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Molecularly Severe *roX1* Mutations Contribute to Dosage Compensation in *Drosophila*

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Summary

Drosophila melanogaster males maintain a constant ratio of X-linked to autosomal gene products by increasing expression from their single X chromosome. This is achieved through the action of a complex composed of protein and *roX* RNA. This complex binds in the body of genes and increases expression through chromatin modification. The X-linked *roX* genes produce RNAs that are essential but redundant for recognition and modification of the male X chromosome. We report that some molecularly severe *roX1* mutations with no detectable transcript accumulation contribute dramatically to male rescue by autosomal *roX1* transgenes. We propose that this represents genetic complementation between a source of *roX* RNA (the autosomal transgene) and the severely mutated X-linked allele.

Keywords

dosage compensation; *roX* RNA; noncoding RNA; complementation; MSL complex; DCC

INTRODUCTION

Drosophila males have a well-studied epigenetic system that increases expression of X-linked genes to maintain a normal ratio between autosomal and X-encoded gene products. Modulation of transcription is accomplished by the Male Specific Lethal (MSL) complex, composed of protein and RNA. A central feature of this system is the ability to selectively modify chromatin at hundreds of X-linked genes. The MSL complex is targeted to transcribed regions of the X chromosome by a combination of X-linked identity elements and co-transcriptional binding (Alekseyenko *et al.*, 2008; Dahlsveen *et al.*, 2006; Gilfillan *et al.*, 2006; Larschan *et al.*, 2007).

The large, noncoding *roX1* and *roX2* RNAs (*RNA on the X*) are essential components of the MSL complex. *roX1* and *roX2* are strikingly male-preferential in expression and redundant in function. *roX* transcripts assemble with the MSL proteins and are required for recognition of the X chromosome. Polytene preparations from *roX1 roX2* males show reduced MSL binding on the X chromosome and ectopic binding at autosomal sites (Meller and Rattner, 2002). A direct relationship is observed between the recovery of male *roX1 roX2* adult

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escapers and the amount of MSL protein remaining on the X chromosome (Deng *et al.*, 2005). *roX*RNA originating from an autosome enables the MSL complex to coat the X chromosome and rescue *roX1 roX2* males (Meller and Rattner, 2002; Meller *et al.*, 1997). *roX*RNA can therefore function from a site of transcription *in trans* to the X chromosome. However, both *roX* genes are X-linked and capable of directing MSL binding and gene regulation in chromatin flanking an autosomal *roX* insertion (Henry *et al.*, 2001; Kelley and Kuroda, 2003; Kelley *et al.*, 1999). The situation of the *roX* genes on the X chromosome is thus expected to contribute to X chromosome recognition. Many studies of this aspect MSL localization have relied on *roX* genes capable of producing functional RNA (Oh *et al.*, 2003; Park *et al.*, 2002). However, there is also evidence that recruitment does not require a functional *roX* gene (Kageyama *et al.*, 2001).

We observed that the rescue of *roX1 roX2* males by autosomal *roX* transgenes depends on the *roX1 roX2* chromosome being rescued. *roX2* is completely deleted from all *roX1 roX2* chromosomes. Although no complete and viable deletions of *roX1* exist, several partially deleted or rearranged *roX1* alleles with no detectable transcript production are in common use. To determine whether *roX1* alleles on the X chromosome influence male rescue by autosomal *roX* transgenes, we measured the survival of males carrying different *roX1 roX2* chromosomes and *roX1* transgenes. We find that the level of rescue is highly dependent on the *roX1* allele present on the X chromosome. *roX1* alleles with little capacity for RNA production have a dramatic influence on male rescue by autosomal *roX1* transgenes and contribute to detectable differences in localization of the MSL proteins. This suggests functional complementation between X-linked *roX1* alleles and autosomal *roX1* transgenes. The influence of *roX1 roX2* chromosomes is most pronounced when rescue by mutated *roX1* transgenes is measured. This is of practical importance as the activity of *roX* transgenes is almost always tested in *roX1^{ex6}roX2* males, which carry an X chromosome that contributes significantly to male rescue (Meller and Rattner, 2002; Park *et al.*, 2007; Stuckenholtz *et al.*, 2003).

RESULTS AND DISCUSSION

Examination of *roX1* mutations and transgenes has identified two regions essential for full function, shown as gray boxes below the gene model in Figure 1a (Stuckenholtz *et al.*, 2003). Deletions removing large portions of the 5' region prevent exclusive recognition of the X chromosome (Deng *et al.*, 2005). This activity is diffusely spread over ~1 kb and may contain redundant elements. A 3' stem loop and flanking repetitive elements are also necessary for full function (Stuckenholtz *et al.*, 2003). Transgenes mutated for the 3' end direct considerable MSL protein to the X chromosome but fail to correctly modify chromatin and only partially rescue *roX1 roX2* males (Kelley *et al.*, in press; Park *et al.*, 2008).

The *roX1* alleles tested in this study are presented in Figure 1a. No *roX1* transcripts are detected in *roX1^{ex6}* or *roX1^{ex84A}* embryos or larvae by RNA blotting or quantitative reverse transcription PCR (qRT PCR), but a transcription start site at -46, representing a minor class of ESTs, is retained in *roX1^{ex6}* (Deng *et al.*, 2005). *roX1^{SMC40A}* lacks 94% of the *roX1* gene, including the entire 3' end, but also retains this transcription start site. *roX1^{SMC17A}* is

genetically the most severe *roX1* mutation available, possibly due to insertion of LacZ and an SV40 poly(A)⁺ site between remaining 3' and 5' sequences. Although *roX1^{SMC17A}* retains a transcription start site at -46 and some *roX1* sequence, adult male escapers are rarely observed (Deng *et al.*, 2005). We previously determined that whole mount in situ hybridization to *roX1* was a more sensitive indicator of transcript accumulation than qRT PCR (Deng *et al.*, 2005). Males carrying *roX1^{ex6}* were compared to wild type males and females using this technique. Consistent with previous observations in our laboratory, a low level of *roX1* is present in the female central nervous system (Fig. 2c). Although there is no known function of *roX1* in females, the staining pattern suggests expression in mitotically active neuroblasts and cells of the developing optic lobe. In contrast, no *roX1* is detected in the nervous system, imaginal discs or salivary glands (not shown) of *roX1^{ex6}* males (Fig. 2e,f). This suggests that either the promoter at -46 is inactive in larvae, or that *roX1^{ex6}* transcript is unstable or produced at such low levels as to be undetectable by in situ hybridization or qRT PCR (Deng *et al.*, 2005).

The *roX1* transgenes tested are presented in Figure 1b. [Hs83-*roX1⁺*], driven by the strong Hs83 promoter, fully rescues MSL localization in *roX1 roX2* mutants. An identical transgene lacking bases 283–2669 ([Hs83-*roX1^{ex7B}*]), and a 4.9 kb genomic fragment containing *roX1* driven by the authentic promoter ([GM-*roX1*]) were also tested (Kelley *et al.*, 1999). Hs83-driven *roX1* cDNAs deleted for the 3' stem loop, [Hs83-*roX1^{OL}*] and [Hs83-*roX1¹⁰*], have been described (Stuckenholtz *et al.*, 2003).

As *roX1* and *roX2* are redundant, transgenes were tested in flies null for *roX2* (Meller and Rattner, 2002). Because male survival is sensitive to environmental conditions that can vary between vials, large numbers of off-spring from each cross were scored. Rescue of *roX1 roX2* males by [Hs83-*roX1⁺*] is high, and rescue by [GM-*roX1*] is intermediate (Table 1). These transgenes retain all essential *roX1* sequences, but [Hs83-*roX1⁺*] is driven by a stronger promoter. Although all *roX1 roX2* X chromosomes are rescued, those carrying *roX1^{ex6}* and *roX1^{SMC40A}* support higher male survival. For example, recovery of *roX1^{ex84A} roX2*;[GM-*roX1*] males is 40% lower than *roX1^{ex6} roX2*;[GM-*roX1*]. We conclude that even fully functional *roX1* transgenes benefit from weak *roX1* alleles on the X chromosome. *roX1^{SMC17A}* retains a transcription start site, as well as all 3' and some 5' *roX1* sequence. A valid concern is that this rearrangement produces antimorphic transcripts that inactivate the MSL complex or compete with *roX1* from transgenes, leading to consistently poor rescue of *roX1^{SMC17A} roX2*. If *roX1^{SMC17A}* is antimorphic, rescue should be especially low for the [GM-*roX1*] transgene, driven by a weak promoter. In contrast, *roX1^{ex84A}* lacks all transcription start sites and makes no detectable transcript. [GM-*roX1*] rescues both chromosomes similarly, but slightly more *roX1^{SMC17A} roX2*;[GM-*roX1*] males are recovered (Table 1). We conclude that *roX1^{SMC17A}* is a severe hypomorph or null for *roX* function.

In spite of deletion of the 3' stem-loop, *roX1^{OL}* partially rescues all *roX1 roX2* chromosomes (Table 1). Rescue of *roX1^{SMC17A} roX2* is lowest, yielding a quarter as many males as *roX1^{ex6} roX2* (Table 1). The *roX1^{OL}* transcript rescues salivary gland development and binds to a limited number of nuclear sites when expressed in *roX1^{SMC17A} roX2* males, but the pattern of localization differs from that observed in males that carry a wild type *roX1*

gene (see Fig. 3). Rather than binding to a single domain, *roX1^{OL}* accumulates at multiple sites (Fig. 3c). In polytene preparations from wild type males, *roX1* is exclusive to the X chromosome (Fig. 3d). Although heavy accumulation of *roX1^{OL}* transcript is observed over the X chromosome of *roX1^{SMC17A}roX2*;[Hs83-*roX1^{OL}*] males, ectopic binding of the transcript is also evident (Fig. 3e). Consistent with the idea that *roX1^{OL}* assembles with MSL proteins but is deficient in directing their localization, binding of MSL1 to the X chromosome of *roX1^{SMC17A}roX2*;[Hs83-*roX1^{OL}*] males is clear, but substantial autosomal accumulation is also detected (Fig. 4f). However, the same *roX1^{OL}* transgene achieves higher male rescue and higher X chromosome targeting of MSL1 when expressed in *roX1^{ex6A}roX2* males (Table 1, Fig. 4d). The exclusivity of X chromosome binding is therefore influenced by the *roX1* allele remaining on the X chromosome. The *roX1¹⁰* transgene is similar to *roX1^{OL}* but lacks additional sequence adjacent to the stem loop. *roX1¹⁰* has weaker rescue but displays a similar pattern of complementation. Greater disparity between *roX1^{ex6}roX2*;[Hs83-*roX1¹⁰*] (18% survival), and *roX1^{SMC17A}roX2*;[Hs83-*roX1¹⁰*] (2.4% survival) is observed with this transgene.

roX1^{ex7B} has an intact 3' stem loop but only 236 bp of 5' *roX1* sequence. [Hs83-*roX1^{ex7B}*] rescues weakly but maintains a similar pattern of complementation, with *roX1^{ex6}roX2* males surviving in greater numbers. In no instance does survival exceed 15%, but rescue of the *roX1^{ex6}roX2* chromosome is almost nine-fold higher than rescue of the *roX1^{SMC17A}roX2* chromosome.

The apparent activity attributable to *roX1^{ex6}*, *roX1^{SMC40A}*, and *roX1^{ex84A}* alleles is surprising given the molecular severity of these mutations. Although they contribute to recognition of the X chromosome, they do so with little ability to produce functional transcripts. Furthermore, these *roX1* alleles have little ability to support male survival on their own (Table 1, right column). We hypothesize that the situation of these alleles on the X chromosome is an important factor. For example, transcription from the *roX1* locus may contribute to X recognition even if the transcript produced is unstable or nonfunctional, as may be the situation with the *roX1^{ex6}* and *roX1^{SMC40A}* alleles. This would support a model of X chromosome recognition that involves co-transcriptional recruitment of chromatin to a nuclear region, such as a nuclear pore, where loading of the MSL complex can occur (Akhtar and Gasser, 2007; Deng and Meller, 2006). However, a functional RNA is also required for effective MSL complex recognition of the X chromosome. Since the influence of X-linked *roX* alleles is most apparent when a transgene supplies *roX*RNA, we propose that this represents a functional complementation between the *roX1* allele on the X chromosome and RNA supplied from the *roX1* transgene.

The contribution of X-linked *roX1* alleles to male survival is most dramatic when deleted transgenes, such as [Hs83-*roX1^{ex7B}*] are tested. As *roX1^{ex6}roX2* is most frequently used to assay the activity of mutated *roX* transgenes, studies relying on this chromosome should be viewed with caution.

METHODS

Flies were maintained at 25° on standard cornmeal-agar fly food in a humidified incubator. The *roX1^{ex6}*, *roX1^{SMC17A}*, and *roX1^{ex84}* mutations have been described (Deng *et al.*, 2005; Meller and Rattner, 2002; Meller *et al.*, 1997). *roX1^{SMC40A}* is an imprecise excision removing 3.6 kb of the *roX1* gene. Elimination of *roX2* is accomplished by combining a lethal deletion removing *roX2* and essential flanking genes, Df(1)52, with a cosmid insertion carrying essential deleted genes but lacking *roX2* [*w⁺4* 4.3]; Meller and Rattner, 2002). For convenience this combination is referred to as *roX2*. The [Hs83-*roX1⁺*] and [Hs83-*roX1^{ex7B}*] transgenes carry *roX1* genomic sequence from 47 to 4164 driven by an Hsp83 promoter (numbering from (Amrein and Axel, 1997). [Hs83-*roX1^{ex7B}*] carries a 2.4 kb deletion identical to the *roX1^{ex7B}* mutation (Deng *et al.*, 2005).

In situ hybridization to dissected tissues was performed as previously described (Deng *et al.*, 2005). In situ hybridization to chromosome squashes used a modified protocol with baked slides and cover slips and RNase-free reagents. Salivary glands were dissected in Ringers and fixed 40 s in PBST with 4% formaldehyde. Glands were then fixed 2 min in 50% acetic acid with 4% formaldehyde, moved to a solution of 50% acetic acid, 17% lactic acid, 4% formaldehyde, squashed and then flattened overnight beneath a weight. Preparations were frozen in liquid N₂, cover slips removed and then plunged into 95% ethanol. Rehydration in 70% and 30% ethanol and a PBST wash was followed by a 15 min fixation in PBST with 4% formaldehyde. Slides were washed 3 times in PBST, digested with 20 µg/ml Proteinase K for 4 min, rinsed twice quickly with 2 mg/ml glycine, and twice with PBST before a 10 min fixation in 4% formaldehyde in PBST. Slides were rinsed 3 times in PBST and pre-hybridized for 3 h at 44°C. They were hybridized overnight with a 1:100 dilution of DIG-labeled antisense *roX1* probe. Slides were washed 40 min in 50% formamide, 5× SSC at 44°C, rinsed in 2× SSC at 44°C and washed at room temperature in 1× SSC in PBST. Slides were blocked 2 h at room temperature with 5 mg/ml BSA in PBST and incubated 3 h in a 1:1,000 dilution of preabsorbed anti-DIG antibody conjugated to alkaline phosphatase (Roche). Five washes in PBST and two washes in alkaline phosphatase buffer were done before developing the color. DNA was visualized by Hoechst counterstaining.

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LITERATURE CITED

- Akhtar A, Gasser SM. The nuclear envelope and transcriptional control. *Nat Rev Genet.* 2007; 8:507–517. [PubMed: 17549064]
- Alekseyenko AA, Peng S, Larschan E, Gorchakov AA, Lee O-K, Karchenko P, McGrath SD, Wang CI, Mardis ER, Park PJ, Kuroda MI. A sequence motif within chromatin entry sites directs MSL establishment on the *Drosophila* X chromosome. *Cell.* 2008; 134:599–609. [PubMed: 18724933]
- Amrein H, Axel R. Genes expressed in neurons of adult male *Drosophila*. *Cell.* 1997; 88:459–469. [PubMed: 9038337]

- Dahlsveen IK, Gilfillan GD, Shelest VI, Lamm R, Becker PB. Targeting determinants of dosage compensation in *Drosophila*. PLoS Genet. 2006; 2:e5. [PubMed: 16462942]
- Deng X, Meller VH. Noncoding RNA in *Drosophila* dosage compensation. Trends Biochem Sci. 2006; 31:526–532. [PubMed: 16890440]
- Deng X, Rattner BP, Souter S, Meller VH. The severity of *roX1* mutations are predicted by MSL localization on the X chromosome. Mech Dev. 2005; 122:1094–1105. [PubMed: 16125915]
- Gilfillan GD, Straub T, de Wit E, Greil F, Lamm R, van Steensel B, Becker PB. Chromosome-wide gene-specific targeting of the *Drosophila* dosage compensation complex. GenesDev. 2006; 20:858–870.
- Henry RA, Tews B, Li X, Scott MJ. Recruitment of the 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. 2001; 276:31953–31958. [PubMed: 11402038]
- Kageyama Y, Mengus G, Gilfillan G, Kennedy HG, Stuckenholtz C, Kelley RL, Becker PB, Kuroda MI. Association and spreading of the *Drosophila* dosage compensation complex from a discrete *roX1* chromatin entry site. Embo J. 2001; 20:2236–2245. [PubMed: 11331589]
- Kelley RL, Kuroda MI. The *Drosophila roX1* RNA gene can overcome silent chromatin by recruiting the Male-Specific Lethal dosage compensation complex. Genetics. 2003; 164:565–574. [PubMed: 12807777]
- Kelley RL, Lee O-K, Shim Y-K. Transcription rate of noncoding *roX1* RNA controls local spreading of the *Drosophila* MSL chromatin remodeling complex. Mech Dev. in press.
- Kelley RL, Meller VH, Gordadze PR, Roman G, Davis RL, Kuroda MI. Epigenetic spreading of the *Drosophila* dosage compensation complex from *roX* RNA genes into flanking chromatin. Cell. 1999; 98:513–522. [PubMed: 10481915]
- Larschan E, Alekseyenko AA, Gortchakov AA, Peng S, Li B, Yang P, Workman JL, Park PJ, Kuroda MI. MSL complex is attracted to genes marked by H3K36 trimethylation using a sequence-independent mechanism. Mol Cell. 2007; 28:121–133. [PubMed: 17936709]
- Meller VH, Rattner BP. The *roX* genes encode redundant male-specific lethal transcripts required for targeting of the MSL complex. EMBO J. 2002; 21:1084–1091. [PubMed: 11867536]
- Meller VH, Wu KH, Roman G, Kuroda MI, Davis RL. *roX1* RNA paints the X chromosome of male *Drosophila* and is regulated by the dosage compensation system. Cell. 1997; 88:445–457. [PubMed: 9038336]
- Oh H, Park Y, Kuroda MI. Local spreading of MSL complexes from *roX* genes on the *Drosophila* X chromosome. Genes Dev. 2003; 17:1334–1339. [PubMed: 12782651]
- Park S-W, Kang YI, Sypula JG, Choi J, Oh H, Park Y. An evolutionarily conserved domain of *roX2* RNA is sufficient for induction of H4-Lys16 acetylation on the *Drosophila* X chromosome. Genetics. 2007; 177:1429–1437. [PubMed: 18039876]
- Park S-W, Kuroda MI, Park Y. Regulation of histone H4 Lys16 acetylation by predicted alternative secondary structures in *roX* non-coding RNAs. Mol Cell Biol. 2008; 28:4952–4962. [PubMed: 18541664]
- Park Y, Kelley RL, Oh H, Kuroda MI, Meller VH. Extent of chromatin spreading determined by *roX* RNA recruitment of MSL proteins. Science. 2002; 298:1620–1623. [PubMed: 12446910]
- Stuckenholtz C, Meller VH, Kuroda MI. Functional redundancy within *roX1*, a noncoding RNA involved in dosage compensation in *D. melanogaster*. Genetics. 2003; 164:1003–1014. [PubMed: 12871910]

FIG. 1.

roX1 mutations and transgenes used in complementation studies. **(a)** *roX1* mutations used in this study. *roX1* and the 3' end of the upstream *opt1* gene are represented by heavy gray arrows. Major *roX1* transcription start sites at 259 and 628 are illustrated by arrows above the gene. A minor transcription start site at -46 is also shown. The 5' and 3' *roX1* regions known to contribute to RNA function are represented by gray boxes below the gene model. Vertical dashed lines correspond to position 1 of a published *roX1* sequence and a poly(A)⁺ site at 3782 (Amrein and Axel, 1997; Meller *et al.*, 1997). *roX1^{ex6}* is deleted for bp 25–1472. *roX1^{SMC40A}* is deleted for bp 224 to 3800. *roX1^{ex84A}* is deleted for bp -170 to 2157. In *roX1^{SMC17A}* bp 203–2362 is replaced by a full length LacZ gene, SV40 poly(A)⁺ site and 3' P-element end (Deng *et al.*, 2005). **(b)** *roX1* transgenes used in this study. The Hs83

promoter is represented by a shaded box. [Hs83-*roXI*⁺] carries genomic *roXI* from 47–4164. GM-*roXI* is driven by the endogenous *roXI* promoter (Kelley *et al.*, 1999). [Hs83-*roXI*^{OL}] and [Hs83-*roXI*¹⁰] have been described (Stuckenholtz *et al.*, 2003). [Hs83-*roXI*^{ex7B}] is identical to [Hs83-*roXI*⁺], but deleted for bp 283–2669.

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FIG. 2. *roX1* transcript is undetectable in *roX1^{ex6}* male tissues. In situ hybridization using an anti-sense *roX1* probe detects *roX1* accumulation in the central nervous system (CNS; **a**) and eye antenna imaginal disc (**b**) of wild type male. Some staining is observed in the CNS (**c**) of a wild type female. Mitotic cells in the developing optic lobe (arrow-heads) are particularly strongly stained. No stain is detected in the eye antenna imaginal disc of a female (**d**). No stain is observed in the CNS (**e**) and eye antenna imaginal disc (**f**) of a *roX1^{ex6}* male. All tissues were processed in parallel and developed for 20 min.

FIG. 3. *roX1*^{OL} transcript partially rescues *roX1*^{SMC17A}*roX2* males but displays ectopic localization. In situ hybridization detects *roX1* accumulation. (a) Salivary gland of a wild type male. (b) Salivary gland from a *roX1*^{SMC17A}*roX2* male. (c) Salivary gland from a *roX1*^{SMC17A}*roX2*[Hs83-*roX1*^{OL}] male. All tissues were hybridized and developed under identical conditions and photographed using a ×40 objective. (d) In situ hybridization to a polytene preparation from a wild type male displays exclusive X chromosome localization

of *roX1*. (e) Polytene preparation from a *roX1^{SMC17A}roX2*;[Hs83-*roX1^{OL}*] male displays *roX1^{OL}* binding to the X chromosome as well as ectopic localization (arrowheads).

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FIG. 4.

Rescue of MSL1 localization is affected by the *roX1* allele on the X chromosome. Males carrying *roX1^{ex6}roX2* (a–d) and *roX1^{SMC17A}roX2* (e,f) are rescued by the fully functional [Hs83-*roX1*⁺] transgene (a,b) or by the partially functional [Hs83-*roX1*^{-OL}] transgene (c–f). The X chromosome is indicated by arrowheads in panels showing Hoechst counterstaining of DNA (a,c, and e). Chromosomes are probed with MSL1 antibodies detected by a Texas Red conjugated secondary antibody (Kelley *et al.*, 1999). Full genotypes

are: $roXI^{ex6}Df(1)52; [w^{+4} \ 4.3]; [Hs83-roXI^{+}]$ (a–b). $roXI^{ex6}Df(1)52; [w^{+4} \ 4.3]; [Hs83-roXI^{OL}]CSF67$ (c–d) and $roXI^{SMC17A}Df(1)52; [w^{+4} \ 4.3]; [Hs83-roXI^{OL}]CSF67$ (e–f).

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Table 1

Survival of roX1 roX2 Males Carrying roX1 Transgenes

Female parent	Male parent	Female progeny ^a	roX1 roX2 Male progeny	
			[w ⁺ roX1]/+	+/+
ywroX1 ^{ex6} roX2	Binsincy/Y;[Hs83-roX1 ⁺]/+	100 (1171)	83.0 (486)	3.8 (22)
wroX1 ^{SMC40A} roX2		100 (883)	87.2 (385)	1.6 (7)
ywroX1 ^{ex84A} roX2		100 (1252)	72.8 * (456)	0 (0)
wroX1 ^{SMC17A} roX2/Binsincy		100 (1191)	66.0 *** (393)	0 (0)
ywroX1 ^{ex6} roX2	Binsincy/Y;[GM-roX1-93E]/+	100 (735)	65.3 (240)	5.7 (21)
wroX1 ^{SMC40A} roX2		100 (596)	58.1 (173)	0.7 (2)
ywroX1 ^{ex84A} roX2		100 (1085)	38.7 *** (210)	0 (0)
wroX1 ^{SMC17A} roX2/Binsincy		100 (1168)	47.8 *** (279)	0 (0)
ywroX1 ^{ex6} roX2	Binsincy/Y;[Hs83-roX1 ^{OL}]/+	100 (773)	54.3 (210)	7.7 (30)
wroX1 ^{SMC40A} roX2		100 (1158)	52.8 (306)	2.6 (15)
ywroX1 ^{ex84A} roX2		100 (1400)	37.4 *** (262)	2.1 (15)
wroX1 ^{SMC17A} roX2/Binsincy		100 (1117)	14.0 *** (78)	0 (0)
ywroX1 ^{ex6} roX2	Binsincy/Y;[Hs83-roX1 ¹⁰]/+	100 (963)	17.9 (86)	3.3 (16)
wroX1 ^{SMC40A} roX2		100 (525)	13.3 (35)	2.7 (7)
ywroX1 ^{ex84A} roX2		100 (595)	11.8 * (35)	2.0 (6)
wroX1 ^{SMC17A} roX2/Binsincy		100 (1311)	2.4 *** (16)	0 (0)
ywroX1 ^{ex6} roX2	Binsincy/Y;[Hs83-roX1 ^{ex7B}]/+	100 (1171)	15.2 (89)	1.4 (8)
wroX1 ^{SMC40A} roX2		100 (896)	13.2 (59)	0.4 (2)
ywroX1 ^{ex84A} roX2		100 (999)	6.4 *** (32)	0 (0)
wroX1 ^{SMC17A} roX2/Binsincy		100 (1304)	1.7 *** (11)	0 (0)
ywroX1 ^{ex84A} roX2/Binsincy ²	yw/Y;[Hs83-roX1 ^{ex7B}]	100 (634)	6.0 (38)	–
ywroX1 ^{ex84A} roX2/Binsincy ²	yw/Y	100 (1102)	–	0.36 (4)

Females carrying roX1 roX2 chromosomes were mated to males carrying autosomal roX1 transgenes. The presence of the transgene in offspring is detected by the w⁺ marker. The recovery of males (%) is based on the number of female progeny.

^aIn tests of roX1^{SMC17A}roX2 survival, only female progeny carrying the roX1 roX2 X chromosome were counted. Rescue of ywroX1^{ex84A}roX2 males from mothers heterozygous for the Binsincy balancer was determined by crossing females to homozygous yw/Y;[Hs83-roX1^{ex7B}] males or to control (yw) males. Rescue from heterozygous mothers was similar to that from homozygous ywroX1^{ex84A}roX2 mothers. XO males resulting from maternal non-disjunction are not included. The number of individuals of each genotype is given in parentheses.

A chi-squared test was performed to determine whether the survival of roX1^{ex6}roX2 males is significantly different from that of other roX1 roX2 males in the presence of a roX1 transgene.

Significance level is indicated by * ($P < 0.05$), ** ($P < 0.01$), and *** ($P < 0.001$).