Evolution of Dosage Compensation in Diptera: The Gene *maleless* Implements Dosage Compensation in Drosophila (Brachycera Suborder) but Its Homolog in Sciara (Nematocera Suborder) Appears to Play No Role in Dosage Compensation

M. Fernanda Ruiz, M. Rosario Esteban, Carmen Doñoro, Clara Goday and Lucas Sánchez

Centro de Investigaciones Biológicas, Velázquez 144, 28006 Madrid, Spain

Manuscript received July 6, 2000
Accepted for publication August 25, 2000

ABSTRACT

In *Drosophila melanogaster* and in *Sciara ocellaris* dosage compensation occurs by hypertranscription of the single male X chromosome. This article reports the cloning and characterization in *S. ocellaris* of the gene homologous to *maleless (mle)* of *D. melanogaster*, which implements dosage compensation. The Sciara *mle* gene produces a single transcript, encoding a helicase, which is present in both male and female larvae and adults and in testes and ovaries. Both Sciara and Drosophila MLE proteins are highly conserved. The affinity-purified antibody to *D. melanogaster* MLE recognizes the *S. ocellaris* MLE protein. In contrast to Drosophila polytene chromosomes, where MLE is preferentially associated with the male X chromosome, in Sciara MLE is found associated with all chromosomes. Anti-MLE staining of Drosophila postblastoderm male embryos revealed a single nuclear dot, whereas Sciara male and female embryos present multiple intranuclear staining spots. This expression pattern in Sciara is also observed before blastoderm stage, when dosage compensation is not yet set up. The affinity-purified antibodies against *D. melanogaster* MSL1, MSL3, and MOF proteins involved in dosage compensation also revealed no differences in the staining pattern between the X chromosome and the autosomes in both Sciara males and females. These results lead us to propose that different proteins in Drosophila and Sciara would implement dosage compensation.

In organisms in which gender is based on one sex being homomorphic and the other heteromorphic for the sex chromosomes, a process has evolved to equalize the output of products encoded by the genes located there. This process has been termed dosage compensation. It is accomplished by different mechanisms in the organisms in which it has been studied so far. In *Drosophila melanogaster*, dosage compensation is achieved in males by hypertranscription of the single X chromosome (Bashaw and Baker 1996; Cline and Meyer 1996; Lucchesi 1996). In *Caenorhabditis elegans*, it is achieved by hypotranscription of the two active X chromosomes in hermaphrodite individuals (Cline and Meyer 1996; Wood *et al.* 1997). In mammals, it is attained by stable inactivation of one of the two X chromosomes of females (Heard *et al.* 1997). In these examples, sets of genes responsible for dosage compensation have been identified. In *D. melanogaster*, the *male-specific-lethals* (*msl*; Kuroda *et al.* 1991; Palmer *et al.* 1993; Bashaw and Baker 1995; Gorman *et al.* 1995; Kelley *et al.* 1995; Zhou *et al.* 1995), the *mof* (Hilfiker *et al.* 1997), and the *roX1* and *roX2* genes (Amrein and Axel 1997; Meller *et al.* 1997; Franke and Baker 1999; Kelley *et al.* 1999) have been cloned and characterized. In *C. elegans*, dosage compensation is implemented by the function of the *dumpy* (*dpy*; Hodgkin 1983; Meyer and Casson 1986; Meneely and Wood 1987; Plenefisch *et al.* 1989; Chaung *et al.* 1994; Hsu *et al.* 1995; Lieb *et al.* 1996) and the *mix-1* (Lieb *et al.* 1998) genes. Finally, in mammals, the *Xist* gene is required for dosage compensation (Brockdorff *et al.* 1991; Kay *et al.* 1993; Brockdorff 1998; Johnston *et al.* 1998).

Little is known of the evolution of dosage compensation mechanisms (reviewed in Chatterjee 1998). Recently, this question has been addressed through the analysis of the staining pattern of affinity-purified antibodies against *D. melanogaster* MSL proteins on polytene chromosomes of other drosophilids (Bone and Kuroda 1996; Marin *et al.* 1996; Steinemann *et al.* 1996). Although in the majority of these species dosage compensation has not been directly demonstrated, sexual determination mechanisms based on chromosome differences, such as those of *D. melanogaster*, argue in favor of the existence of dosage compensation by hypertranscription of the sex chromosomes in the male (heterogametic) sex. Some of these anti-MSL antibodies specifically label the male X chromosome.

A previous study showed that in *Sciara ocellaris* (Diptera order, Nematocera suborder), where females are XX and males are XO, dosage compensation occurs and is achieved by hypertranscription of the single X chromo-

Corresponding author: Lucas Sánchez, Centro de Investigaciones Biológicas, Velázquez 144, 28006 Madrid, Spain.
E-mail: lsanchez@cib.csic.es

some in males (da Cunha *et al.* 1994). The same seems to occur in *Rhynchosiara americana* (Nematocera suborder; Casartelli and Santos 1969). Since in drosophilids (higher Diptera) and sciarids (lower Diptera) dosage compensation has been solved in a similar manner, *S. ocellaris* is employed as an experimental model in the study of how the genetic basis underlying dosage compensation might have evolved in dipteran species. To this end, the search in *S. ocellaris* for the homologs of the genes controlling dosage compensation in *D. melanogaster* has been undertaken.

The following briefly summarizes the molecular mechanism of dosage compensation in *D. melanogaster* (reviewed in Bashaw and Baker 1996; Cline and Meyer 1996; Lucchesi 1996). Among the *msl* genes, the gene *mle* encodes a protein containing motifs characteristic of members of a helicase superfamily (Kuroda *et al.* 1991). The gene *msl-1* encodes a protein that contains an acidic N terminus characteristic of proteins involved in transcription and chromatin modeling (Palmer *et al.* 1993). Recently, it has been reported that the main role of MSL1 protein is to serve as the backbone for assembly of the MSL complex (Scott *et al.* 2000). The gene *msl-3* encodes a protein that contains two chromodomains found in proteins known to participate in transcriptional repression by chromatin packing and in proteins known to function as transcriptional activators (Gorman *et al.* 1995; Koonin *et al.* 1995). Finally, the *msl-2* gene encodes a protein that has a RING finger and a metallothionein-like domain in addition to positively and negatively charged amino acid residue clusters and a coil-coil domain that may be involved in protein-protein interactions (Bashaw and Baker 1995; Kelley *et al.* 1995; Zhou *et al.* 1995). The *mof* gene encodes a putative acetyl transferase that plays a direct role in the specific histone acetylation associated with dosage compensation (Hilfiker *et al.* 1997). The *roX1* and *roX2* genes produce RNAs that lack any significant open reading frame (ORF; Amrein and Axel 1997; Meller *et al.* 1997). The products of all these genes have been found to be preferentially associated with many sites along the polytene X chromosome in males, but not in females, as is the case for the H4 histone acetylated at lysine 16 (H4Ac16; Turner *et al.* 1992). The sex-specific association of the MSL proteins, as well as the association of the H4Ac16 protein, is dependent on the simultaneous presence of the wild-type products of all *msl* genes. These results, together with the fact that the *msl* mutations show no additive effects (Bachiller and Sánchez 1989; Gorman *et al.* 1993), have led to the proposal that the MSL proteins form a heteromultimeric complex that specifically interacts with the male X chromosome. The MSL complex acetylates histone H4 at lysine 16 (Akhtar and Becker 2000; Smith *et al.* 2000). Consequently, this chromosome acquires a chromatin structure, reflected by its pale bloated appearance, that allows easier access of the transcription machinery and therefore its hypertranscription.

This article reports on the cloning and characterization of the gene *maleless (mle)* of *S. ocellaris* and its comparison with *mle* of *D. melanogaster*. In addition, an analysis in *S. ocellaris* of the homologs to the other MSL proteins of *D. melanogaster* was undertaken. The results lead us to propose that different proteins in Drosophila and Sciara would implement dosage compensation.

MATERIALS AND METHODS

Fly culture: *S. ocellaris* was raised at 25° following the procedure of Perondini and Dessen (1985).

Construction of a genomic library from *S. ocellaris*: This was performed according to Pirrotta (1986).

Cloning of the gene *mle* of *S. ocellaris*: The *S. ocellaris* genomic library was screened with full-length *D. melanogaster mle* cDNA (Kuroda *et al.* 1991). Hybridization conditions were 42° for 18–20 hr, 5× SSC, 0.1% SDS, 25% formamide, 1× Denhardt's, and 0.1 mg/ml of denatured salmon sperm DNA. Washes were repeated three times (20 min each) at 50° in 0.5× SSC and 0.1% SDS. Identification of positive clones, plaque purification, preparation of phage DNA, Southern blot analysis, identification of cross-hybridization fragments, subcloning of the restriction fragments into plasmid pBluescript KS^-, and isolation of plasmid DNA were performed using the protocols described by Maniatis *et al.* (1982).

Transcript analyses: RNA preparation from frozen adult males and females, electrophoretical fractionation of total RNA, and blotting on nylon membranes were performed as described by Maniatis *et al.* (1982) and Campuzano *et al.* (1986). Blots were hybridized with a riboprobe synthesized from the *Sal*I 1.2-kb genomic fragment following the protocol "Riboprobe *in vitro* Transcription Systems" of Promega (Madison, WI). The hybridization conditions were 55° for 20–30 hr in 5× SSC, 0.25% SDS, 5× Denhardt's, 50% formamide, and 0.1 mg/ml of denatured salmon sperm DNA. Four washes were performed for 15 min each, the first two at room temperature in 2× SSC and 0.1% SDS, the remainder at 65° in 0.2× SSC and 0.1% SDS. After exposure of the filters for autoradiography, the *mle* probe was then prehybridized and subsequently hybridized with the *D. melanogaster* rDNA probe (pDm238) (Roiha *et al.* 1981), which recognizes the *S. ocellaris* rRNA (da Cunha *et al.* 1994). Hybridization was performed overnight at 42° in 5× SSC, 0.1% SDS, 50% formamide, 5× Denhardt's and 0.1 mg/ml of denatured salmon sperm DNA. The filters were washed two times (5 min each one) at room temperature in 2× SSC and 0.1% SDS and twice (15 min each) at 60° in 0.1× SSC and 0.1% SDS.

RT-PCR analyses: From the comparison between the DNA genomic sequences corresponding to *mle* of *S. ocellaris* (7874 bp) and the *mle* cDNA sequence of *D. melanogaster*, the putative exons and introns for the *S. ocellaris mle* gene were established. This was performed using the program "Splice Site Prediction by Neural Network" (Brunak *et al.* 1991). To verify the existence of these exons, RT-PCR analyses were performed in *S. ocellaris*. Ten micrograms of total RNA from Sciara larvae (males and females), previously digested with RQI RNase-free DNase (Promega), were reversed transcribed with AMV (Promega). Twenty percent of the synthesized cDNA was amplified by PCR. The conditions of the first round were 95° for 5 min—one cycle, and 95° for 1 min, 45° or 49° for 1 min, 72° for 1 min—35 cycles, ended by a single incubation at 72° for 10 min. RT-PCR products were analyzed by electrophoresis

in 1.5% agarose. The fragments were extracted from the agarose and reamplified using the same primers and conditions of the first amplification. The reamplified products were then sequenced twice using the same two primers. A total of 20 overlapping RT-PCRs were performed that together allowed the analysis of 4.2 kb. As a control, non-RT-PCR amplifications from the RNA preparations treated with the RQI RNase-free DNase were performed for each pair of primers used. Fragments were never amplified in the non-RT-PCR reactions.

For the analysis of the expression pattern of the gene *mle*, 10 μg of total RNA from frozen material, previously digested with RQI RNase-free DNase (Promega), were reversed transcribed with AMV (Promega). Twenty percent of the synthesized cDNA was amplified by PCR. The conditions of the first round were 95° for 5 min—one cycle, and 94° for 1 min, 54° for 1 min, 72° for 1 min—25 cycles, ended by a single incubation at 72° for 10 min. Ten percent of the PCR product was reamplified using the same conditions. The RT-PCR products were analyzed by electrophoresis in 2% agarose, blotted onto nylon membranes, and hybridized with a *S. ocellaris mle* probe. The hybridization conditions were 16–20 hr at 68° in 5× SSC, 1% SDS, 2% powder milk, and 0.1 mg/ml of denatured salmon sperm DNA. Four washes were performed, the first two at room temperature for 5 min each in 2× SSC and 0.1% SDS, and the others at 65° for 20 min each in 0.1× SSC and 0.1% SDS. Two pairs of primers were used. The first pair (P4-P5) corresponded to primers from exons 3 and 4, which amplify a fragment of 186 bp. The second pair corresponded to primers (P9-P10) from exons 7 and 8, which amplify a fragment of 164 bp. As a control, non-RT-PCR amplifications from the RNA preparation treated with the RQI RNase-free DNase were performed. Fragments were never amplified in the non-RT-PCR reactions.

DNA sequencing: DNA genomic fragments and amplified cDNA fragments from RT-PCR analyses were sequenced using an automatic 377 DNA sequencer (Applied Biosystem, Foster City, CA). The primers used for RT-PCR analysis were also used for sequencing the corresponding amplified cDNA fragments.

Western blots: Samples of total proteins from larvae and adults of *D. melanogaster* and *S. ocellaris* were prepared by homogenization in RIPA lysis buffer (150 mm NaCl, 1% NP-40, 0.5% sodium deoxycholate, 0.1% SDS, 50 mm Tris, pH 7.5) or NP-40 lysis buffer (150 mm NaCl, 1% NP-40, 50 mm Tris, pH 8.0) with 2 mm phenylmethylsulfonyl fluoride, 1 μm iodoacetic acid, and 100 μg/ml of leupeptin, pepstatin, aprotinin, and benzamidine. SDS-polyacrylamide gels (8%; Laemmli 1970) were blotted onto nitrocellulose (Towbin *et al.* 1979), blocked with 5% BSA, 10% nonfat dried milk, and 0.05% Tween-20 in PBS, and probed with the anti-MLE (1:2000; an affinity-purified antibody, MLE25, to the *D. melanogaster* MLE protein; Palmer *et al.* 1994) for 3 hr at room temperature. After washing in 0.05% Tween-20 in PBS (TPBS), filters were incubated with the secondary antibody anti-rabbit IgG conjugated to alkaline phosphatase (1:3000; Bio-Rad) for 2 hr at room temperature. Filters were washed in TPBS and developed with 5-bromo-4-chloro-3-indolylphosphate/nitro blue tetrazolium as previously described (Harlow and Lane 1988).

Immunostaining analyses of polytene chromosomes: *D. melanogaster* and *S. ocellaris* polytene chromosome preparations were performed as published previously (Kuroda *et al.* 1991). Primary and secondary antibodies were diluted in PBN (1% BSA, 0.5% NP-40 in PBS) and used as follows: anti-MLE (1:200), anti-MOF (1:100), anti-MSL1 (1:100), and anti-MSL2 (1:100), all of them followed by FITC-conjugated anti-rabbit (1:50; Sigma, St. Louis); anti-MSL3 (1:100) followed by Rhd-anti-goat (1.50; Sigma). Incubation with primary antibody was performed overnight at 4°. Slides were washed three times in PBN and incubated with the secondary antibody for 45 min at room temperature. After washing with PBN, slides were stained with 0.01 μg/ml Hoechst 33258 in PBS for 2 min, mounted with antifading solution [1 mg/ml *p*-phenylenediamine in a 1:10 (v/v) mixture of PBS:glycerol] and sealed with rubber cement. Secondary antibodies by themselves do not show hybridization to the chromosomes (data not shown).

Immunostaining analysis of nuclei from Sciara and Drosophila embryos: Wild-type *D. melanogaster* (Oregon-R) and *S. ocellaris* embryos at syncytial preblastoderm and postblastoderm developmental stages were dechorionated and fixed in methanol following described procedures (Warn and Warn 1986; de Saint Phalle and Sullivan 1996). Alternatively, for both species the embryonic nuclei content was spread and squashed on microscope slides as described (CSS technique; Goday *et al.* 1999). In this case, the buffer solution used to crack the embryos consisted of 6 mm $MgCl_2$, 1% citric acid, and 1% Triton X-100. Slides were fixed in cold methanol (−20° for 10 min) followed by cold acetone (−20° for 30 sec). After fixation, slides and the embryo suspensions were processed identically. Essentially, they were washed in PBS (3 × 5 min) and then in PBS containing 1% Triton-X and 0.5% acetic acid for 10 min. They were then incubated with 2% BSA for 1 hr at room temperature. Primary MLE antibody (1:100) was incubated at 4° overnight. After washing in PBS 3 × 5 min they were incubated with a secondary Cy3-conjugated anti-rabbit antibody (1:400) at 4° for at least 4 hr. DNA was visualized with 4′6-diamidino-2-phenylindole (DAPI) staining (0.1 μg/ml) and preparations mounted in antifading solution. Observations were made under epifluorescence optics with a Zeiss axiophot microscope equipped with a Photometrics CCD camera.

Comparison of DNA and protein sequences: This was performed using the Fasta program (version 3.0t82; Pearson and Lipman 1988).

RESULTS

The *mle* gene of *S. ocellaris* and its encoded protein: Southern blots of genomic *S. ocellaris* DNA, digested with either *Eco*RI or *Bam*HI, were hybridized under different conditions with the 3.5-kb *Eco*RI fragment of *D. melanogaster mle* cDNA probe, which encodes the amino-terminal domain plus the helicase domains. The aim was to determine whether *S. ocellaris* contains sequences homologous to the *D. melanogaster mle* gene and to check the hybridization conditions appropriate for the isolation of the putative homologous *mle* gene of *S. ocellaris*. Figure 1A shows the Southern blot hybridized under the conditions described in materials and methods. Two unique *Eco*RI and *Bam*HI bands of 10- and 2.7-kb, respectively, were detected. The same hybridization conditions were used to screen, with the *D. melanogaster mle* cDNA probe, a genomic library of *S. ocellaris* synthesized in λ-EMBL4 (see materials and methods). A positive phage (λ-mle-sci) that contained an insert of 16 kb of genomic DNA was isolated (Figure 1D). Southern blots of λ-mle-sci DNA, digested with *Eco*RI, *Bam*HI, or *Sal*I, were performed. They were hybridized with the *D. melanogaster* 3.5-kb *Eco*RI fragment of *mle* cDNA and used as a probe in the screening of the Sciara genomic library. The other probe was the 0.5-kb *Eco*RI fragment of the *D. melanogaster mle* cDNA, which contains glycine-rich

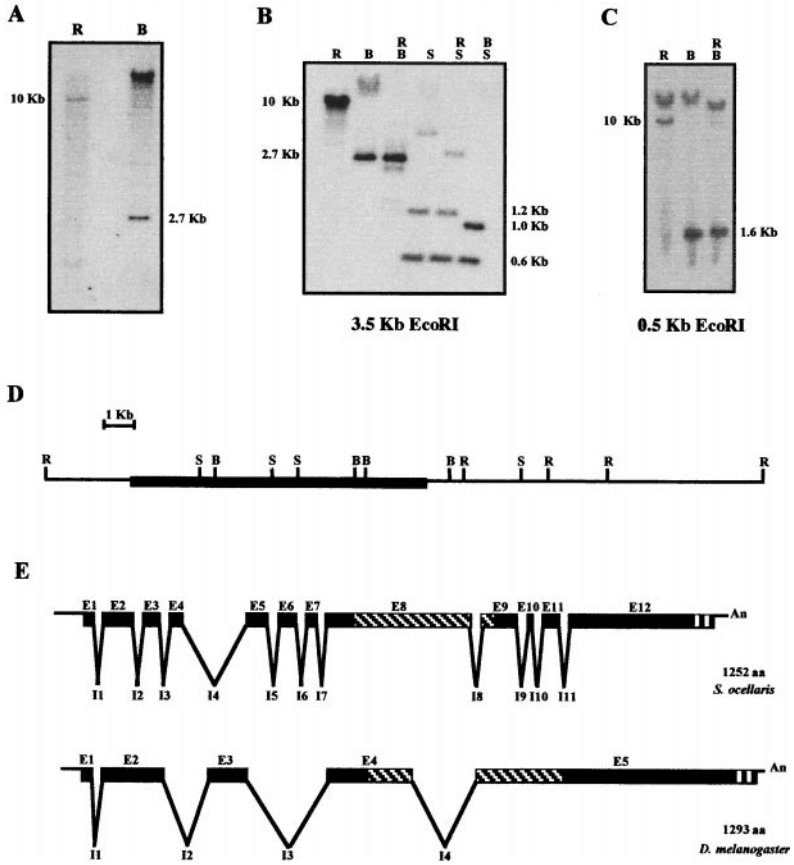

FIGURE 1.—(A) Genomic Southern blots of *S. ocellaris* probed with the 3.5-kb *Eco*RI fragment of *D. melanogaster mle* cDNA probe, which encodes the amino-terminal domain plus the helicase domains. (B) Southern blots of λ-mle-sci DNA hybridized with the *D. melanogaster* 3.5-kb *Eco*RI fragment of *mle* cDNA used as a probe in the genomic Southern blot of Sciara. (C) Southern blot of λ-mle-sci DNA hybridized with the 0.5-kb *Eco*RI fragment of the *D. melanogaster mle* cDNA, which contains the glycine-rich sequence repeats at the carboxy-terminal region. (D) Restriction map of the genomic insert of λ-mle-sci DNA. The broad line represents the fragment containing the whole transcription unit of the *S. ocellaris mle* gene. R, *Eco*RI; B, *Bam*HI; and S, *Sal*I. (E) The molecular organization of Sciara *mle* gene and its comparison with the gene *mle* of *D. melanogaster*. E and I stand for exon and intron, respectively. An, the poly(A) sequence. The cross-hatching corresponds to the region containing the helicase domains.

sequence repeats in the carboxy-terminal region. A 10-kb *Eco*RI and a 2.7-kb *Bam*HI fragment hybridized with the 3.5-kb probe (Figure 1B). These bands coincide with those observed in the Southern blot of Sciara genomic DNA (Figure 1A). A 1.6-kb *Bam*HI fragment hybridized with the 0.5-kb *Eco*RI probe (Figure 1C). This and the 2.7-kb *Bam*HI fragment are within the 10-kb *Eco*RI fragment (Figure 1D). The 2.7-kb *Bam*HI genomic fragment of the λ-mle-sci phage was then subcloned in pBluescript KS^- plasmid and sequenced. The sequence was compared with sequences stored in GenBank and showed homology with genes encoding helicases. The highest degree of homology was with the gene *mle* of *D. melanogaster*. One of the ORFs of this sequence encodes a partial protein that showed very high homology with the region of the *D. melanogaster* MLE protein containing the helicase domains. These results suggest that the λ-mle-sci phage have the *S. ocellaris* genomic sequence homologous to the *D. melanogaster mle* gene.

The following procedure was then undertaken to characterize the gene *mle* of *S. ocellaris*. First, the sequencing of the λ-mle-sci phage from both ends of the 2.7-kb *Bam*HI fragment was extended. A total of 7874 bp was sequenced. Second, to determine the putative ORF encoded by this sequence, a theoretical analysis of the more frequent splicing sites of this sequence was performed (see MATERIALS AND METHODS). The sequence was subject to different putative splicing pathways. Each spliced product was then translated and compared to the MLE protein of *D. melanogaster*. It was found that the biggest putative ORF corresponded to a primary RNA with 10 introns and 11 exons and encoded a protein with high homology to the MLE protein of *D. melanogaster*. Third, to ascertain the molecular organization of the *S. ocellaris mle* gene, overlapping RT-PCR fragments spanning the biggest ORF were synthesized from total RNA of male and female larvae. These were subsequently sequenced (see MATERIALS AND METHODS). Finally, 5′-primer extension analysis was performed to ensure that the whole transcription unit of the Sciara *mle* gene had been isolated. The transcription start site lies 242 bp upstream of the beginning of the ORF, within the 10-kb *Eco*RI fragment (data not shown). The 3′ end of the transcription unit is also within this fragment (the polyadenylation signal is present in the sequence; accession no. Y18119).

The molecular organization of the Sciara *mle* gene and its comparison with the *mle* gene of *D. melanogaster* is shown in Figure 1E. It contains 12 exons and 11 introns (one exon and one intron more than in the theoretical analysis). The introns are very small (between 58 and 72 bp) with the exception of intron 4, which is formed of 533 bp. This contrasts with the gene *mle* of *D. melanogaster*, which contains 5 exons and 4 introns (KURODA *et al.* 1991). With respect to the distribution of the introns, the first intron in both species

matches exactly, as do *D. melanogaster* intron 2 and *S. ocellaris* intron 4.

The putative Sciara MLE protein was compared with other proteins in the Protein Data Bank. It showed homology with proteins belonging to the RNA- or DNA-dependent ATPase helicase superfamily of proteins (DEAH family). The highest degree of homology was found with the MLE protein of *D. melanogaster* (63%) and with bovine (Zhang *et al.* 1995; 52%) and human (Lee and Hurwitz 1993; 50%) RNHA helicase A. Less extensive homology (between 25 and 29% of identity) has been found with the yeast pre-mRNA splicing factors PRP2 (Chen and Lin 1990), PRP16 (Burgess *et al.* 1990), and PRP22 (Company and Arenas 1991). A more detailed comparison between the Drosophila and Sciara MLE proteins is presented in Figure 2. The Sciara MLE protein is shorter (1252 amino acids) than the Drosophila MLE protein (1293 amino acids). To better compare the degree of conservation between these proteins and to analyze the distribution of the conservative changes, the proteins were divided into three regions: N-terminal, helicase, and C-terminal regions (see legend to Figure 2). The N-terminal region showed 76.9% similarity (51.2% identical, 25.7% conservative amino acid changes), the helicase region showed 89.3% similarity (75.4% identical, 13.9% conservative amino acid changes), and the C-terminal region showed 84.8% similarity (61.2% identical, 23.6% conservative amino acid changes). The highest degree of conservation was in the helicase domains (shaded and marked with roman numerals in Figure 2). The amino acids of these domains are identical in both proteins, except for a few conservative changes in domains Ia, II, IV, and V, where it is possible to find either Ile or Val, or either Ser or Ala. In addition, both proteins share two main features that distinguish them from other helicases. One is the putative ATP-binding domain sequence DE*I*H (second helicase domain) instead of the DE*A*H sequence found in other helicases. The other feature is that they contain an additional 54 amino acids between domains III and IV, absent in most DEAH family members. In the majority, spacing between domains is generally conserved. Furthermore, these proteins have glycine-rich sequence repeats at their carboxy-terminal ends that could be involved in RNA binding (Lee and Hurwitz 1993). The number of these repeats varies between the two proteins. Finally, they also contain an additional RNA-binding domain termed the double-stranded RNA-binding domain (dsRBD; Gibson and Thompson 1994). This is located in the N-terminal region outside the DExH domain (shown in italic in Figure 2). This domain might endow these helicases with the capacity to function as monomers, unlike other DExH helicases. This domain is conserved, showing 87.1% similarity (66% identical, 21.1% conservative amino acid changes). All these results allow the conclusion that the gene *mle* of *S. ocellaris* has been isolated.

Transcript analysis of the gene *mle* of *S. ocellaris* and its expression pattern: In *D. melanogaster*, the gene *mle* encodes at least two species of RNA. Transcript *mle-25* has 4.0 kb and encodes the 1293-amino-acid MLE protein. The transcript *mle-12* has 4.4 kb and encodes a truncated MLE protein containing the first 226 amino acids as a consequence of a stop codon (Kuroda *et al.* 1991). In *S. ocellaris*, a single *mle* transcript of ~4.2 kb is present in both male and female adults (Figure 3A). In male and female larvae the same transcript is also present (data not shown). The quantity of *mle* RNA is higher in female than in male adults. This difference cannot be attributed to a different amount of loaded RNA in each lane. In fact, densitometric analysis of the *mle* RNA and the control rRNA in males and females revealed that females contain about seven times more *mle* RNA than males. This indicates a maternal expression of the gene *mle* in *S. ocellaris*.

In *D. melanogaster*, the gene *mle* is expressed in both males and females at all developmental stages (Kuroda *et al.* 1991). It is also expressed in the germ cells (Rastelli *et al.* 1995; Rastelli and Kuroda 1998) in agreement with the requirement of this gene in the male germline (Bachiller and Sánchez 1986). To determine the expression pattern of *mle* in *S. ocellaris*, RT-PCR analyses of total RNA from different sources were performed. Two sets of primers were used: one pair corresponded to sequences of exons 3 and 4, the second pair to sequences of exons 7 and 8. It was found that *mle* is expressed at larval and adult stages in both sexes (Figure 3B). Further, it is expressed in adult testis as well as adult ovarian tissue (Figure 3B), confirming maternal expression of this gene.

Association of the MLE protein with polytene chromosomes in males of *S. ocellaris*: In *D. melanogaster*, the staining of the polytene chromosomes with the affinity-purified MLE antibody (anti-MLE) showed that the MLE protein virtually covers the single male X chromosome. In contrast, the female X chromosome and the autosomes of both sexes show a weak anti-MLE staining (Kuroda *et al.* 1991). This indicates that the MLE protein is mainly associated with the single male X chromosome, as expected for a gene involved in dosage compensation.

Due to the high degree of conservation between the MLE proteins of Drosophila and Sciara, it was expected that this antibody might recognize the *S. ocellaris* MLE protein. This was tested by performing Western blots of total protein extracts from *S. ocellaris* probed with the anti-MLE antibody (Figure 4). The affinity-purified anti-MLE detected a protein of ~144 kD in *D. melanogaster* males and females that corresponds to the MLE protein. In *S. ocellaris* males and females, it detected a unique protein of ~140 kD, which corresponds to the size of the MLE protein predicted from the transcript analysis (see above). In Western blots of total protein extracts from *S. ocellaris* larvae, the same unique band was also

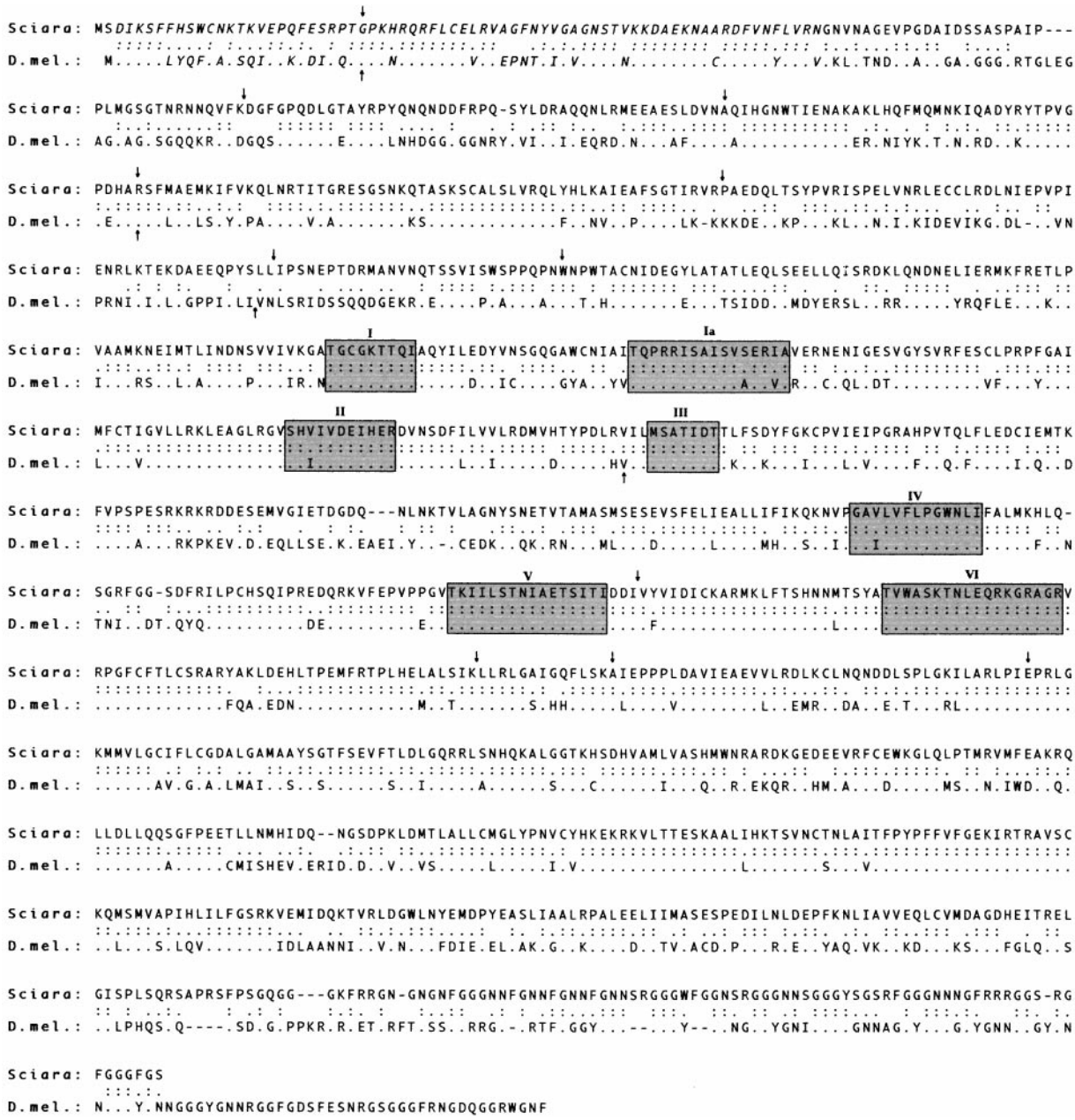

FIGURE 2.—Alignment of the Sciara and Drosophila (D. mel.) MLE proteins. The amino acid sequences were aligned using the FASTA program (version 3.0t82). The seven different helicases motifs are shaded and marked with roman numerals. The dsRBD is shown in italics. For comparisons (see text), the proteins were divided into three regions: N-terminal, helicase, and C-terminal regions. For each region, four categories of comparisons were performed: matching of amino acids (double dots in the alignment), conservative amino acid changes (single dots in the alignment), nonconservative amino acid changes (absence of dots in the alignments), and gaps (–, introduced in the alignments to maximize homology). To define the regions in which the proteins were divided, the Sciara MLE protein was taken as a reference: (1) N-terminal region, the first 382 amino acids; (2) helicase region, 383–780 amino acids; and (3) C-terminal region, 781 to the end of the protein. Arrows indicate the positions of the introns.

detected (data not shown). These results confirm that the gene isolated in *S. ocellaris* corresponds to the homolog of the gene *mle* of *D. melanogaster*. Furthermore, they indicate that the affinity-purified anti-MLE can be used to analyze the distribution of the MLE protein in *S. ocellaris* polytene chromosomes. To this end, the staining of the *S. ocellaris* polytene chromosomes in males was performed. As a control, the polytene chromosomes of *D. melanogaster* males were simultaneously stained. The results are shown in Figure 5. As expected, in *D. melanogaster* the anti-MLE mainly stained the X chromosome, which showed a heavily banded appearance, while the autosomes showed only a few weak bands (Figure 5B). In *S. ocellaris*, no differences in the staining pattern

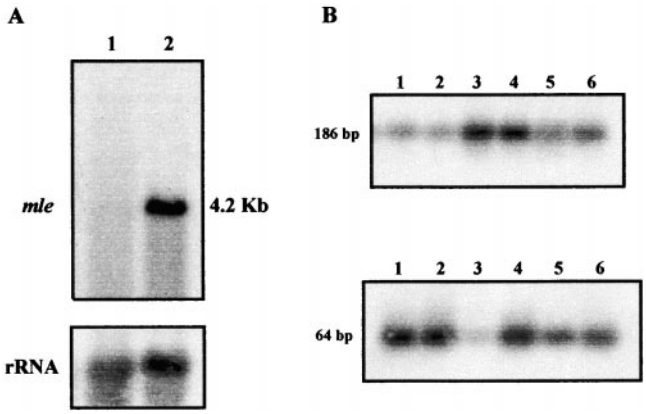

FIGURE 3.—(A) Northern blot of total RNA from male (lane 1) and female (lane 2) adults of *S. ocellaris*. (B) RT-PCR analyses of total RNA from different sources: 1, male larvae; 2, female larvae; 3, head and thorax of male adults; 4, head and thorax of female adults; 5, adult testis; 6, adult ovaries. Two sets of primers were used: one pair corresponded to sequences of exons 3 and 4, which amplify a fragment of 186 bp; the second pair corresponded to sequences of exons 7 and 8, which amplify a fragment of 164 bp. The RT-PCR products were hybridized with a *S. ocellaris mle* probe. For details, see MATERIALS AND METHODS.

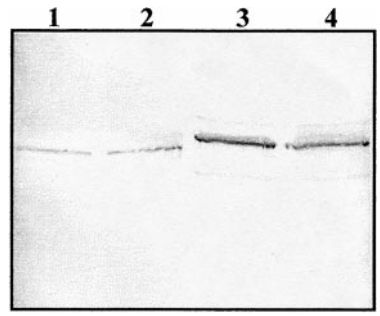

FIGURE 4.—Western blot of total protein extracts from males (1) and females (2) of *S. ocellaris* and from males (3) and females (4) of *D. melanogaster*. The probe was the affinity-purified antibody against the *D. melanogaster* MLE protein (PALMER *et al.* 1994).

between the X chromosome and the autosomes were observed: the staining pattern consisted of highly fluorescent bands on all chromosomes (Figure 5A). The same result was obtained in females (data not shown). These results indicate that, in contrast to that observed in *D. melanogaster*, in *S. ocellaris* the MLE protein is not mainly associated with the male X chromosome.

Association of the MLE protein with chromosomes from Sciara and Drosophila embryos: The localization of MLE protein in *D. melanogaster* male and female embryos by immuno-staining using the anti-MLE antibody has been analyzed (RASTELLI *et al.* 1995; FRANKE *et al.* 1996). This antibody labels the nuclei of the male embryos, revealing a single subnuclear spot that corresponds to the X chromosome. In female embryos, however, the antibody does not stain the nuclei. The male-specific staining depends on the presence of the other MSL proteins. These results indicate that in *D. melanogaster* dosage compensation is already operating in embryos after blastoderm stage and that MLE and the other MSL proteins are responsible for this process (RASTELLI *et al.* 1995; FRANKE *et al.* 1996; MCDOWELL *et al.* 1996).

The localization of the MLE protein in *S. ocellaris* embryos was studied here. To this end, *S. ocellaris* embryos at pre- and postblastoderm developmental stages were stained with the anti-MLE. As a control, *D. melanogaster* embryos at pre- and postblastoderm stages were simultaneously stained (no analysis about staining of *D. melanogaster* embryos before blastoderm stage with the antibodies against the MSL proteins has been reported). The anti-MLE staining pattern in all preblastoderm and postblastoderm *S. ocellaris* embryos is similar and consists in multiple staining spots dispersed within the nuclei (Figure 6, A and B). Identical results have been obtained using whole-mount or squashed embryo preparations (see MATERIALS AND METHODS). As expected, in *D. melanogaster* half of the embryos (male embryos) at postblastoderm stage show the staining pattern of a single intranuclear signal (Figure 6D). No nuclear staining, however, was found in any of the *D. melanogaster* embryos at preblastoderm stage (Figure 6C). These results indicate that the localization of the MLE protein in Drosophila and Sciara embryos is different. In Drosophila, MLE shows nuclear localization only after blastoderm stage, when dosage compensation is known to start. However, in Sciara embryos, MLE already shows nuclear localization at preblastoderm stage, when dosage compensation does not exist yet. Furthermore, in contrast to Drosophila, in Sciara MLE appears as multiple nuclear spots, indicating that its binding is not restricted to the X chromosome.

Analysis of other MSL proteins in *S. ocellaris*: As mentioned above, the MSL proteins form a heteromultimeric complex that specifically interacts with the *D. melanogaster* male X chromosome. This sex-specific association of the MSL proteins is dependent on the simultaneous presence of the wild-type products of all *msl* genes. In view of this, an analysis in *S. ocellaris* of the homologs to the other MSL proteins of *D. melanogaster* was undertaken.

The affinity-purified antibodies against *D. melanogaster* MSL3 protein (anti-MSL3) and MOF (anti-MOF) detect a single band, each one in Western blots of total protein extracts from *S. ocellaris* larvae (data not shown). Positive hybridization (unique bands) has been observed in Southern blots of genomic *S. ocellaris* DNA when the *D. melanogaster msl-3* and *mof* cDNAs were used as probes (data not shown). This suggests that anti-MSL3 and anti-MOF likely detect the homologous MSL3 and MOF proteins in *S. ocellaris*. The staining of polytene chromosomes from *S. ocellaris* male larvae with anti-MSL3 and

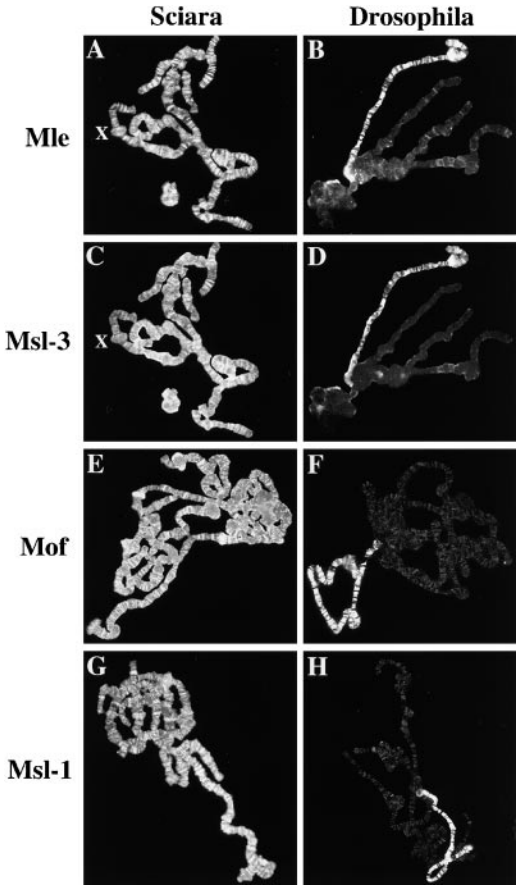

FIGURE 5.—Association of the affinity-purified antibodies against the *D. melanogaster* MSL proteins with polytene chromosomes of *S. ocellaris* (A, C, E, G) and *D. melanogaster* (B, D, F, H) males (for details see MATERIALS AND METHODS). In *S. ocellaris*, highly fluorescent bands are detected over all chromosomes and no difference is observed between the X chromosome and the autosomes. In *D. melanogaster*, the X chromosome is brightly labeled. X stands for the X chromosome.

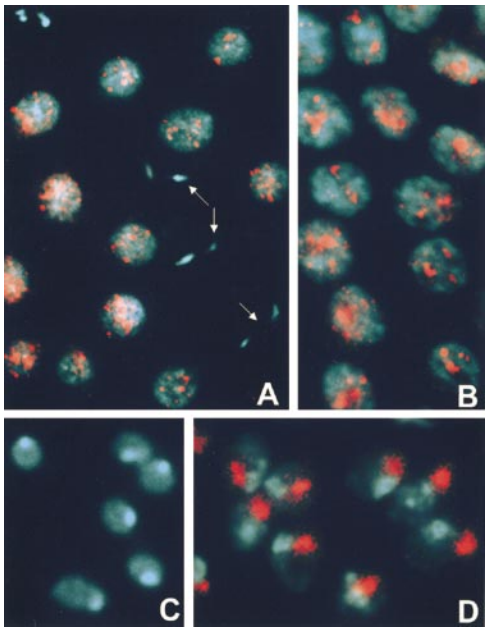

FIGURE 6.—Indirect immunofluorescence staining of anti-MLE (red) and DAPI chromatin staining (blue) in *S. ocellaris* (A and B) and *D. melanogaster* (C and D) embryonic interphase cells. At *S. ocellaris* preblastoderm (A) and postblastoderm (B) stages, anti-Mle antibody decorates multiple intranuclear sites. Arrows in (A) point to paternal-derived X chromosomes that have been excluded from the daughter nuclei due to chromosome elimination occurring at preblastoderm cycles. At *D. melanogaster* preblastoderm stages (A) no anti-MLE staining is observed while in postblastoderm male embryonic cells (D) a single intranuclear labeling is present.

anti-MOF was performed. As a control, the polytene chromosomes of *D. melanogaster* males were simultaneously stained. As expected, in *D. melanogaster* the two antibodies mainly stained the X chromosome, which showed a heavily banded appearance, while the autosomes were not stained (Figure 5, D and F). In *S. ocellaris*, no differences in the staining pattern between the X chromosome and the autosomes were observed for the two antibodies (Figure 5, C and E). Since the anti-MLE was made in rabbit and anti-MSL3 in goat, the double staining of polytene chromosomes of *S. ocellaris* and *D. melanogaster* males was performed with the two antibodies. It was found that both co-localize in all *S. ocellaris* chromosomes (Figure 5, A and C) as they do in the X chromosome of *D. melanogaster* males (Figure 5, B and D). These results indicate that, in contrast to that observed in *D. melanogaster*, the putative MSL3 and MOF proteins of *S. ocellaris* are not mainly associated with the male X chromosome, like MLE protein.

The *D. melanogaster msl-1* cDNA detects unique bands in Southern blots of *S. ocellaris* genomic DNA, although no positive band was detected in Western blots of total protein extracts from *S. ocellaris* using the affinity-purified antibody against *D. melanogaster* MSL1 protein (anti-MSL1; data not shown). The anti-MSL1, however, recognizes all polytene chromosomes of *S. ocellaris* males (Figure 5G) and females (not shown), whereas it brightly labels the male X chromosome of *D. melanogaster* as expected (Figure 5H). These results suggest that in *S. ocellaris* the putative homologous MSL3, MOF, and MSL1 proteins are not specifically located in the male X chromosome, as in *D. melanogaster*, but that they are distributed along all chromosomes, like the MLE protein.

No positive signal in polytene chromosomes of *S. ocellaris* was observed with the affinity-purified antibody against *D. melanogaster* MSL2 protein. In addition, we failed to find sequences homologous to the *D. melanogaster msl-2* gene in *S. ocellaris* Southern blots, even at the lowest stringent hybridization conditions. This suggests that *S. ocellaris* might not have a gene homologous to the *D. melanogaster msl-2* gene (see DISCUSSION).

DISCUSSION

The results support the contention that the *mle* gene of *S. ocellaris* has been isolated. First, unique sequences

(a single 10-kb *Eco*RI band) were detected in Southern blots of genomic *S. ocellaris* DNA hybridized with the full-length *D. melanogaster mle* cDNA. Second, it was determined that the whole transcription unit of the Sciara *mle* gene is within the 10-kb *Eco*RI fragment. Third, when the putative Sciara MLE protein was compared with other proteins of the Protein Data Bank, it showed the highest degree of homology with the MLE protein of *D. melanogaster*. A more detailed comparison between the Drosophila and Sciara MLE proteins revealed that the helicase domains are identical in both proteins, except for a few conservative changes. Both proteins have glycine-rich sequence repeats at their carboxy-terminal ends, and they also contain the dsRBD located in the N-terminal region outside the DExH domain. Furthermore, both proteins share two main features that distinguish them from other helicases. One is the putative ATP-binding domain sequence DE*I*H, instead of the DE*A*H sequence found in other helicases. The other is that they contain an additional stretch of 54 amino acids between domains III and IV, absent in most DEAH family members. Finally, Western blots of total protein extracts from *S. ocellaris* probed with the affinity-purified antibody against the *D. melanogaster* MLE (anti-MLE) detected a unique protein of ~140 kD, which corresponds to the size of the Sciara MLE protein predicted from transcript analysis.

Comparative function of *mle* in Drosophila and Sciara: A key property of the genes implementing dosage compensation (effector genes) is that their products are expected to be specifically associated with the sex chromosomes in one of the two sexes: the one in which dosage compensation takes place. This has been supported by observations made in organisms in which dosage compensation has been studied (see Introduction for references). In *D. melanogaster*, where dosage compensation is achieved in males by hypertranscription of its single X chromosome, the products of the genes responsible for dosage compensation are specifically associated with the male X chromosome. In *C. elegans*, where dosage compensation is achieved by hypotranscription of the two active X chromosomes in hermaphrodite individuals, the products of the genes accomplishing dosage compensation are specifically associated with both hermaphrodite X chromosomes. In mammals, where dosage compensation is attained by stable inactivation of one of the two X chromosomes in females, the product of the gene *XIST*, which triggers dosage compensation, specifically acts upon the X chromosome that becomes inactivated. Finally, when dosage compensation was analyzed in other Drosophila genus species, it was found that the specific association of the MSL products to the male X chromosome is conserved (Bone and Kuroda 1996; Marin *et al.* 1996; Steinemann *et al.* 1996).

The polytene chromosomes of *S. ocellaris* and *D. melanogaster* showed a different pattern of staining for the affinity-purified antibody against *D. melanogaster* MLE protein (anti-MLE), which also recognizes the *S. ocellaris* MLE protein. In *D. melanogaster*, MLE is preferentially associated with the male X chromosome and not with the female X chromosomes nor the autosomes of either sex. This is expected for the product of an effector gene involved in dosage compensation. On the contrary, MLE is distributed similarly in the X chromosomes and autosomes of *S. ocellaris* males and females. The *D. melanogaster* and *S. ocellaris* embryos also showed a different pattern of staining for anti-MLE. Half of the *D. melanogaster* embryos (male embryos) present a single nuclear dot in each cell, which presumably represents binding to the X chromosome (Rastelli *et al.* 1995; Franke *et al.* 1996; results reported here). However, all *S. ocellaris* embryos (males and females) present multiple staining spots dispersed within the nuclei of each cell, which excludes a restricted binding to the X chromosome and supports the presence of MLE in the autosomes also. Importantly, this expression pattern in *S. ocellaris* is also observed before blastoderm stage, when dosage compensation is not yet set up, the nuclei are still dividing and migrating to the periphery of the egg, and the elimination of the paternal X chromosomes is taking place. In *D. melanogaster*, on the contrary, the nuclei of preblastoderm embryos do not show positive staining with anti-MLE. This is expected since MLE is involved in dosage compensation and this process is established after blastoderm. All these results suggest that dosage compensation might be implemented by different effector genes in *D. melanogaster* and *S. ocellaris*, at least with respect to the gene *mle*, although in both species dosage compensation is achieved by hypertranscription of the single male X chromosome.

The question arises as to what the role of the MLE protein might be in Sciara. The role of MLE in Drosophila dosage compensation (*mle* function) is not yet completely understood. It has been suggested that its possible function in the MSL complex could be to destabilize the chromatin structure through its DNA helicase activity. Alternatively, but not mutually exclusively, it might interact with the nascent RNA transcript or with a putative structural RNA through its RNA helicase activity, thus stimulating the transcription rate (Eisen and Lucchesi 1998). In this respect, it is worth mentioning that the MLE protein is removed from the chromosome by RNase treatment, whereas the other MSL proteins are not (Richter *et al.* 1996). Recently, it has been reported that the incorporation of the *roX1* and *roX2* RNAs to the MSL complex requires the MLE helicase (Meller *et al.* 2000). The function of gene *mle* is also required for the activity of voltage-gated sodium channels (*nap* function, from *n*o *a*ction *p*otential; Kernan *et al.* 1991). These channels are involved in initiation and propagation of action potentials, which are the neuronal signals that govern locomotor activity in most animals. It has been proposed that the MLE protein, which is a dsRNA helicase, might be needed to resolve the dsRNA structure of the *para* Na^+ channel transcript (Reenan *et al.*

2000). The majority of the *mle* mutations affect both functions. However, two mutations have been identified, which exclusively affect one of these two fucntions: the mle^{RK} mutation affects the *mle* function but not the *nap* function, whereas the nap^{6} mutation affects the *nap* function but not the *mle* function (Kernan *et al.* 1991). This indicates that the two functions are potentially independent, though both activities are performed by the same protein. The molecular characterization of these mutations revealed that mle^{RK} results from the substitution of the amino acid proline (at position 385) by leucine (Kernan *et al.* 1991) and that nap^{6} is a consequence of the replacement of the third amino acid threonine in the TGCGKTTQI ATP-binding domain by serine (Kernan *et al.* 1991). Further, when the amino acid lysine (K) of the TGCGKTTQI domain was substituted by asparagine (N; Richter *et al.* 1996) or by glutamic acid (Lee *et al.* 1997), NTPase/helicase activity was abolished and dosage compensation was affected. These results revealed important regions for the two activities of the MLE protein. The Sciara MLE protein has conserved the proline, the threonine plus the physical distance (30 amino acids) between them, as well as the lysine amino acid of the TGCGKTTQI domain.

In Drosophila, MLE becomes a specialized helicase involved in dosage compensation, since loss-of-function *mle* mutations affect the viability of males, whereas the viability of females is unaffected (Tanaka *et al.* 1976; Belote and Lucchesi 1980). Because in Sciara dosage compensation is achieved by hypertranscription of the male X chromosome, it is reasonable to think that this would require the function of a helicase with DNA or RNA unwinding activity. Since MLE appears to play no role in Sciara dosage compensation, it is proposed here that in the phylogenetic lineage that gave rise to Drosophila, the *mle* gene was co-opted to become a helicase specifically involved in dosage compensation (see below), whereas its homolog in Sciara performs a more general function. On the other hand, in the phylogenetic lineage that gave rise to Sciara, a different helicase was co-opted to exert the function of *mle* in Drosophila. The *nap* function of *mle* in Sciara remains open.

Other putative functions of the MLE proteins have been reported. This is the case of the RNA helicase A (RHA), which is the human homologue of the Drosophila MLE protein. To examine the role(s) of RHA in mammalian development, RHA knockout mice were generated. Homozygosity for the mutant *RHA* allele led to early embryonic lethality. Histological analysis, combined with terminal deoxynucleotidyltransferase-mediated UTP end labeling reactions of *RHA*-null embryos, revealed marked apoptotic cell death, specifically in embryonic ectodermal cells during gastrulation (Lee *et al.* 1998). These observations indicate that RHA is necessary for early embryonic development and suggest the requirement of RHA for the survival and differentiation of embryonic ectoderm.

Two *D. melanogaster* transgenic lines carrying the gene *mle* from *S. ocellaris* were generated by Virginia Boulais in Brian Oliver's laboratory. We performed tests to determine whether this transgene was able to provide the *mle* function in *D. melanogaster* flies mutant for *mle*. None of the two transgenic lines supplied the *mle* function. An analysis of the transgenic flies revealed that the transgene is transcribed but either the mRNA is not translated or the transgenic protein is not stable and is immediately degraded because it is not detected in Western blots (data not shown). Therefore, we cannot know whether the Sciara MLE protein is able to interact with the other *D. melanogaster* MSL proteins to form a functional MSL complex, or whether the differences between both Sciara and Drosophila MLE proteins are preventing such interaction. In any case, with respect to the function of MLE in Sciara, the results presented in this article indicate that this protein plays no role in Sciara dosage compensation.

Expression of *mle* in the germline of Sciara and Drosophila: The gene *mle* is expressed in the ovaries of both *D. melanogaster* and *S. ocellaris*. The expression of *mle* in Drosophila ovaries has nothing to do with the normal development of the female germline since germ cells mutant for *mle* are capable of generating normal functional oocytes (Bachiller and Sánchez 1986). A maternal effect of *mle* mutation has been reported, which is manifested by the slightly earlier lethal phase of males born to mothers mutant for this gene, homozygous for *mle* (Tanaka *et al.* 1976; Belote and Lucchesi 1980). In these mutant males, the assembly of the MSL complex is delayed (Rastelli *et al.* 1995). This maternal effect, however, is not important since the early syncytial stages before zygotic transcription starts are normal in both males and females coming from mothers mutant for *mle*. In addition, the lack of maternal MLE is irrelevant to males that receive a wild-type *mle* copy from the father (Tanaka *et al.* 1976; Belote and Lucchesi 1980). There are no mutations in the Sciara *mle* gene, which allow checking the requirement of its maternal expression in zygotic development. However, following the proposal that this gene might exert a general function, it is suggested here that the maternal Sciara MLE might be needed for the early developmental stages of the zygotes. Early development of Sciara and Drosophila takes place in a syncytium, where nuclei divide quickly and migrate into the periphery of the egg. These divisions occur before transcription of the zygotic genome starts and use the maternal products stored by the oocyte during oogenesis. Some of these products must be helicases whose activities are certainly required for DNA replication of the fast-dividing nuclei. It is suggested that the Sciara MLE helicase could be one of these maternal molecules used in these early divisions. This is supported by the distribution of MLE in multiple-staining nuclear spots in *S. ocellaris* embryos at the syncytium stage, when the nuclei are still dividing and migrat-

ing to the periphery of the egg and the elimination of the paternal X chromosomes is taking place. In Drosophila this function would not be carried out by MLE but by another helicase.

Contrary to that which happens in the female germline, *mle* is required for normal development of the male germline in Drosophila (Belote and Lucchesi 1980; Bachiller and Sánchez 1986). The role of *mle* in spermatogenesis remains unknown but it presumably has nothing to do with dosage compensation. In this respect, it is worth mentioning that MLE is not localized to the X chromosome of *D. melanogaster* male germ cells (Rastelli and Kuroda 1998). It has been proposed that MLE might be required for RNA processing in Drosophila spermatogenesis, representing this activity the ancient function of *mle* and its homologs in *C. elegans* and mammals (cited in Rastelli and Kuroda 1998). The gene *mle* is also expressed in the Sciara male germline. Whether this gene exerts the same function in both Drosophila and Sciara male germlines remains uncertain. It may well be possible that *mle* retains a common function in spermatogenesis but has a different function in the soma of these two species.

Function of other homologous MSL proteins in Sciara: The putative homologous *msl-1*, *msl-2*, *msl-3*, and *mof* genes of *S. ocellaris* have not yet been isolated and characterized. Nevertheless, positive hybridization (unique bands) has been observed in Southern blots of genomic *S. ocellaris* DNA when the *D. melanogaster msl-1*, *msl-3*, and *mof* cDNAs were used as probes. In addition, positive hybridization of *S. ocellaris* polytene chromosomes was also observed with the affinity-purified antibodies against *D. melanogaster* MSL1, MSL3, and MOF proteins. Thus, these antibodies are presumably identifying the homologous Sciara MSL1, MSL3, and MOF proteins. In the three cases, the affinity-purified antibodies, which specifically stain the X chromosome of *D. melanogaster* males, showed no differences in the staining pattern between the X chromosome and the autosomes in both *S. ocellaris* males and females, similarly to the staining pattern obtained with anti-MLE. Therefore, it is proposed here that as for MLE, the MSL1, MSL3, and MOF proteins play no role in Sciara dosage compensation. Thus, different proteins in Drosophila and Sciara would implement this process.

To this respect, the results concerning the MSL2 protein become relevant. No positive hybridization was observed when *D. melanogaster msl-2* cDNA was used as a probe in Southern blots of *S. ocellaris* genomic DNA, even under the lowest possible stringency conditions. Furthermore, no positive signal was observed in polytene chromosomes of *S. ocellaris* with the affinity-purified antibody against the *D. melanogaster* MSL2 protein. These results suggest that *S. ocellaris* has no sequences homologous to the *D. melanogaster msl-2* gene. Interestingly, the gene *msl-2* plays a key role in Drosophila dosage compensation. As mentioned in the Introduction, the MSL proteins in Drosophila form a heteromultimeric complex that specifically interacts with the male X chromosome. Although the *msl* genes are transcribed in both sexes, the assembly of the MSL complex occurs only in males, because only they have the MSL2 protein. The production of MSL2 protein is prevented in females by the exclusive presence in them of the Sex-lethal protein. In fact, ectopic expression of *msl-2* is sufficient to assemble the MSL complex in females (Kelley *et al.* 1995, 1997; Bashaw and Baker 1997). The failure to find sequences homologous to the *D. melanogaster msl-2* gene in *S. ocellaris* agrees with the proposal that different effector genes in Sciara and Drosophila may implement dosage compensation.

We are grateful to Mitzi Kuroda for proving the affinity-purified antibodies against the *D. melanogaster* MSL1, MSL2, MSL3, and MOF proteins and the *D. melanogaster mle*, *msl-1*, *msl-3*, *msl-2*, and *mof* cDNA clones. We are grateful to Brian Oliver and Virginia Boulais for generating the *D. melanogaster* transgenic lines carrying the Sciara *mle* gene. We are grateful to M. Kuroda, A. L. P. Perondini, and P. P. López for their comments on a previous version of this manuscript. This work was supported by grants UE95-0035 and PB95-1236 to L. Sánchez and PB96-0810 to C. Goday from D.G.I.C.Y.T., Ministerio de Educación y Cultura of Spain, and CI1*-CT 94-0071 BR from European Union to L. Sánchez and C. Goday.

LITERATURE CITED

Akhtar, A., and P. B. Becker, 2000 Activation of transcription through histone H4 acetylation by MOF, and acetyltransferase essential for dosage compensation in Drosophila. Mol. Cell **5:** 367–375.

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

Bachiller, D., and L. Sánchez, 1986 Mutations affecting dosage compensation in *Drosophila melanogaster*: effects in the germline. Dev. Biol. **118:** 379–384.

Bachiller, D., and L. Sánchez, 1989 Further analysis on the *male-specific lethal* mutations that affect dosage compensation in *Drosophila melanogaster*. Roux's Arch. Dev. Biol. **98:** 34–38.

Bashaw, G. J., and B. S. Baker, 1995 The *msl-2* dosage compensation gene encodes a putative DNA-binding protein whose expression is sex specifically regulated by *Sex lethal*. Development **121:** 245–258.

Bashaw, G. J., and B. S. Baker, 1996 Dosage compensation and chromatin structure in Drosophila. Curr. Opin. Genet. Develop. **6:** 496–501.

Bashaw, G. J., and B. S. Baker, 1997 The regulation of the Drosophila msl-2 gene reveals a function for *Sex-lethal* in translational control. Cell **89:** 789–798.

Belote, J. M., and J. C. Lucchesi, 1980 Male-specific lethal mutations of *Drosophila melanogaster*. Genetics **96:** 165–186.

Bone, J. R., and M. I. Kuroda, 1996 Dosage compensation regulatory proteins and the evolution of sex chromosomes in Drosophila. Genetics **144:** 705–713.

Brockdorff, N., 1998 The role of Xist in X-inactivation. Curr. Opin. Genet. Dev. **8:** 328–333.

Brockdorff, N., A. Ashworth, G. F. Kay, P. Cooper, S. Smith *et al.*, 1991 Conservation of position and exclusive expression of mouse *Xist* from the inactive X chromosome. Nature **351:** 329–331.

Brunak, S., J. Engelbrecht and S. Knudsen, 1991 Prediction of human donor and acceptor sites from the DNA sequence. J. Mol. Biol. **220:** 49–65.

Burgess, S., J. Couto and C. Guthrie, 1990 A putative ATP binding protein influences fidelity of branchpoint recognition in yeast splicing. Cell **60:** 705–717.

Campuzano, S., L. L. Balcells, R. Villares, L. Carramolino, L. García-Alonso *et al.*, 1986 Excess function *Hairy-wing* muta-

tions caused by gypsy and copia insertions within structural genes of the *achaete-scute* locus of Drosophila. Cell **44:** 303–312.

Casartelli, C. B. R., and E. P. Santos, 1969 DNA and RNA synthesis in *Rhynchosiara angelae* and the problem of dosage compensation. Caryologia **22:** 203–211.

Chatterjee, R. N., 1998 Mechanism and evolutionary origins of gene dosage compensation, pp. 167–214 in *Genome Analysis in Eukaryotes: Developmental and Evolutionary Aspects*, edited by R. N. Chatterjee and L. Sánchez. Springer/Narosha Publishing House, New Dehli.

Chaung, P.-T., D. G. Albertson and B. J. Meyer, 1994 DYP-27: a chromosome condensation protein homolog that regulates C. elegans dosage compensation through association with the X chromosome. Cell **79:** 459–474.

Chen, J.-H., and R.-J. Lin, 1990 The yeast PRP2 protein, a putative RNA-dependent ATPase, shares extensive sequence homology with two other pre-mRNA splicing factors. Nucleic Acids Res. **18:** 6447.

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

Company, M. J., and A. J. Arenas, 1991 Requirement of the RNA helicase-like protein PRP22 for release of messenger RNA from spliceosomes. Nature **349:** 487–493.

da Cunha, P. R., B. Granadino, A. L. P. Perondini and L. Sánchez, 1994 Dosage compensation in sciarids is achieved by hypertranscription of the single X chromosome. Genetics **138:** 787–790.

de Saint Phalle, B., and W. Sullivan, 1996 Incomplete sister chromatid separation is the mechanism of programmed chromosome elimination during early *Sciara coprophila* embryogenesis. Development **122:** 3775–3784.

Eisen, A., and J. C. Lucchesi, 1998 Unraveling the role of helicases in transcription. Bioessays **20:** 634–641.

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.

Gibson, T. J., and J. D. Thompson, 1994 Detection of dsRNA-binding domains in RNA helicase A and *Drosophila* maleless: implications for monomeric RNA helicases. Nucleic Acids Res. **22:** 2552–2556.

Goday, C., Y. Panzera and M. R. Esteban, 1999 A simple cytological technique to analyze nuclear divisions during preblastodermic development in Drosophila. Chromosome Res. **7:** 445–448.

Gorman, M., M. Kuroda and B. S. Baker, 1993 Regulation of the sex-specific binding of the Maleless dosage compensation protein to the male X chromosome in Drosophila. Cell **72:** 39–49.

Gorman, M., A. Franke and B. S. Baker, 1995 Molecular characterization of the *male-specific lethal-3* gene and investigations of the regulation of dosage compensation. Development **121:** 463–475.

Harlow, E., and D. Lane, 1988 *Antibodies: A Laboratory Manual.* Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Heard, E., P. Clerc and P. Avner, 1997 X-chromosome inactivation in mammals. Annu. Rev. Genet. **31:** 571–610.

Hilfiker, A., D. HilfikerKleiner, A. Pannuti and J. C. Lucchesi, 1997 mof, a putative acetyl transferase gene related to the Tip60 and MOZ human genes and to the SAS genes of yeast, is required for dosage compensation in Drosophila. EMBO J. **16:** 2054–2060.

Hodgkin, J., 1983 X chromosome dosage and gene expression *Caenorhabditis elegans*: two unusual dumpy genes. Mol. Gen. Genet. **192:** 452–458.

Hsu, D., P.-T. Chuang and B. J. Meyer, 1995 DPY-30, a nuclear protein essential early in embryogenesis for *Caenorhabditis elegans* dosage compensation. Development **121:** 3323–3334.

Johnston, C. M., T. B. Nesterova, E. J. Formstone, A. E. T. Newall, S. M. Duthie *et al.*, 1998 Developmentally regulated Xist promoter switch mediates initiation of X inactivation. Cell **94:** 809–817.

Kay, G. F., G. D. Penny, D. Patel, A. Ashworth, N. Brockdorff *et al.*, 1993 Expression of *Xist* during mouse development suggests a role in the initiation of X chromosome inactivation. Cell **72:** 171–182.

Kelley, R. L., I. Solovyeva, L. M. Lyman, R. Richman and M. I. Kuroda, 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., J. W. Wang, L. Bell and M. I. Kuroda, 1997 *Sex lethal* controls dosage compensation in Drosophila by a non-splicing mechanism. Nature **387:** 195–199.

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.

Kernan, M. J., M. I. Kuroda, R. Kreber, B. S. Baker and B. Ganetzky, 1991 *nap*^{ts}, a mutation affecting sodium channel activity in Drosophila, is an allele of *mle*, a regulator of X chromosome transcription. Cell **66:** 949–959.

Koonin, E. V., S. Zhou and J. C. Lucchesi, 1995 The chromo superfamily: new members, duplication of the chromo domain and possible role in delivering transcription regulators to chromatin. Nucleic Acids Res. **23:** 4229–4233.

Kuroda, M. I., M. J. Kernan, R. Kreber, B. Ganetzky and B. S. Baker, 1991 The Maleless protein associates with the X chromosome to regulate dosage compensation in *Drosophila*. Cell **66:** 935–947.

Laemmli, U. K., 1970 Cleavage of structural proteins during assembly of the head of bacteriophage T4. Nature **277:** 680–685.

Lee, C-G., and J. Hurwitz, 1993 Human RNA helicase A is homologous to the Maleless protein of *Drosophila*. J. Biol. Chem. **268:** 16822–16830.

Lee, C-G., K. A. Chang, M. I. Kuroda and J. Hurwitz, 1997 The NTPase/helicase activities of Drosophila maleless, an essential factor in dosage compensation. EMBO J. **16:** 2671–2681.

Lee, C-G., V. C. Soares, C. Newberger, K. Manova, E. Lacy *et al.*, 1998 RNA helicase A is essential for normal gastrulation. Proc. Natl. Acad. Sci. USA **95:** 13709–13713.

Lieb, L., E. Capowski, P. Meneely and B. J. Meyer, 1996 DYP-26, a link between dosage compensation and meiotic chromosome segregation in the nematode. Science **274:** 1732–1736.

Lieb, J. D., M. R. Albrecht, P. T. Chuang and B. J. Meyer, 1998 MIX-1: an essential component of the *C. elegans* mitotic machinery executes x chromosome dosage compensation. Cell **92:** 265–277.

Lucchesi, J. C., 1996 Dosage compensation in drosophila and the 'complex' world of transcriptional regulation. Bioessays **18:** 541–547.

Maniatis, T., E. F. Fritsch and J. Sambrook, 1982 *Molecular Cloning: A Laboratory Manual.* Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Marin, I., A. Franke, G. J. Bashaw and B. S. Baker, 1996 The dosage compensation system of Drosophila is co-opted by newly evolved x chromosomes. Nature **383:** 160–163.

McDowell, K. A., A. Hilfiker and J. C. Lucchesi, 1996 Dosage compensation in Drosophila: the X chromosome binding of MSL-1 and MSL-2 in female embryos is prevented by the early expression of the *Sxl* gene. Mech. Dev. **57:** 113–119.

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.

Meller, V. H., P. R. Gordadze, Y. Park, X. Chu, C. Stuckenholz *et al.*, 2000 Ordered assembly of *roX* RNAs into MSL complexes on the dosage-compensated X chromosome in Drosophila. Curr. Biol. **10:** 136–143.

Meneely, P. M., and W. B. Wood, 1987 Genetic analysis of X-chromosome dosage compensation in *Caenorhabditis elegans*. Genetics **117:** 25–41.

Meyer, B. J., and L. P. Casson, 1986 *Caenorhabditis elegans* compensation for the difference in X chromosome dosage between the sexes by regulating transcript levels. Cell **47:** 871–881.

Palmer, M. J., V. A. Mergner, R. Richman, J. E. Manning, M. I. Kuroda *et al.*, 1993 The *male-specific lethal-one (msl-1)* gene of *Drosophila melanogaster* encodes a novel protein that associates with the X chromosome in males. Genetics **134:** 545–557.

Palmer, M. J., R. Richman, R. Richter and M. I. Kuroda, 1994 Sex specific regulation of the *male-specific lethal-1* dosage compensation gene in Drosophila. Genes Dev. **8:** 698–706.

Pearson, W. R., and D. J. Lipman, 1988 Improved tools for biological sequence comparison. Proc. Natl. Acad. Sci. USA **85:** 2444–2448.

Perondini, A. L. P., and E. M. B. Dessen, 1985 Polytene chromosomes and the puffing patterns in the salivary glands of *Sciara ocellaris*. Rev. Brasil. Genet. **8:** 465–478.

Pirrotta, V., 1986 *Cloning Drosophila Genes*. IRL Press, Oxford.
Plenefisch, J. D., L. DeLong and B. J. Meyer, 1989 Genes that implement the hermaphorite mode of dosage compensation in *Caenorhabditis elegans*. Genetics **121:** 57–76.
Rastelli, L., and M. I. Kuroda, 1998 An analysis of maleless and histone H4 acetylation in Drosophila melanogaster spermatogenesis. Mech. Dev. **71:** 107–117.
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.
Reenan, R. A., C. J. Hanharan and B. Ganetzky, 2000 The *mle*^{napts} RNA helicase mutation in Drosophila results in a splicing catastrophe of the *para* Na^+ channel transcript in a region of RNA editing. Neuron **25:** 139–149.
Richter, L., J. R. Bone and M. I. Kuroda, 1996 RNA-dependent association of the Drosophila maleless protein with the male X chromosome. Genes Cells **1:** 325–336.
Roiha, H., J. R. Miller, L. C. Woods and D. M. Glover, 1981 Arrangements and rearrangements of sequences flanking the two types of rDNA insertion in *D. melanogaster*. Nature **290:** 49–53.
Scott, M. J., L. L. Pan, S. B. Cleland, A. L. Knox and J. Heinrich, 2000 MSL1 plays a central role in assembly of the MSL complex, essential for dosage compensation in Drosophila. EMBO J. **19:** 144–155.
Smith, E. R., A. Pannuti, W. Gu, A. Steurnagel, R. G. Cook *et al.*, 2000 The Drosophila MSL complex acetylates histone H4 at lysine 16, a chromatin modification linked to dosage compensation. Mol. Cell. Biol. **20:** 312–318.
Steinemann, M., S. Steinemann and B. M. Turner, 1996 Evolution of dosage compensation. Chromosome Res. **4:** 185–190.
Tanaka, A., A. Fukunaga and K. Oishi, 1976 Studies on the sex-specific lethals of *Drosophila melanogaster*. II. Further studies on a *male-specific lethal* gene, *maleless*. Genetics **84:** 257–266.
Towbin, H., T. Staehelin and J. Gordon, 1979 Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets, procedure and some applications. Proc. Natl. Acad. Sci. USA **76:** 4350–4354.
Turner, B. M., A. J. Birley and J. Lavender, 1992 Histone H4 isoforms acetylated at specific lysine residues define individual chromosomes and chromatin domains in Drosophila polytene nuclei. Cell **69:** 375–384.
Warn, R. M., and A. Warn, 1986 Microtubule arrays present during the syncytial and cellular blastoderm stages of the early Drosophila embryo. Exp. Cell Res. **163:** 201–210.
Wood, W. B., A. Streit and W. Q. Li, 1997 Dosage compensation: X-repress yourself. Curr. Biol. **7:** R227–R230.
Zhang, S., H. Maacke and F. Grosse, 1995 Molecular cloning of the gene encoding nuclear DNA helicase II. A bovine homologue of human RNA helicase A and Drosophila Mle protein. J. Biol. Chem. **270:** 16422–16427.
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, undergoes sex-specific regulation and encodes a protein with a RING finger and a metallothionein-like cysteine cluster. EMBO J. **14:** 2884–2895.

Communicating editor: T. Schüpbach