* sequences or flanking DNA contribute to the pairing-sensitive silencing seen in some GMroX1 lines. The only pairing-sensitive inserts located near endogenous PcG-binding sites are at 84E and 93E (Rastelli *et al.* 1993). We did note that PREs often contain binding sites for the GAGA/Pipsqueak factors (Horard *et al.* 2000; Americo *et al.* 2002) and the major MSL-binding site within the *roX1* gene also contains several conserved GAGA sites (Kageyama *et al.* 2001 and data not shown).

Another explanation for male-specific activation might be that the vigorous transcription of *roX1* in males somehow prevents surrounding DNA from being packaged into silent chromatin similar to that reported for transgenes responding to GAL4 (Zink and Paro 1995; Ahmad and Henikoff 2001). Although regulation of *roX* transcription is not well understood, let us assume that MSL proteins drive *roX1* expression only in males. Transcription of *roX1* RNA *in cis* may indeed be a prerequisite for the nearby region to escape silencing, but we postulate a narrow role. We have previously suggested that MSL proteins assemble onto nascent *roX* transcripts and that this favors spreading into adjacent chromatin (Park *et al.* 2002). We propose that locally active RNA polymerase is not sufficient. For instance, strong transcription of Pax6-GFP in the eye does not guarantee expression of an adjacent *white* gene (Horn *et al.* 2000). We postulate that in the cases reported here *roX1* transcription is significant only in that it produces MSL complexes able to spread into flanking chromatin, modifying nucleosomes along the way.

Repressive nature of flanking chromatin: The MSL complex can overcome different mechanisms of silencing. The GMroX1-80C line is subject to severe PEV. The surprising aspect of this insert is the strength of silencing in females where neither the presence of a Y chromosome nor the presence of *Su(var)* mutations allowed any mini-*white* expression. Yet in males, the MSL complex can spread from *roX1* sequences through centric heterochromatin and into the euchromatic proximal arms of 3L and 3R, activating mini-*white* along the way (Park *et al.* 2002). The insertion in the *iroquois* cluster demonstrates that the MSL complex can overcome Polycomb-mediated silencing in the ventral half of the eye. The MSL complex can also overcome silencing due to insertion in dispersed repeats (75C and 84E).

The insertion in one of the ~110 tandem copies of the histone gene cluster at 39DE is particularly interesting. Mini-*white* is sometimes poorly expressed within long repeats (Dorer and Henikoff 1994). A second explanation rests on close proximity of the histone cluster to centric heterochromatin. A high histone gene copy number had been thought necessary to supply cells with enough histone proteins during each replication cycle. However, the discovery that *Drosophila hawaiiensis* carries 20 copies of the histone genes and *D. hydei* carries only 5–10 copies called for a new explanation (Fitch *et al.* 1990). In species with low copy number, the histone genes are located far from heterochromatin. However, the histone genes in *D. melanogaster* are adjacent to centric heterochromatin. Selection for increased copy number may have compensated for low expression per gene copy (Fitch *et al.* 1990).

The silencing mechanism remains a mystery for most inserts. The idea that flanking genes are kept silent because they are not needed for eye development is difficult to test because few of the surrounding genes are well characterized. Ectopic expression of the *zfh1* gene, interrupted by GMroX1-99F, disrupts eye development (Lai *et al.* 1991), but *zfh1* expression in wild-type eye discs has not been reported. The GMroX1-64A insert landed upstream of the *scrt* gene encoding a Zn finger transcription factor regulating neuron development (Roark *et al.* 1995). Although the *scrt* gene is expressed in the developing neurons of the eye, the insert is located in an enhancer element driving expression in the central nervous system (Emery and Bier 1995). The

scrt gene appears to be silent in the nonneuronal pigment cells where mini-*white* should be expressed. Our failure to detect any interaction between mutations in the *pr-set7* histone H4 methyltransferase gene and the mosaic transgenes cannot rule out a role for histone methylation in euchromatic gene silencing. Lowering methylation by 50% may be insufficient to change the mosaic pattern in eyes, or a different methylase may be responsible.

In summary, the MSL complex is a versatile chromatin-remodeling machine able to act on many different chromatin substrates. This might be expected for a regulator that must normally act on several thousand unrelated genes expressed in different tissues throughout development. However, this behavior raises the question of how males can keep appropriate segments of the X silent in tissues in which a gene product is not needed and might even be harmful. Presumably the MSL complex is tightly regulated on the X so that only active genes are upregulated. Others have shown that the MSL complex can radically alter the morphology of the X when certain chromatin-modifying factors, such as ISWI or NURF, are mutated (Deuring *et al.* 2000; Badenhorst *et al.* 2002; Corona *et al.* 2002). Perhaps such proteins normally restrict the action of the MSL complex. In addition, chromosomes may be organized into loops or domains of activity *in vivo* so that the MSL complex can respect domain boundaries if it spreads along the chromosome *in cis* (Gu *et al.* 2000). The *roX1* transgenes studied here may subvert such regulation by placing a MSL-binding/assembly site internal to domain boundary elements.

Because of its extreme sensitivity to a chromatin environment, mini-*white*-based *P* elements are being replaced with *yellow*$^{+}$ or PAX6-EGFP marked vectors for mutagenesis screens (Roseman *et al.* 1995; Horn *et al.* 2000; Yan *et al.* 2002). However, the GMroX1 transposon may be useful in screens to assay for repressive chromatin environments. Simply comparing the eye color of brothers and sisters from the same stock would quickly identify euchromatic inserts subject to subtle chromatin effects.

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LITERATURE CITED

Ahmad, K., and S. Henikoff, 2001 Modulation of a transcription factor counteracts heterochromatic gene silencing in *Drosophila.* Cell **104:** 839–847.

Americo, J., M. Whiteley, J. L. Brown, M. Fujioka, J. B. Jaynes *et al.*, 2002 A complex array of DNA-binding proteins required for pairing-sensitive silencing by a polycomb group response element from the *Drosophila engrailed* gene. Genetics **160:** 1561–1571.

Amrein, H., and R. Axel, 1997 Genes expressed in neurons of adult male *Drosophila.* Cell **88:** 459–469.

Badenhorst, P., M. Voas, I. Rebay and C. Wu, 2002 Biological function of the ISWI chromatin remodeling complex NURF. Genes Dev. **16:** 3186–3198.

Bashaw, G. J., and B. S. Baker, 1995 The *msl-2* dosage compensation gene of *Drosophila* encodes a putative DNA-binding protein whose expression is sex specifically regulated by *Sex-lethal.* Development **121:** 3245–3258.

Bhadra, U., M. Pal-Bhadra and J. A. Birchler, 1999 Role of the *male specific lethal* (*msl*) genes in modifying the effects of sex chromosomal dosage in Drosophila. Genetics **152:** 249–268.

Birve, A., A. K. Sengupta, D. Beuchel, J. Larsson, J. A. Kennison *et al.*, 2001 Su(z)12, a novel *Drosophila* polycomb group gene that is conserved in vertebrates and plants. Development **128:** 3371–3379.

Chan, C.-S., L. Rastelli and V. Pirrotta, 1994 A *Polycomb* response element in the *Ubx* gene that determines an epigenetically inherited state of repression. EMBO J. **13:** 2553–2564.

Chang, K. A., and M. I. Kuroda, 1998 Modulation of MSL1 abundance in female Drosophila contributes to the sex specificity of dosage compensation. Genetics **150:** 699–709.

Cleard, F., and P. Spierer, 2001 Position-effect variegation in *Drosophila*: the modifier Su(var)3-7 is a modular DNA-binding protein. EMBO Rep. **2:** 1095–1100.

Cline, T. W., and B. J. Meyer, 1996 Vive la difference: males vs. females in flies vs. worms. Annu. Rev. Genet. **30:** 637–702.

Corona, D. F. V., C. R. Clapier, P. B. Becker and J. W. Tamkun, 2002 Modulation of ISWI function by site-specific histone acetylation. EMBO Rep. **3:** 242–247.

Cryderman, D. E., M. H. Cuaycong, S. C. R. Elgin and L. L. Wallrath, 1998 Characterization of sequences associated with position-effect variegation at pericentric sites in *Drosophila* heterochromatin. Chromosoma **107:** 277–285.

Cryderman, D. E., E. J. Morris, H. Biessmann, S. C. Elgin and L. L. Wallrath, 1999 Silencing at *Drosophila* telomeres: nuclear organization and chromatin structure play critical roles. EMBO J. **18:** 3724–3735.

Deuring, R., L. Fanti, J. A. Armstrong, M. Sarte, O. Papoulas *et al.*, 2000 The ISWI chromatin-remodeling protein is required for gene expression and the maintenance of higher order chromatin structure in vivo. Mol. Cell **5:** 355–365.

Dorer, D. R., and S. Henikoff, 1994 Expansions of transgene repeats cause heterochromatin formation and gene silencing in *Drosophila.* Cell **77:** 993–1002.

Eissenberg, J. C., T. C. James, D. M. Foster-Hartnett, T. Hartnett, V. Ngan *et al.*, 1990 Mutation in a heterochromatin-specific chromosomal protein is associated with suppression of position-effect variegation in *Drosophila melanogaster.* Proc. Natl. Acad. Sci. USA **87:** 9923–9927.

Emery, J. F., and E. Bier, 1995 Specificity of CNS and PNS regulatory subelements comprising pan-neural enhancers of the *deadpan* and *scratch* genes is achieved by repression. Development **121:** 3549–3560.

Fauvarque, M.-O., and J.-M. Dura, 1993 *polyhomeotic* regulatory sequences induce developmental regulator-dependent variegation and targeted P-element insertions in *Drosophila.* Genes Dev. **7:** 1508–1520.

Fitch, D. H., L. D. Strausbaugh and V. Barrett, 1990 On the origins of tandemly repeated genes: Does histone gene copy number in *Drosophila* reflect chromosomal location? Chromosoma **99:** 118–124.

Franke, A., and B. S. Baker, 1999 The *roX1* and *roX2* RNAs are essential components of the compensasome, which mediates dosage compensation in *Drosophila.* Mol. Cell **4:** 117–122.

Franke, A., A. Dernburg, G. J. Bashaw and B. S. Baker, 1996 Evidence that MSL-mediated dosage compensation in *Drosophila* begins at blastoderm. Development **122:** 2751–2760.

Gomez-Skarmeta, J. L., R. Diez del Corral, E. De La Calle-Mustienes, D. Ferres-Marco and J. Modolell, 1996 Araucan and caupolican, two members of the novel Iroquois complex, encode homeoproteins that control proneural and vein-forming genes. Cell **85:** 95–105.

Grewal, S. I., and S. C. Elgin, 2002 Heterochromatin: new possibili-

ties for the inheritance of structure. Curr. Opin. Genet. Dev. **12:** 178–187.

Gu, W., X. Wei, A. Pannuti and J. C. Lucchesi, 2000 Targeting the chromatin-remodeling MSL complex of *Drosophila* to its sites of action on the X chromosome requires both acetyl transferase and ATPase activities. EMBO J. **19:** 5202–5211.

Halder, G., P. Callaerts, S. Flister, U. Walldorf, U. Kloter *et al.*, 1998 Eyeless initiates the expression of both sine oculis and eyes absent during *Drosophila* compound eye development. Development **125:** 2181–2191.

Henry, R. A., B. Tews, X. Li and M. J. Scott, 2001 Recruitment of the male-specific lethal (MSL) dosage compensation complex to an autosomally integrated *roX* chromatin entry site correlates with an increased expression of an adjacent reporter gene in male *Drosophila*. J. Biol. Chem. **276:** 31953–31958.

Horard, B., C. Tatout, S. Poux and V. Pirrotta, 2000 Structure of a polycomb response element and in vitro binding of polycomb group complexes containing GAGA factor. Mol. Cell. Biol. **20:** 3187–3197.

Horn, C., B. Jaunich and E. A. Wimmer, 2000 Highly sensitive, fluorescent transformation marker for *Drosophila* transgenesis. Dev. Genes Evol. **210:** 623–629.

Kageyama, Y., G. Mengus, G. Gilfillan, H. G. Kennedy, C. Stuckenholz *et al.*, 2001 Association and spreading of the Drosophila dosage compensation complex from a discrete *roX1* chromatin entry site. EMBO J. **20:** 2236–2245.

Kassis, J. A., E. P. VanSickle and S. M. Sensabaugh, 1991 A fragment of *engrailed* regulatory DNA can mediate transvection of the *white* gene in Drosophila. Genetics **128:** 751–761.

Kehl, B. T., K.-O. Cho and K.-W. Choi, 1998 *mirror*, a *Drosophila* homeobox gene in the *iroquois* complex, is required for sensory organ and alula formation. Development **125:** 1217–1227.

Kelley, R. L., I. Solovyeva, L. M. Lyman, R. Richman, V. Solovyev *et al.*, 1995 Expression of Msl-2 causes assembly of dosage compensation regulators on the X chromosomes and female lethality in *Drosophila*. Cell **81:** 867–877.

Kelley, R. L., V. H. Meller, P. R. Gordadze, G. Roman, R. L. Davis *et al.*, 1999 Epigenetic spreading of the *Drosophila* dosage compensation complex from *roX* RNA genes into flanking chromatin. Cell **98:** 513–522.

Lai, Z. C., M. E. Fortini and G. M. Rubin, 1991 The embryonic expression patterns of *zfh-1* and *zfh-2*, two *Drosophila* genes encoding novel zinc-finger homeodomain proteins. Mech. Dev. **34:** 123–124.

McNeill, H., C. H. Yang, M. Brodsky, J. Ungos and M. A. Simon, 1997 *mirror* encodes a novel PBX-class homeoprotein that functions in the definition of the dorsal-ventral border in the *Drosophila* eye. Genes Dev. **11:** 1073–1082.

Meller, V. H., 2000 Dosage compensation: making 1X equal 2X. Trends Cell Biol. **10:** 54–59.

Meller, V. H., and B. P. Rattner, 2002 The *roX* genes encode redundant male-specific lethal transcripts required for targeting of the MSL complex. EMBO J. **21:** 1084–1091.

Meller, V. H., K. H. Wu, G. Roman, M. I. Kuroda and R. L. Davis, 1997 *roX1* RNA paints the X chromosome of male *Drosophila* and is regulated by the dosage compensation system. Cell **88:** 445–457.

Netter, S., M. O. Fauvarque, R. Diez del Corral, J. M. Dura and D. Coen, 1998 *white*$^{+}$ transgene insertions presenting a dorsal/ventral pattern define a single cluster of homeobox genes that is silenced by the polycomb-group proteins in *Drosophila melanogaster*. Genetics **149:** 257–275.

Nishioka, K., J. C. Rice, K. Sarma, H. Erdjument-Bromage, J. Werner *et al.*, 2002 PR-Set7 is a nucleosome-specific methyltransferase that modifies lysine 20 of histone H4 and is associated with silent chromatin. Mol. Cell **9:** 1201–1213.

Park, Y., R. L. Kelley, H. Oh, M. I. Kuroda and V. H. Meller, 2002 Extent of chromatin spreading determined by *roX* RNA recruitment of MSL proteins. Science **298:** 1620–1623.

Patton, J. S., X. V. Gomes and P. K. Geyer, 1992 Position-independent germline transformation in *Drosophila* using a cuticle pigmentation gene as a selectable marker. Nucleic Acids Res. **20:** 5859–5860.

Pirrotta, V., 1988 Vectors for P-mediated transformation in *Drosophila*, pp. 437–456 in *Vectors: A Survey of Molecular Cloning Vectors and Their Uses*, edited by R. L. Rodriguez and D. T. Denhardt. Butterworths, Boston.

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.

Rastelli, L., R. Richman and M. I. Kuroda, 1995 The dosage compensation regulators MLE, MSL-1 and MSL-2 are interdependent since early embryogenesis in *Drosophila*. Mech. Dev. **53:** 223–233.

Roark, M., M. S. Sturtevant, J. Emery, H. Vaessin, E. Grell *et al.*, 1995 *scratch*, a pan-neural gene encoding a zinc finger protein related to *snail*, promotes neuronal development. Genes Dev. **9:** 2384–2398.

Robertson, A. M., C. R. Preston, R. W. Phillis, D. Johnson-Schlitz, W. K. Benz *et al.*, 1988 A stable genomic source of *P* element transposase in *Drosophila melanogaster*. Genetics **118:** 461–470.

Roseman, R. R., E. A. Johnson, C. K. Rodesch, M. Bjerke, R. N. Nagoshi *et al.*, 1995 A *P* element containing *suppressor of Hairy-wing* binding regions has novel properties for mutagenesis in *Drosophila melanogaster*. Genetics **141:** 1061–1074.

Sigrist, C. J. A., and V. Pirrotta, 1997 Chromatin insulator elements block the silencing of a target gene by the *Drosophila* polycomb response element (PRE) but allow *trans* interactions between PREs on different chromosomes. Genetics **147:** 209–221.

Sun, F.-L., M. H. Cuaycong, C. A. Craig, L. L. Wallrath, J. Locke *et al.*, 2000 The fourth chromosome of *Drosophila melanogaster*: interspersed euchromatic and heterochromatic domains. Proc. Natl. Acad. Sci. USA **97:** 5340–5345.

Sun, Y. H., C. J. Tsai, M. M. Green, J. L. Chao, C. T. Yu *et al.*, 1995 *white* as a reporter gene to detect transcriptional silencers specifying position-specific gene expression during *Drosophila melanogaster* eye development. Genetics **141:** 1075–1086.

Wakimoto, B. T., 1998 Beyond the nucleosome: epigenetic aspects of position-effect variegation in *Drosophila*. Cell **93:** 321–324.

Wallrath, L. L., 1998 Unfolding the mysteries of heterochromatin. Curr. Opin. Genet. Dev. **8:** 147–153.

Wallrath, L. L., and S. C. R. Elgin, 1995 Position effect variegation in *Drosophila* is associated with an altered chromatin structure. Genes Dev. **9:** 1263–1277.

Wallrath, L. L., V. P. Guntur, L. E. Rosman and S. C. Elgin, 1996 DNA representation of variegating heterochromatic P-element inserts in diploid and polytene tissues of *Drosophila melanogaster*. Chromosoma **104:** 519–527.

Yan, C. M., K. W. Dobie, H. D. Le, A. Y. Konev and G. H. Karpen, 2002 Efficient recovery of centric heterochromatin *P*-element insertions in *Drosophila melanogaster*. Genetics **161:** 217–229.

Zhou, S., Y. Yang, M. J. Scott, A. Pannuti, K. C. Fehr *et al.*, 1995 *Male-specific-lethal 2*, a dosage compensation gene of *Drosophila* that undergoes sex-specific regulation and encodes a protein with a RING finger and a metallothionein-like cluster. EMBO J. **14:** 2884–2895.

Zink, D., and R. Paro, 1995 *Drosophila* polycomb-group regulated chromatin inhibits the accessibility of a trans-activator to its target DNA. EMBO J. **14:** 5660–5671.

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