Dosage Compensation in Sciarids Is Achieved by Hypertranscription of the Single X Chromosome in Males

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ABSTRACT

Dosage compensation refers to the process whereby females and males with different doses of sex chromosomes have similar amounts of products from sex chromosome-linked genes. We analyzed the process of dosage compensation in Sciarus ocellaris, Diptera of the suborder Nematocera. By autoradiography and measurements of X-linked rRNA in females (XX) and males (XO), we found that the rate of transcription of the single X chromosome in males is similar to that of the two X chromosomes in females. This, together with the bloated appearance of the X chromosome in males, support the idea that in sciarids dosage compensation is accomplished by hypertranscription of the X chromosome in males.

In organisms in which females and males differ in the number of sex chromosomes, one sex having one and the other sex having two, a process has evolved to eliminate the difference in the doses of the sex chromosome-linked genes in the two sexes. This mechanism is called dosage compensation. This process is accomplished by different mechanisms in the three organisms in which it has been studied so far: Drosophila melanogaster (Kuroda et al. 1993), Caenorhabditis elegans (Hsu and Meyer 1993) and mammals (Borsani and Ballabio 1995). In D. melanogaster, the two X chromosomes of the females are active and dosage compensation is achieved in males by hypertranscription of its single X chromosome. In C. elegans, dosage compensation is achieved by hypotranscription of the two active X chromosomes in the hermaphrodites. Finally, in mammals, dosage compensation is attained by stable inactivation of one of the two X chromosomes in females. In the three organisms, a set of genes have been identified, which are responsible for dosage compensation: the male-specific-lethal (msl) genes in D. melanogaster (Belote and Lucchesi 1980a,b; Uchida et al., 1981) the dumpy genes in C. elegans (Hodgkin 1983; Meyer and Casson 1986; Meneely and Wood 1987) and the Xist gene in mammals (Brockdorff et al. 1991; Kay et al. 1993).

In D. melanogaster, the study of the msl genes has been pursued further. Two of these genes, mle (Kuroda et al. 1991) and msl-1 (Palmer et al. 1993) have been cloned. The gene mle encodes a protein containing motifs characteristic of members of a helicase superfamily. The gene msl-1 encodes a protein that contains an acidic N terminus characteristic of proteins involved in transcription and chromatin modeling. Both MLE and MSL-1 proteins are associated with many sites along the polytenic X chromosome in males, but not in females, as is the case also for the histone H4 acetylated at lysine 16 (Turner et al. 1992). These results, together with the fact that the msl mutations do not show additive effects (Bachiller and Sánchez 1989; Gorman et al. 1993), led to the proposal that the MSL proteins might be components of a heteromultimeric complex that specifically interacts with the male X chromosome. Consequently, this chromosome would acquire a chromatin structure, reflected by its pale bloated appearance, that allows a better accessibility to the transcription machinery components. In this context, it is worth mentioning that the inactive X chromosome in female mammals is distinguished by a lack of histone H4 acetylation (Jepesen and Turner 1993); thus supporting the idea that the mechanism of dosage compensation is primarily related to chromatin structure.

Little is known on the evolution of the dosage compensation mechanisms. A prerequisite is the elucidation of the genetic complexes governing dosage compensation in different species. Apart from the three organisms mentioned above, dosage compensation has not been thoroughly studied in other organisms. A previous study showed that in Rhynchosiaera americana uridine incorporation in the single X chromosome of male salivary glands was similar to that observed on the double X of female larvae (Casartelli et al. 1969). Here we report on the analysis of dosage compensation in sciarids, Diptera of the suborder Nematocera. We used Sciarus ocellaris, where females are XX and males are XO, as an experimental model. We found that in sciarids dosage compensation occurs and is achieved by hypertranscription of the single male X chromosome.

MATERIALS AND METHODS

S. ocellaris were raised following the procedure of Rocha et al. (1979).

 Autoradiographic analysis: The larval stage was identified by the size and morphology of the eyespot (Perondini and...
we hybridized, and we measured different exposure times to chloroform-isoamyl alcohol (49:1), the mixture was stirred and centrifuged for 20 min at 4°C. Prehybridization (6 hr) and hybridization (16 hr) were carried out at 42°C. After hybridization, filters were washed three times in 1 × SSC, 5 × Denhardt's solution, 0.25% sodium dodecyl sulfate (SDS), 100 μg/ml of denatured salmon DNA and 50% formamide. A Molecular Dynamics Computer Densitometer, model 300A, was used to scan the autoradiographic analysis. The template activity of the polytenic chromosomes from "e" but not from "h" phase larvae (data not shown). Thus, polytenization of the salivary gland chromosomes had already finished at the "h" phase. Figure 1 shows the autoradiographic analysis of [3H]uridine incorporation into salivary gland chromosomes of female and male larvae at the "h" phase. Each dot represents the number of grains along the X chromosome (ordinate) relative to the number of grains along the autosome A (abscissa) within the same nucleus. As a measure of the X chromosome transcription activity in relation to the transcription of the autosome A (control), we calculated the slope of the best-fit straight line by regression analysis in both females and males. The statistical analysis comparing both slopes is shown in Table 1. No significant difference (P > 0.1) was observed between the sexes. This indicates that the rate of RNA synthesis for the single X chromosome in males equals that of the two X chromosomes in females; i.e., dosage compensation occurs in S. ocellaris. Despite these results, however, dosage compensation could be the consequence of a higher DNA content (double amount) in the male X chromosome with respect to each of the two female X chromosomes. One more round of X chromosome polytenization could necessary to ascertain that polytenization of the salivary gland chromosomes had already finished; otherwise, any difference in template activity could be attributed to a different degree of polyteny. To determine whether polytenization had already ceased at the "h" phase of the fourth larval instar, the capacity of the chromosomes to incorporate [3H]thymidine was analyzed by autoradiography. As a control, we used salivary gland chromosomes from younger larvae at the "e" phase of the fourth larval instar. Incorporation of [3H]thymidine occurred only in the polytenic chromosomes from "e" but not from "h" phase larvae (data not shown). Thus, polytenization of the salivary gland chromosomes had already finished at the "h" phase. Figure 1.—Scatter diagram of the number of grains over the X chromosome vs. number of grains over the autosome A, corresponding to the autoradiography for measuring [3H]uridine incorporation in female (white dot) and male (black dot) polytenic chromosomes.
specifically occur in males, so that the X male DNA content would equal the DNA content of both female X chromosomes. As a measure of the DNA content, we compared the amount of Hoechst 33258 that binds to the polytenic X chromosome relative to the amount of Hoechst that binds to the autosomal A within the same nucleus, in both sexes. For this purpose, salivary glands of "h" phase larvae were incubated with Hoechst and the amount incorporated monitored by densitometry. In males the X-DNA/A-DNA ratio value was half the value found in females (Figure 2A). This difference was significant (P < 0.01), whereas the DNA content of the autosomal A was not significantly different between both sexes (data not shown). Therefore, male polytenic nuclei contain half the amount of X chromosome DNA than female polytenic nuclei. Consequently, the compensated X chromosome RNA synthesis in male relative to female polytenic nuclei cannot be attributed to a similar DNA content of the X chromosomes in both sexes.

Although incorporation of [3H]uridine along polytenic chromosomes indicated that dosage compensation exists in sciarids, we performed a more precise study of gene transcription by directly assaying the production of specific X-linked RNAs. Initially, we tried the genes G-6-PDH and sgs-4, known to be located in the X chromosome of D. melanogaster and showing dosage compensation (Luccchesi and Manning 1987), as well as the autosomal genes Adh and sgs-3 as control. Unfortunately, we could not detect the RNA of these four genes in Sciara by using the Drosophila probes. Then, we chose the rDNA genes, which are localized in the proximal end of the X chromosome in Sciara (Desse and Perondini 1991). The rDNA genes are greatly conserved; therefore, rDNA from D. melanogaster was used as a probe (pDM238) (Rohda et al. 1981). Since rDNA genes are expressed in all cells and at all developmental stages, as a control, we analyzed transcripts from an autosomal gene that shows a similar expression pattern. We chose the histone genes, which are also greatly conserved. To quantify the histone RNA we used an heterologous probe (pKG-11) from Chironomous thummi thummi (Hankeln and Schmidt 1990). As in S. ocellaris the localization of histone genes was not known, we performed an "in situ" hybridization of C. t. thummi histone-DNA on Sciara ocellaris polytenic chromosomes, finding that they hybridized to a specific band of the autosome C (data not shown). We measured the amount of rRNA relative to the amount of histone RNA, as described in MATERIAL AND METHODS, in sisters and brothers from the same generation, so it would be unlikely that the rDNA copies would vary between them. No significant differences (P > 0.05) were found for the rRNA/histone RNA value between both sexes (Figure 2B), indicating that the rDNA genes in S. ocellaris are dosage compensated.

All these results demonstrated that the rate of transcription of the single X chromosome in males is about twice the rate of transcription of either of the two X chromosomes of females. This can be accomplished by either decreasing in females or increasing in males the transcription of the X-linked genes. Our results, however, cannot formally distinguish between those two alternative mechanisms. In D. melanogaster, the polytenic X chromosome of males is as wide as the two paired X chromosomes of females, although males contain half the amount of X chromosome DNA than females do. This bloated appearance of the male X chromosome is lost in males mutant for msl mutations, which specifically reduce the transcriptional activity of the male X. Those msl mutant males show a narrower and more intensely stained X chromosome (Belote and Luccchesi

<table>
<thead>
<tr>
<th>Sex</th>
<th>No. of nuclei</th>
<th>Chromosome A</th>
<th>X chromosome</th>
<th>b</th>
<th>t Student</th>
</tr>
</thead>
<tbody>
<tr>
<td>Females</td>
<td>33</td>
<td>187.1</td>
<td>129.5</td>
<td>0.695</td>
<td>1.444</td>
</tr>
<tr>
<td>Males</td>
<td>29</td>
<td>161.4</td>
<td>114.1</td>
<td>0.721</td>
<td>(P &gt; 0.1)</td>
</tr>
</tbody>
</table>

**TABLE 1**
Statistical analysis of [3H]UTP incorporation in the X and A chromosomes of females and males

**Figure 2.**—(A) Analysis of the DNA content of the X chromosome in relation to the DNA content of the autosomal A in salivary glands of "h" phase female and male larvae, corresponding to the measurements of 15 nuclei for each sex. (B) Analysis of the amount of rRNA and histone-RNA in females and males, corresponding to the measurements of 15 samples for each sex. The bars represent the 95% confidence intervals.
The bloated appearance of the male polytene X chromosome in *S. ocellaris* (Figure 3) supports the idea of increased transcriptional activity in males rather than decreased transcriptional activity in females.

In conclusion, we have demonstrated that in *S. ocellaris* the rate of transcription of the X chromosome in males is greater than the rate of transcription of either of the two X chromosomes in females, in spite of the fact that males contain half the amount of X chromosome DNA than females do. These results, together with the specific pale bloated appearance of the male polytenic X chromosome, argue that in *S. ocellaris*, as in the case of *D. melanogaster*, dosage compensation is achieved by hypertranscription of the single X chromosome in males, rather than decreased X-linked gene transcription in females. How much the molecular basis of the mechanism of dosage compensation in Diptera has been conserved during evolution is currently under study. We are trying to isolate and characterize in *S. ocellaris* the genes homologous of the *msl* genes of *D. melanogaster*.

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