A Role for siRNA in X-Chromosome Dosage Compensation in *Drosophila melanogaster*

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ABSTRACT Sex-chromosome dosage compensation requires selective identification of X chromatin. How this occurs is not fully understood. We show that small interfering RNA (siRNA) mutations enhance the lethality of *Drosophila* males deficient in X recognition and partially rescue females that inappropriately dosage-compensate. Our findings are consistent with a role for siRNA in selective recognition of X chromatin.

MALES of many species carry a euchromatic, gene-rich X chromosome and a gene-poor, heterochromatic Y chromosome (Charlesworth 1991). This creates a potentially lethal imbalance in the X to autosomal (X:A) ratio in one sex (Gupta *et al.* 2006; Nguyen and Disteche 2006; Deng *et al.* 2011). Dosage compensation is an essential process that equalizes X-linked gene expression between XY males and XX females, thereby maintaining a constant ratio of X:A gene products. Strategies to accomplish this differ between species, but share the need for coordinated regulation of an entire chromosome (Lucchesi *et al.* 2005). In flies, the male-specific lethal (MSL) complex, composed of five MSL proteins and noncoding *roX* (*RNA on the X chromosome*) RNA, binds with great selectivity to the X chromosome of males (Deng and Meller 2006a). The MSL complex directs H4K16 acetylation to the body of X-linked genes, increasing transcription by enhancing RNA polymerase II processivity (Smith *et al.* 2001; Larschan *et al.* 2011).

Recruitment of the MSL complex is postulated to occur at X-linked *c*hromatin *e*ntry *s*ites (CES) (Kelley *et al.* 1999; Alekseyenko *et al.* 2008; Straub *et al.* 2008). CES contain 21-bp *M*SL *r*ecognition *e*lements (MREs), which are modestly enriched on the X chromosome (Alekseyenko *et al.* 2008). The MSL complex then spreads to nearby transcribed genes (Larschan *et al.* 2007; Sural *et al.* 2008). While this model elegantly describes the local distribution of the MSL complex, it fails to explain the exclusive recognition of X chromatin that is a hallmark of *Drosophila* dosage compensation.

The initiation of dosage compensation and hypertranscription of X-linked genes is dependent on *roX* RNA (Meller 2003; Deng and Meller 2006b). The X-linked *roX* genes, *roX1* and *roX2*, are redundant for these functions (Meller and Rattner 2002). Mutation of a single *roX* gene is without phenotype, but simultaneous mutation of *roX1* and *roX2* reduces X-localization of the MSL complex, resulting in a reduction in X-linked gene expression and male-specific lethality (Meller and Rattner 2002; Deng and Meller 2006b).

Because the *roX* RNAs are necessary for exclusive X-localization of the MSL proteins, genetic modifiers of *roX1 roX2* lethality may identify novel pathways that contribute to X-recognition. We previously reported that a maternally imprinted Y chromosome is a potent suppressor of *roX1 roX2* lethality (Menon and Meller 2009). The expression of Y-linked protein-coding genes is restricted to the germline, making it unlikely that these genes influence the somatic process of dosage compensation. Furthermore, the Y chromosome itself is nonessential for dosage compensation (reviewed by Lucchesi 1973). We postulate that, in spite of the fact that Y-linked genes are unnecessary for dosage compensation, the Y-chromosome imprint modulates a pathway involved in this process.

Repetitive sequences, which are abundant on the Y chromosome, have been proposed to influence somatic gene expression (Lemos *et al.* 2008, 2010; Jiang *et al.* 2010; Piergentili 2010). Small RNA pathways are potential mediators of this effect. To pursue the idea that small RNA might play a role in dosage compensation, we conducted a directed

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screen of RNAi pathways. Mutations in the small interfering RNA (siRNA) pathway were found to enhance *roX1 roX2* lethality. siRNA mutations disrupt localization of the MSL complex in *roX1 roX2* mutants and partially rescue female flies that inappropriately dosage-compensate, leading to toxic overexpression of X-linked genes. Our findings are consistent with participation of siRNA in recognition of X chromatin.

Materials and Methods

Fly culture and genetics

Flies were maintained at 25° on standard cornmeal–agar fly food. Unless otherwise noted, mutations are described in Lindsley and Zimm (1992). *roX1* mutations and a complex *roX2* deletion (Df(1)52; [w^+4Δ4.3]) have been described (Meller *et al.* 1997; Meller and Rattner 2002; Deng *et al.* 2005). A viable deletion of *roX2* (*roX2*Δ) was accomplished by FLP-mediated recombination between CG11695^{f01356} and nod^{f04008}. Description of *dcr2*^{f06544}, *ago2*^{dop1}, *ago2*^{414}, *r2d2*^{1}, *D-elp1*^{c00296}, *loqs*^{f00791}, *ago1*^{k00281}, *spn-E*^{1}, *aub*^{QC42}, *aub*^{HN}, and *piwi*^{06843} can be found at http://flybase.org. *ago2*^{414} was provided by R. Carthew, and all other mutations were provided by the Bloomington Drosophila Stock Center.

RNAi mutations were outcrossed for six generations to minimize genetic background effects. All stocks were constructed with the Y chromosome from the laboratory reference *yw* strain to eliminate confounding effects attributable to different Y chromosomes that we and others have observed (Lemos *et al.* 2008). After rebalancing, all mutations were confirmed by PCR or phenotype. Matings to determine the effect of RNAi pathway mutations on *roX1*^{ex33} *roX2*Δ male and *yw* female survival are detailed in Figure S2.

qRT-PCR

Accumulation of *roX1*^{ex40} transcript was measured by qRT-PCR as previously described (Deng *et al.* 2009). Briefly, RNA was prepared from three groups of 50 third instar male larvae. One microgram of RNA was reverse-transcribed using random hexamers and ImProm-II reverse transcriptase (Promega). Two technical replicates of each biological replicate were amplified with 300 nM of the primers TTTTTGTCCCACCCGAATAA and CCTTTTAATGCGTTTTCCGA. Expression of *roX1*^{ex40} was normalized to autosomal *Dmn*, amplified with 300 nM of primers GACAAGTTGAGCCGCCTTAC and CTTGGTGCTTAGATGACGCA.

Results and Discussion

The *roX1*^{ex33} *roX2*Δ X chromosome supports ~20% eclosion of adult male escapers. *roX1*^{ex33} *roX2*Δ females were mated to males heterozygous for mutations in the Piwi-interacting RNA, small interfering RNA (siRNA), and microRNA (miRNA) pathways (*RNAi*$^{-}$/+). The survival of sons with reduced RNA interference (RNAi) function (*roX1*^{ex33} *roX2*Δ ; *RNAi*$^{-}$/+) was divided by that of their brothers with intact RNAi (*roX1*^{ex33} *roX2*Δ ; +/+) to reveal enhancement or suppression of male lethality. Mutations in *Dcr-2*, *Ago2*, *Loqs*, and *D-elp1* were found to lower the survival of *roX1*^{ex33} *roX2*Δ males by 30, 55, 50, and 70%, respectively (Figure 1A).

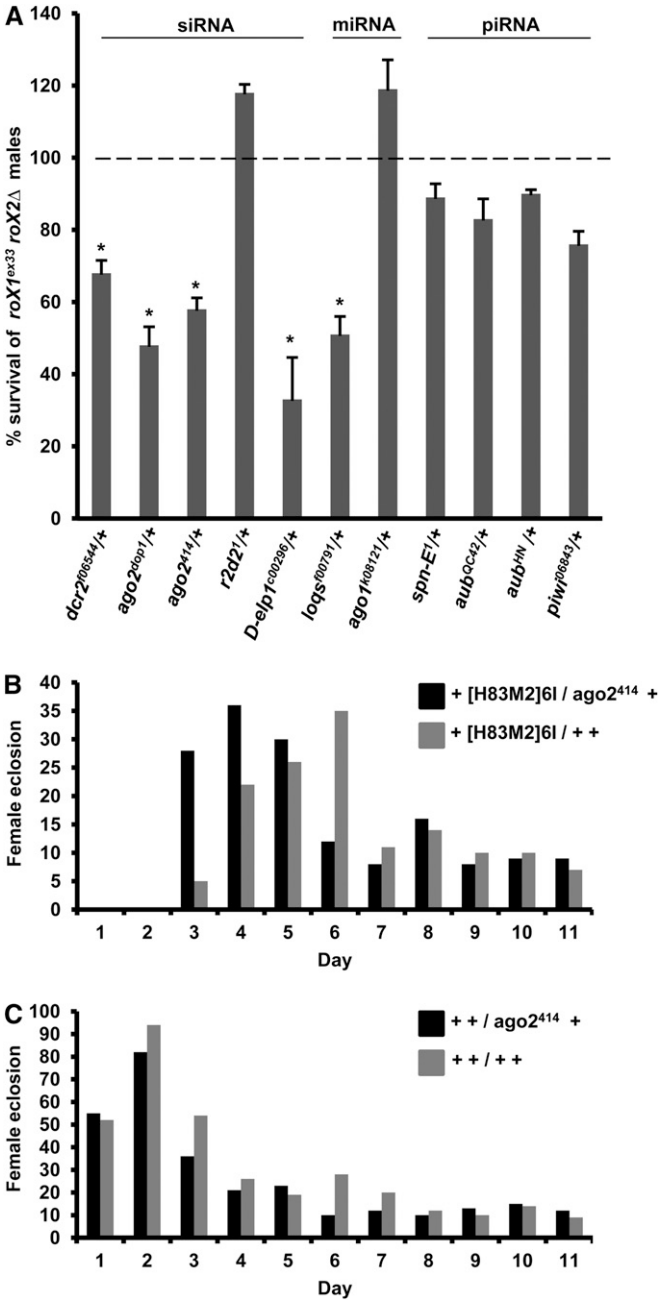

Figure 1 siRNA mutations enhance *roX1 roX2* male lethality. (A) Eclosing *roX1*^{ex33} *roX2*Δ males carrying RNAi mutations divided by their brothers with full RNAi function. SEM is represented by error bars. An asterisk indicates Student's two-sample *t*-test significance of ≤0.05. (B) Ago2 reduction partially rescues the developmental delay of females expressing MSL2. Females carry the [H83M2]6I transgene and express MSL2. Solid bars represent females heterozygous for *ago2*^{414}; shaded bars represent females with wild-type *ago2*. (C) Ago2 reduction does not influence the eclosion of otherwise wild-type females. Solid bars depict females heterozygous for *ago2*^{414}; shaded bars are their sisters with wild-type *ago2*.

Dcr-2 and *D-elp1* play a role in endogenous siRNA (endo-siRNA) production and transposon silencing, and *Ago2* is a member of the RNAi-induced silencing complex (Carthew and Sontheimer 2009; Lipardi and Paterson 2009; Siomi and Siomi 2009). While *loqs* has a prominent role in miRNA biogenesis, an isoform of Loqs has been implicated in the biogenesis of endo-siRNA from structured loci and transposons (Okamura *et al.* 2008; Zhou *et al.* 2009; Marques *et al.* 2010). All of the candidate genes therefore affect siRNA production or function. Reduction of the canonical siRNA gene *r2d2* did not enhance *roX1 roX2* male lethality. R2D2 affects strand selection during loading of siRNA onto Ago2 (Liu *et al.* 2003; Tomari *et al.* 2004). It is possible that this is unnecessary for dosage compensation or that the level of R2D2 is not limiting when a single copy of the gene is mutated.

To confirm that siRNA selectively affects dosage compensation, we asked whether reduction of Ago2 rescued females that inappropriately deploy the dosage compensation machinery, leading to toxic overexpression of both X chromosomes. Ectopic expression of *male-specific lethal 2* (*msl2*) induces dosage compensation in females (Kelley *et al.* 1995). MSL2 expression, driven by the [H83M2]6I transgene, reduces female survival and delays the peak of eclosion until day 6 (shaded bars, Figure 1B) (Kelley *et al.* 1995). In contrast, eclosion of sisters not expressing MSL2 peaks on day 2 (shaded bars, Figure 1C). Eclosion of [H83M2]6I females with one mutated *ago2* allele is advanced by 2 days, peaking on day 4 (solid bars, Figure 1B). Reduction of Ago2 in otherwise wild-type females had no discernible effect on eclosion timing (Figure 1C). The enhancement of *roX1 roX2* male lethality by siRNA mutations and partial rescue of MSL2-expressing females by reduction of Ago2 identifies a role for small RNA in *Drosophila* dosage compensation.

The $roX1^{ex40}$ internal deletion mutant supports full male survival, presumably because it retains essential 5′ and 3′ *roX1* regions in a transcript of reduced size (Deng *et al.* 2005). Localization of the MSL complex on polytene chromosomes of $roX1^{ex40}$ *roX2*Δ males is similar to that observed in wild-type flies. $roX1^{ex40}$ therefore has a molecularly detectable but subphenotypic defect. Loss of Ago2 has no effect on male survival by itself, but when Ago2 is eliminated in $roX1^{ex40}$ *roX2*Δ males, survival is reduced to 8% (Figure 2A). Loss of Loqs reduces $roX1^{ex40}$ *roX2*Δ male survival by >50% (Figure 2B). $roX1^{ex40}$ *roX2*Δ males with reduced D-Elp1 levels have full viability, but *D-elp1* lethality precludes homozygote testing. We took advantage of the synthetic lethality between $roX1^{ex40}$ *roX2*Δ and siRNA mutations to explore how siRNA contributes to dosage compensation.

To address the possibility that siRNA mutations act by modulating the level of *roX* RNA, quantitative RT-PCR (qRT-PCR) was used to measure $roX1^{ex40}$ transcript in $ago2^{414}$ or $D\text{-}elp1^{c00296}$/+ males. Accumulation of $roX1^{ex40}$ RNA was unaffected by these mutations (Supporting Information, Figure S1A). We also considered the possibility that

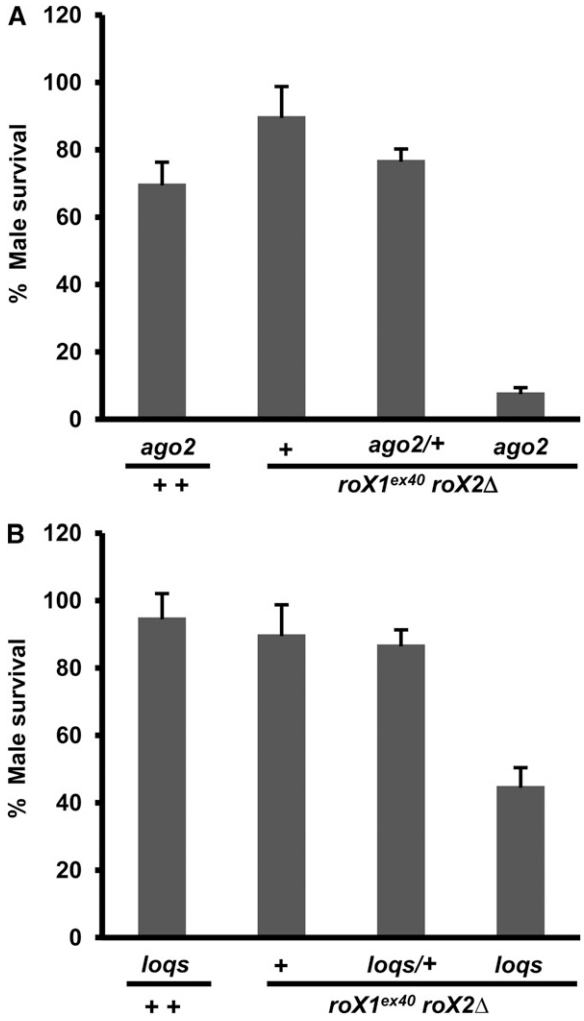

Figure 2 $roX1^{ex40A}$ *roX2*Δ is synthetic lethal with siRNA mutations. (A) Loss of Ago2 reduces the survival of $roX1^{ex40}$ *roX2*Δ adult males. The number of males recovered was the following: $ago2^{414}$, 245; $roX1^{ex40}$ *roX2*Δ, 274; $roX1^{ex40}$ *roX2*Δ; $ago2^{414}$/+, 1356; and $roX1^{ex40}$ *roX2*Δ; $ago2^{414}$, 45. (B) Loss of Loqs reduces $roX1^{ex40}$ *roX2*Δ adult male survival. The total number of males recovered was the following: $loqs^{f00791}$, 230; $roX1^{ex40}$ *roX2*Δ, 274; $roX1^{ex40}$ *roX2*Δ; $loqs^{f00791}$/+, 708; and $roX1^{ex40}$ *roX2*Δ; $loqs^{f00791}$, 166. Survival of $roX1^{ex40}$ *roX2*Δ; $ago2^{414}$ and $roX1^{ex40}$ *roX2*Δ; $loqs^{f00791}$ males was determined by mating $roX1^{ex40}$ *roX2*Δ; $ago2^{414}$ /TM3*SbTb* males and females or $roX1^{ex40}$ *roX2*Δ; $loqs^{f00791}$/In *(*2LR*)Bc Gla* males and females. Survival of $ago2^{414}$ and $loqs^{f00791}$ males was determined by observation of *yw*; $ago2^{414}$/TM3*SbTb* and *yw*; $loqs^{f00791}$/In(2LR)*Bc Gla* stocks.

siRNA indirectly influences the level of an MSL protein. Protein blotting revealed no reduction in core members of the MSL complex in males lacking Ago2 or with reduced D-elp1 (Figure S1, C–F). This conclusion is supported by whole-genome expression studies in S2 cells following Ago2 knockdown (Rehwinkel *et al.* 2006). As suggested by the lack of a male phenotype, the $roX1^{ex40}$ *roX2*Δ chromosome alone did not affect MSL protein levels (Figure S1, C–F). Disruption of dosage compensation in *roX1 roX2* males with reduced siRNA therefore does not involve reduction in the core components of the MSL complex.

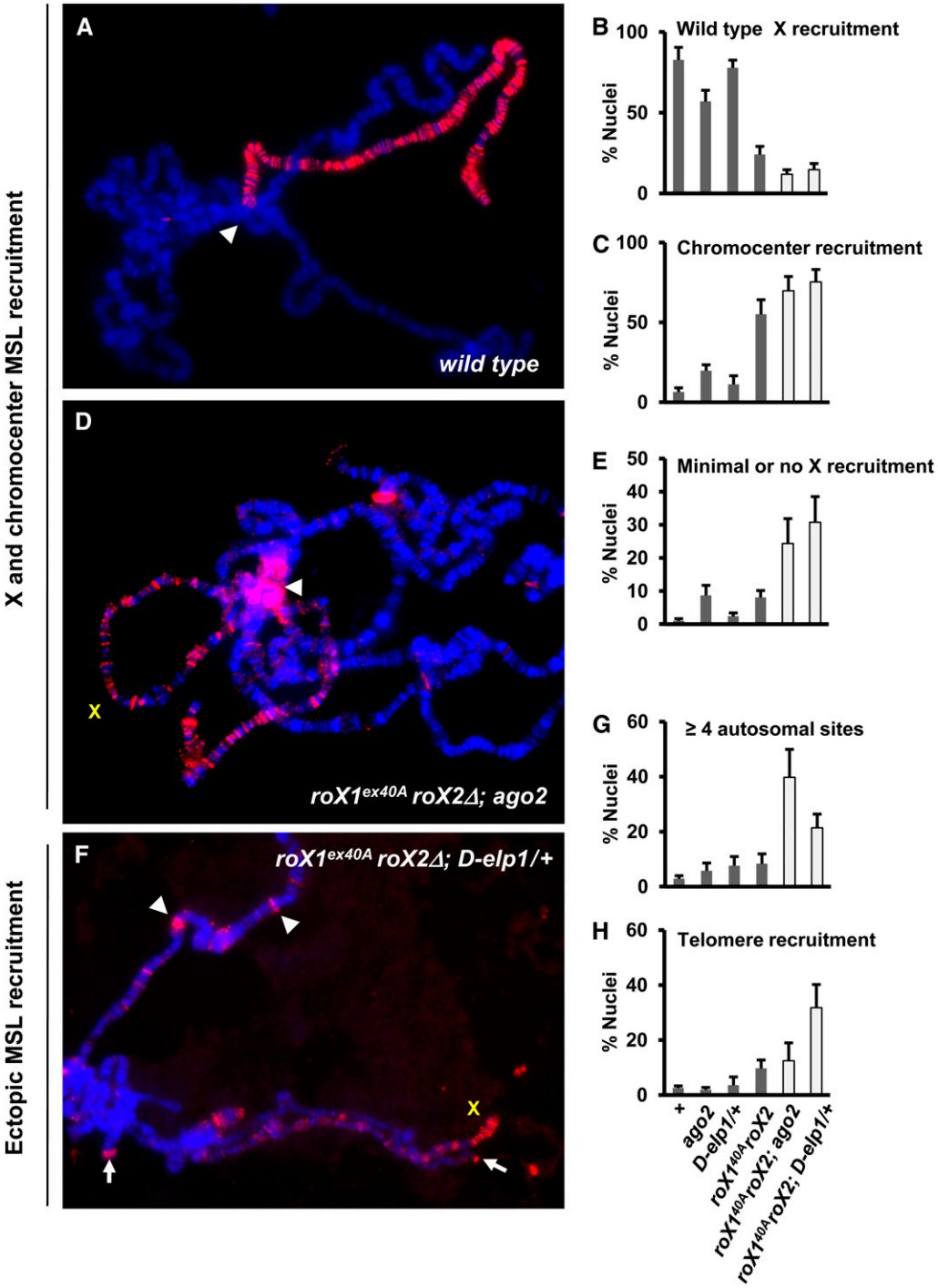

Figure 3 MSL1 localization is disrupted in *roX1*^{ex40} *roX2*Δ males mutated for *ago2* or *D-elp1*. (A) MSL1 localization is exclusive to the X chromosome in a polytene preparation from a wild-type male larva. (B) Percentage of nuclei of each genotype that display wild-type MSL1 recruitment to the X chromosome. (C) Percentage of nuclei with ectopic MSL1 binding at the chromocenter (compare arrowheads in A and D). (D) Minimal MSL1 recruitment to the X chromosome and strong chromocenter recruitment in a *roX1*^{ex40} *roX2*Δ; *ago2*^{414} male. (E) Percentage of nuclei with minimal or no MSL1 recruitment to the X chromosome (sum of categories "+" and "no MSL recruitment," Table S1A). (F) Ectopic autosomal MSL1 binding in a *roX1*^{ex40} *roX2*Δ; *D-elp1*/+ male. (G) Percentage of nuclei with four or more distinct autosomal MSL1-binding sites (arrowheads in F). (H) Percentage of nuclei with MSL1 recruitment to a telomere (arrows in F). Polytene chromosome preparations were immunostained for MSL1 as previously described (Kelley *et al.* 1999). MSL1 is detected by Texas Red, and DNA is detected by DAPI. One hundred fifty to 300 nuclei of each genotype were scored for MSL1 recruitment. Genotypes were obscured during scoring to eliminate bias. Full genotypes are the following: *yw* reference strain (wild type); *ago2*^{414}; *D-elp1*^{c00296}/+; *roX1*^{ex40} *roX2*Δ; *roX1*^{ex40} *roX2*Δ; and *ago2*^{414} (open bars); and *roX1*^{ex40} *roX2*Δ; *D-elp1*^{c00296}/+ (open bars). SEM is depicted by error bars. Categories of MSL1 recruitment are detailed in Table S1.

The synthetic lethality between *roX1*^{ex40} *roX2*Δ and siRNA mutations suggested that siRNA could contribute to X-identification or to recruitment of the MSL complex to the X chromosome. If this is the case, loss of siRNA alone might disrupt MSL localization, which is exclusive to the X chromosome in wild-type males (Figure 3A). Reduction of D-Elp1 did not discernibly affect MSL1 localization to the polytene X chromosome of otherwise wild-type males (Figure 3B). A slight disruption of X-localization was detected in *ago2* mutants, but this was only marginally higher than that observed in wild-type controls (Figure 3, B, C, and E; Table S1).

Ectopic MSL1 binding on the autosomes at the chromocenter and at the telomeres is a sensitive metric for disruption of MSL localization. Although MSL1 recruitment in *roX1*^{ex40} *roX2*Δ males is superficially similar to wild type, examination of a large number of nuclei revealed a reduction of MSL recruitment to the X chromosome in some nuclei and elevated ectopic localization, particularly at the chromocenter (Figure 3, B and C; Table S1). This supports the idea that

roX1^{ex40} has a defect in function. However, mislocalization of MSL1 was notably more severe in chromosome preparations from *roX1*^{ex40} *roX2*Δ; *ago2*^{414} and *roX1*^{ex40} *roX2*Δ; *D-elp1*^{c00296}/+ males. The number of nuclei exhibiting minimal or no recruitment of MSL1 to the X chromosome is enhanced over threefold by the loss or reduction of these siRNA proteins (Figure 3E). These same genotypes displayed a threefold increase in ectopic autosomal MSL1 localization (Figure 3, D, F, and G; Table S1). Despite increased mislocalization of the MSL complex, *roX1*^{ex40} *roX2*Δ; *D-elp1*^{c00296}/+ male viability appears unaffected, and the viability of *roX1*^{ex40} *roX2*Δ males with reduced levels of Ago2 or Loqs is also high (Figure 2, A and B). It is possible that this disparity is because the accumulation of mutated *roX1* transcripts, including *roX1*^{ex40}, is lower in the salivary gland than in other tissues (Figure S1B; see figure 3 in Deng *et al.* 2005). In spite of reduced transcript in the salivary gland, the *roX1*^{ex40A} mutant directs considerable X-localization of the MSL complex, in accord with the ability of *roX1*^{ex40} *roX2*Δ males to tolerate a partial, but not a complete, reduction in RNAi. Taken together, these studies reveal a role for siRNA in the process of dosage compensation in *Drosophila*. The genetic interaction between mutations affecting siRNA and *roX1 roX2* chromosomes, as well as the enhancement of ectopic MSL mislocalization, suggests that siRNA contributes to X recognition or chromatin binding of the MSL complex.

Small RNA has been implicated in numerous chromatin-based processes, but the present study is the first to link small RNA to *Drosophila* dosage compensation. Small RNA typically acts through gene silencing (Pal-Bhadra *et al.* 2004; Verdel *et al.* 2004; Brower-Toland *et al.* 2007; Wang and Elgin 2011). For example, *Ago2* and *Dcr2* mutations suppress position-effect variegation in flies, suggesting a function in heterochromatic repression (Deshpande *et al.* 2005; Fagegaltier *et al.* 2009). Ago2 and Dcr2 exert a repressive effect on expression of euchromatic genes by modulating transcriptional elongation (Cernilogar *et al.* 2011). In contrast, dosage compensation selectively elevates transcription of a large portion of the fly genome. The siRNA mutations examined in this study dramatically enhance the male-specific lethality of *roX1 roX2* chromosomes and promote delocalization of the MSL complex from the X chromosome. This suggests that siRNA modulates the stability of MSL binding or contributes to recognition of the X chromosome. While evidence that Ago2 or other siRNA factors directly activate gene expression is lacking, a few studies have demonstrated increased silencing at some loci upon loss of Ago2 and Piwi (Yin and Lin 2007; Moshkovich and Lei 2010). It is possible that siRNA influences dosage compensation not through direct action at compensated genes, but by contributing to interphase chromosome architecture or organization of the nucleus. This would be consistent with the role of RNAi at insulators (Lei and Corces 2006; Moshkovich *et al.* 2011). Intriguingly, the male X chromosome displays an interphase conformation distinct from that in females (Grimaud and Becker 2009).

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A Role for siRNA in X-Chromosome Dosage Compensation in *Drosophila melanogaster*

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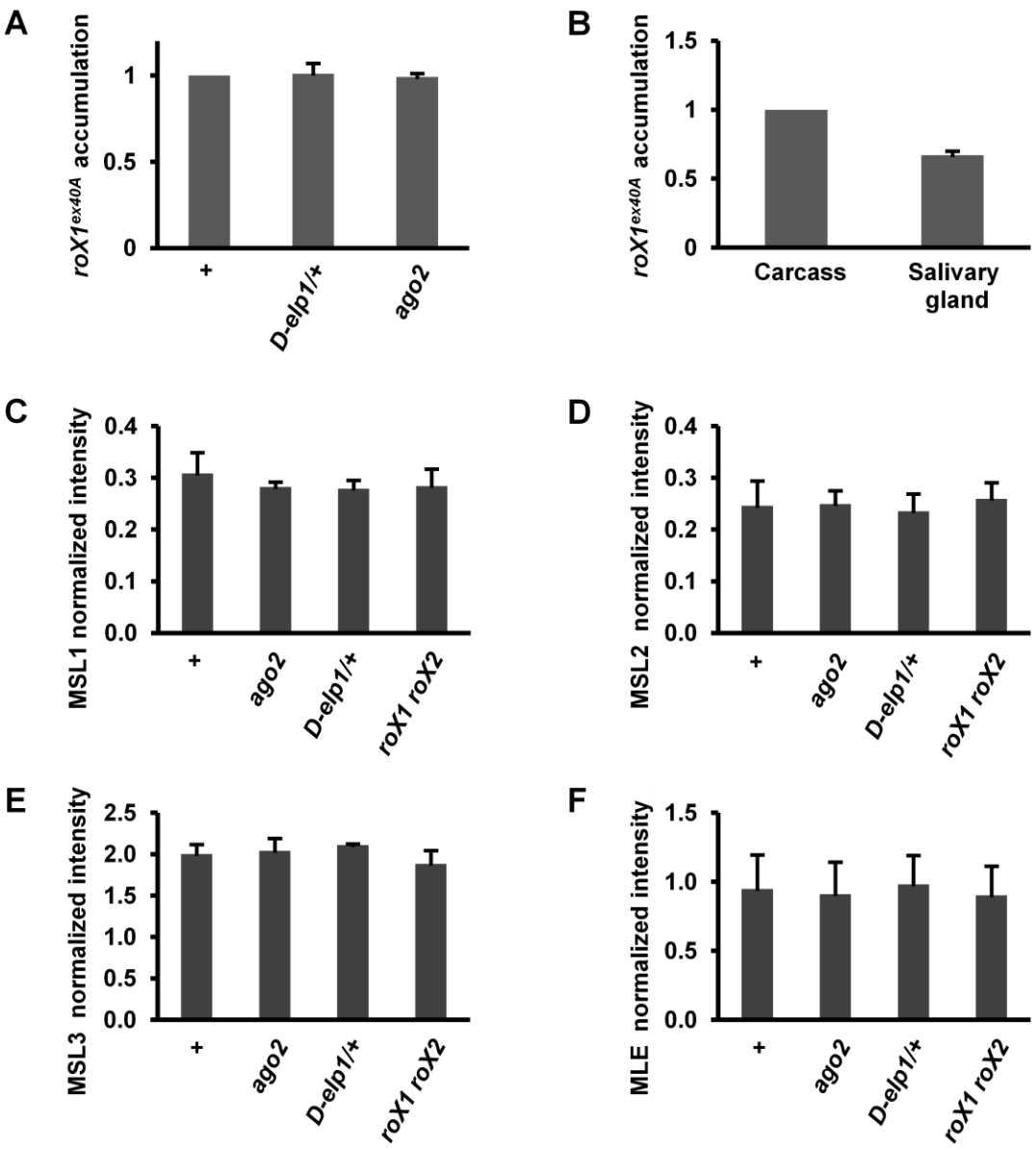

FIGURE S1 Mutation of *ago2* or *D-elp1* does not affect accumulation of molecules in the MSL complex. (A) Accumulation of *roX1*^{ex40} transcript was determined in *roX1*^{ex40} *roX2*Δ (value set to 1), *roX1*^{ex40} *roX2*Δ*; D-elp1*^{c00296}/+ and *roX1*^{ex40} *roX2*Δ*; ago2*^{414} male larvae by quantitative RT-PCR (qRT PCR). (B) Accumulation of *roX1*^{ex40} transcript in salivary glands and carcass (value set to 1) of *roX1*^{ex40} *roX2*Δ male larvae. Expression in A and B is normalized to the autosomal gene *Dmn*. Bars represent the standard error of three biological replicates. (C–F) Quantification of MSL levels from protein blots of (C) MSL1 (n=2), (D) MSL2 (n=4), (E) MSL3 (n=3) and (F) MLE (n=3) in wild type, *ago2*^{414} , *D-elp1*^{c00296}/+ and *roX1*^{ex40A} *roX2*Δ adult males. β-tubulin and d-FMR1 were the loading controls. Quantification was performed by scanning blots and integrating signal density using Image J software (http://rsbweb.nih.gov/ij/). Protein signal was normalized to loading controls. A dilution series established that signal remained within linear range. Protein blotting was performed on extracts from groups of 10 or 20 adult males homogenized in 100 or 200 µl of Laemmli buffer with 1mM PMSF. Homogenates were boiled and centrifuged at 10,000 rpm for 2 min to obtain crude lysates. Equal volumes of lysate were separated on 7% polyacrylamide gels and transferred to nitrocellulose (Micron Separations Inc.). Membranes were blocked with 0.5% fish gelatin and 2-5% BSA in PBST or TBST. Primary and secondary antibodies were diluted in the respective blocking solutions. Primary antibodies to MSL1, MSL2, MSL3 and MLE were a gift from M. Kuroda. Antibodies to β tubulin and dFMR1 are from the Developmental Studies Hybridoma Bank. Alkaline phosphatase conjugated secondary antibodies (Sigma) were used for detection by NBT/BCIP chromogenic system.

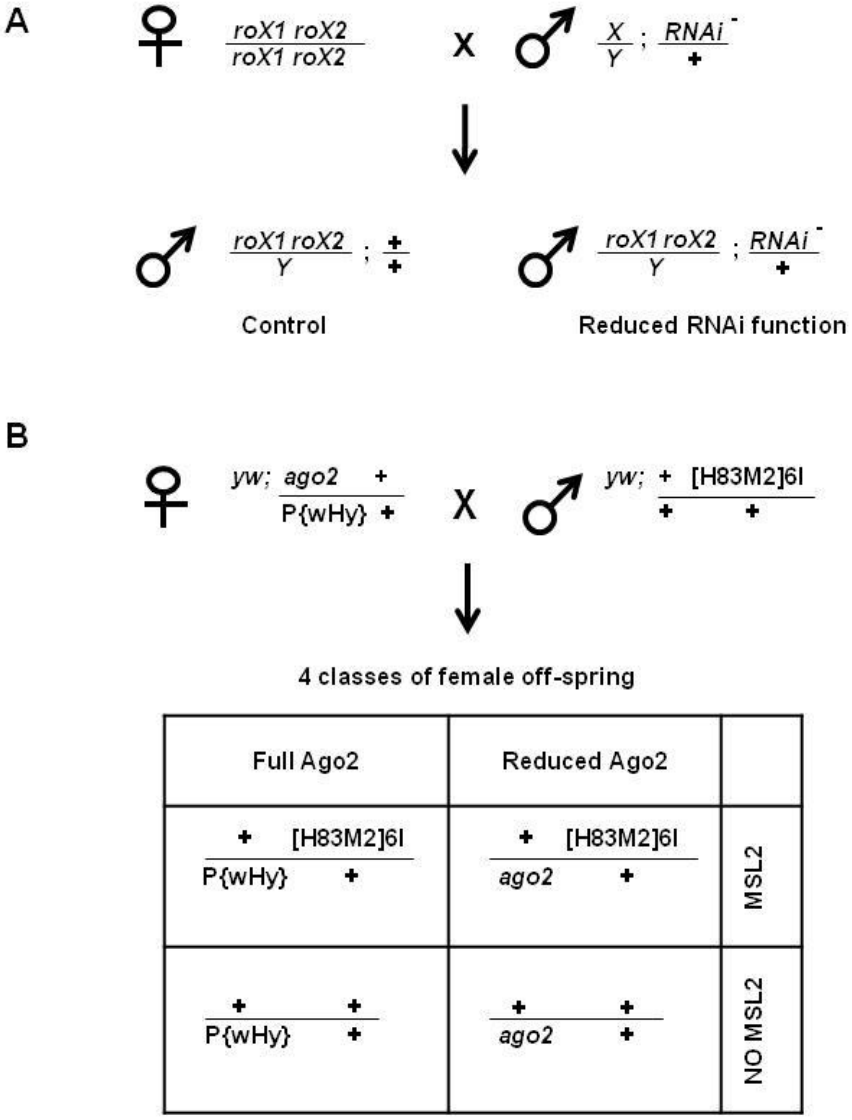

FIGURE S2 Matings to determine effect of RNAi mutations on male and female survival. (A) Screen for genetic interaction between *roX1*^{ex33} *roX2*Δ and RNAi mutants. *roX1*^{ex33} *roX2*Δ females were mated to males heterozygous for RNAi mutations, producing *roX1*^{ex33} *roX2*Δ sons with wild type (control) and reduced RNAi (experimental). (B) Mating performed to determine the effect of reduced Ago2 on female development. Females with a *yw* (wild type) X chromosome and trans-heterozygous for *ago2*^{414} and P{wHy}^{DG23507}, a marker situated 5 kb proximal to *ago2*, were mated to males heterozygous for [H83M2]6I. Equal numbers of the four classes of female zygotes are predicted. Daughters inheriting [H83M2]6I express MSL2, leading to developmental delay (top row); presented in Fig. 1B. Their sisters lacking [H83M2]6I (bottom row) are plotted in Fig. 1C. Daughters with full Ago2 (left) and their sisters with reduced Ago2 (right) were distinguished by *y*$^{+}$, present in P{wHy}.

Table S1 MSL1 recruitment to polytene chromosomes

A

X chromosome MSL1 recruitment	+	*ago2*	*D-elp1/+*	*roX1 roX2*	*roX1 roX2; ago2*	*roX1 roX2; D-elp1/+*
++++	82.66 (177)	56.96 (156)	77.76 (138)	24.13 (81)	11.86 (28)	14.73 (22)
+++	16.33 (26)	34.35 (113)	19.84 (36)	65.90 (184)	63.77 (141)	54.49 (84)
+	0.62 (1)	8.07 (18)	2.4 (5)	7.05 (18)	19.47 (36)	26.96 (42)
No stain	0.39 (1)	0.62 (2)	0 (0)	1.03 (4)	4.91 (11)	3.83 (6)
Total nuclei counted	205	289	179	287	216	154

B

Chromocenter MSL1 recruitment	+	*ago2*	*D-elp1/+*	*roX1 roX2*	*roX1 roX2; ago2*	*roX1 roX2; D-elp1/+*
No recruitment	93.54 (192)	80.22 (223)	88.76 (161)	44.93 (119)	30.21 (56)	24.64 (37)
Recruitment	6.46 (15)	19.78 (59)	11.23 (18)	55.07 (163)	69.79 (153)	75.36 (116)
Total nuclei counted	207	282	179	282	209	153

C

Ectopic MSL1 recruitment	+	*ago2*	*D-elp1/+*	*roX1 roX2*	*roX1 roX2; ago2*	*roX1 roX2; D-elp1/+*
No autosomal recruitment	67.83 (134)	73.36 (194)	55.88 (110)	61.48 (168)	35.92 (67)	52.70 (79)
1-2 autosomal bands	14.70 (59)	18.89 (63)	36.49 (58)	30.15 (89)	24.29 (51)	25.88 (40)
≥4 autosomal bands	2.89 (14)	5.81 (17)	7.63 (11)	8.37 (25)	39.80 (91)	21.43 (34)
telomere recruitment	2.59 (8)	1.95 (8)	3.58 (6)	9.70 (29)	12.48 (37)	31.79 (48)
Total nuclei counted	215	282	185	311	246	201

Scoring of polytene nuclei stained for MSL1 from wild type (+), $ago2^{414}$, $roX1^{ex40}$ *roX2*Δ, $roX1^{ex40}$ *roX2*Δ; $ago2^{414}$/+ and $roX1^{ex40}$ *roX2*Δ; *D-elp1*^{c00296} /+ male larvae. (A) MSL1 recruitment to X chromosome is categorized as ++++ (wild type), +++ (moderate) and + (minor). Examples of ++++ and + are presented in Fig. 3. (B) MSL1 recruitment to the chromocenter. (C) MSL1 recruitment to ectopic autosomal sites and telomeres. The percentage of nuclei in each category is represented, followed by the total number of nuclei in parentheses.