Fine-Scale Analysis of X Chromosome Inactivation in the Male Germ Line of Drosophila melanogaster

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Abstract

Inactivation of the X chromosome in the male germ line has been suggested to contribute to the excess of gene movement off the X chromosome and the paucity of X-linked male-biased genes that have been observed in Drosophila species. Recent experimental work has demonstrated the transcriptional inactivation of the X chromosome during spermatogenesis, but it is not known if some regions of the X escape inactivation. To test this, we analyzed the expression of 112 precisely-mapped, testis-specific reporter gene insertions along the X chromosome. All of the reporter gene insertions showed low levels of expression that were significantly less than those of autosomal insertions, suggesting that the X chromosome is globally inactivated in the male germ line. There was no evidence for regions of the X chromosome escaping inactivation, including cytological region 19, which appears to be a hot spot for newly evolved, testis-expressed genes.

Key words: gene expression, X chromosome, spermatogenesis, MSCI.

Main Text

In the early 1970s, it was proposed that the X chromosome is transcriptionally inactivated during spermatogenesis in species with heterogametic (XY) males (Lifschytz and Lindsley 1972). This hypothesis was put forth to explain the observation that Drosophila melanogaster males with X-autosome translocations are sterile, whereas those with autosome-autosome translocations are fertile. More recently, inactivation of the X chromosome in the male germ line (also referred to as meiotic sex chromosome inactivation or MSCI) has been suggested to contribute to observed patterns of Drosophila genome organization, including the excess of retrotransposed genes that have moved from the X chromosome to the autosomes (Betrán et al. 2002) and the paucity of genes with male-biased expression on the X chromosome (Paris et al. 2003; Ranz et al. 2003). Molecular genetic studies have produced conflicting results regarding the existence of MSCI in Drosophila (Mckee and Handel 1993; Hoyle et al. 1995; Rastelli and Kuroda 1998; Sturgill et al. 2007). However, two recent studies using transgenic reporter genes (Hense et al. 2007) and microarray analysis of dissected testes (Vibranovski et al. 2009, 2010) provided compelling evidence for MSCI in the male germ line of D. melanogaster. These studies, however, could not exclude the possibility that some regions of the X chromosome escape MSCI and continue to be expressed throughout spermatogenesis. For example, it has been proposed that the region around cytological band 19, which appears to be a hot spot for new gene evolution, may escape inactivation (Chen et al. 2007). This region shows a general enrichment of testis-expressed genes (Boutanaev et al. 2002), including the newly evolved genes Sdic, CG15323, and hydra (Nurminsky et al. 1998; Levine et al. 2006; Chen et al. 2007). The orthologous region in D. yakuba also appears to be a hot spot for de novo gene evolution (Begun et al. 2007). To test for regions of the X chromosome that escape MSCI, we used the approach of Hense et al. (2007) to generate a large number of independent insertions of a testis-specific gene construct on the D. melanogaster X chromosome and create a fine-scale map of X chromosome inactivation in the male germ line.

We used genetic crosses to a transposase-expressing stock to produce 107 new X-chromosomal insertions of the P[wFl-ocn-lacZ] reporter gene construct, which contains the promoter of the D. melanogaster testis-specific ocnus gene fused to the lacZ gene of Escherichia coli (Hense et al. 2007). The precise chromosomal location of each insertion was determined by inverse polymerase chain reaction (PCR) (Bellen et al. 2004). Reporter gene expression of the 107 mapped insertions, plus five insertions previously mapped by Hense et al. (2007), was measured by a β-galactosidase enzymatic assay (fig. 1). For comparison, β-galactosidase activity of 22 independent autosomal insertions of the same construct (15 from Hense et al. (2007) and seven newly generated in this study) was determined in flies heterozygous for the insertion (i.e., transgene copy number was the same for X-linked and autosomal insertions). Overall, we detected low (but significantly nonzero; one sample t-test, $P < 0.0001$) expression of the X-linked transgenes in males, which, as expected, was significantly greater than that detected in females (Mann–Whitney–Wilcoxon test [MWW], $P < 0.0001$). There was some variation in male expression among transgenes inserted at different locations, but all of the X-linked transgenes showed much lower expression than the autosomal transgenes (fig. 1). The difference in expression between X-linked and autosomal transgenes was highly significant (MWW, $P < 0.0001$). Thus, we find no evidence for any region of the X chromosome escaping X inactivation in the male germ line.
Some of the variation in expression among X-linked transgenes may be explained by the local context into which they inserted. In particular, X-linked transgenes inserted into intergenic regions tended to have higher expression than those inserted into parts of transcriptional units, including the 5′ untranslated region (UTR), exons, and introns (table 1). However, there was no significant difference in transgene expression among any of these regions (MWW, \( P > 0.09 \) for all comparisons). The four transgenes with the highest expression were spread across the X chromosome (at positions 6.76, 8.28, 16.73, and 19.25 Mb), with two located in intergenic regions and two located in 5′ UTRs. The insertion at 16.73 Mb lies \( \sim 500 \) bp upstream of the gene CG13004, which shows male-biased expression according to the Sebida database (Gnad and Parsch 2006) and testis-enriched expression according to FlyAtlas (Chintapalli et al. 2007). However, none of the other three insertions was within \( 10 \) kb of a male-biased or testis-expressed gene. Overall, the observed variation in expression among X-linked insertions is unlikely to represent variation in X chromosome inactivation, as the coefficient of variation for X-linked insertions (13.2%) was less than that for autosomal insertions (18.5%).

Previous work indicated that there was a good concordance between transgene expression measured as protein abundance (β-galactosidase enzymatic activity) and mRNA abundance measured by quantitative reverse transcription–PCR (qRT–PCR) (Hense et al. 2007). To confirm this, we used qRT–PCR to measure transcript abundance of seven X-linked and seven autosomal transgenes. A significantly positive correlation between protein and mRNA abundance was observed (fig. 2), and there was significantly less transgene mRNA present in flies with X-linked insertions (MWW; \( P = 0.016 \)), indicating that the enzymatic assays accurately reflect transcript abundance.

**Table 1.** Comparison of X-Linked and Autosomal Insertion Sites.

<table>
<thead>
<tr>
<th>Location</th>
<th>X-Linked Insertions</th>
<th>X-Linked Expression*</th>
<th>Autosomal Insertions</th>
<th>Autosomal Expression*</th>
</tr>
</thead>
<tbody>
<tr>
<td>5′ UTR</td>
<td>65</td>
<td>2.34</td>
<td>9</td>
<td>9.77</td>
</tr>
<tr>
<td>Exon</td>
<td>6</td>
<td>2.36</td>
<td>1</td>
<td>9.15</td>
</tr>
<tr>
<td>Intron</td>
<td>12</td>
<td>2.18</td>
<td>1</td>
<td>9.54</td>
</tr>
<tr>
<td>Intergenic</td>
<td>29</td>
<td>2.52</td>
<td>9</td>
<td>8.36</td>
</tr>
<tr>
<td>Unknown</td>
<td>0</td>
<td>—</td>
<td>2</td>
<td>7.57</td>
</tr>
<tr>
<td>Total</td>
<td>112</td>
<td>2.37</td>
<td>22</td>
<td>8.96</td>
</tr>
</tbody>
</table>

* Measured as mean units of β-galactosidase activity.
The proposed hot spot for new gene evolution at cytological band 19 lies between nucleotide positions 19.8 and 21.2 Mb of the X chromosome (FlyBase release 5.30; Tweedie et al. 2009). Four of our transgene insertions fall within this interval, including an insertion at position 20,915,774 that is ~1 kb away from 3' end of the gene Sdc1. The expression of this transgene, and of the three other insertions in region 19, was not higher than the average of all X-linked transgenes (fig. 1). Thus, we conclude that escape from X chromosome inactivation is not a general property of this genomic region and cannot explain why it is enriched for newly evolved, testis-expressed genes. It is possible that some of the genes in this region are expressed in somatic cells of the testis and, thus, are not subject to MSCI. However, experimental studies of Sdc1 and hydra indicate that they are expressed in germ line cells (Nurminsky et al. 1998; Chen et al. 2007).

In summary, our results are consistent with global inactivation of the X chromosome in the male germ line of D. melanogaster. The 112 transgene insertions cover the X chromosome with an average spacing of 194 kb. None of these insertions shows an expression level that is as high as an autosomal insertion. This holds for cytological region 19, which appears to be a hot spot for new gene evolution. A limitation to the present study is that it is restricted to a single promoter from an autosomal testis-expressed gene. Thus, although we can rule out that there are large chromosomal neighborhoods that escape MSCI, we cannot exclude the possibility that local-acting regulatory elements linked to particular X-chromosomal genes allow them to escape inactivation in the male germ line. Further studies with additional promoter sequences are needed to address this possibility.

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References


