The Ceratitis capitata homologue of the Drosophila sex-determining gene sex-lethal is structurally conserved, but not sex-specifically regulated

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Abstract

In Drosophila, Sxl functions as a binary switch in sex determination. Under the control of the primary sex-determining signal, it produces functional protein only in XX animals to implement female development. Here we report that, in contrast to Drosophila, the Sxl homologue in the Medfly, Ceratitis capitata, expresses the same mRNAs and protein isoforms in both XX and XY animals irrespective of the primary sex-determining signal. Also, experiments with two inducible transgenes demonstrate that the corresponding Ceratitis SXL product has no significant sex-transforming effects when expressed in Drosophila. Similar results have been obtained for the Sxl homologue of Musca domestica (Meise, M., Hilfiker-Kleiner, D., Brunner, C., D?bendorfer, A., N¿thiger, R. and Bopp, D. (1998) Development 125, 1487-1494). Our findings suggest that Sxl acquired its master regulatory role in sex determination during evolution of the Acalyptratae group, most probably after phylogenetic divergence of the genus Drosophila from other genera of this group.
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Author’s correction


A revised version of Table 1 is given below.

Table 1. Viability rate of male and female transgenic flies when kept at 25°C or when subjected to heat pulses at 37°C

<table>
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<th>Males showing defects</th>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>25°C</td>
<td>hs 37°C</td>
<td>25°C</td>
</tr>
<tr>
<td>CcSwA1</td>
<td>A11</td>
<td>2</td>
<td>1</td>
<td>103% (229)</td>
<td>90% (162)</td>
<td>95% (215)</td>
</tr>
<tr>
<td>CcSwA1</td>
<td>A11</td>
<td>2</td>
<td>2</td>
<td>95% (176)</td>
<td>48% (73)</td>
<td>93% (182)</td>
</tr>
<tr>
<td>CcSwE3</td>
<td>E31</td>
<td>2</td>
<td>1</td>
<td>90% (191)</td>
<td>91% (201)</td>
<td>110% (179)</td>
</tr>
<tr>
<td>CcSwE3</td>
<td>E31</td>
<td>2</td>
<td>2</td>
<td>109% (208)</td>
<td>89% (185)</td>
<td>92% (236)</td>
</tr>
</tbody>
</table>

The total number of flies, carrying one or two copies of the transgene, is indicated in parentheses.

The percentage of viability of transgenic flies bearing one or two copies of the transgene is calculated referring respectively to the non-transgenic siblings or to those bearing one copy.

Crosses:

w1118/BsY; A1/Cyo × w1118/w1118
w1118/BsY; A1/Cyo × A1/Cyo
w1118/BsY; E3/Cyo × w1118/w1118
w1118/BsY; E3/Cyo × A1/Cyo

Female viability Male viability Males showing defects

25°C hs 37°C 25°C hs 37°C
The *Ceratitis capitata* homologue of the *Drosophila* sex-determining gene *Sex-lethal* is structurally conserved, but not sex-specifically regulated

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**SUMMARY**

In *Drosophila*, *Sxl* functions as a binary switch in sex determination. Under the control of the primary sex-determining signal, it produces functional protein only in XX animals to implement female development. Here we report that, in contrast to *Drosophila*, the *Sxl* homologue in the Medfly, *Ceratitis capitata*, expresses the same mRNAs and protein isoforms in both XX and XY animals irrespective of the primary sex-determining signal. Also, experiments with two inductive transgenes demonstrate that the corresponding *Ceratitis* SXL product has no significant sex-transforming effects when expressed in *Drosophila*. Similar results have been obtained for the *Sxl* homologue of *Musca domestica* (Meise, M., Hilfiker-Kleiner, D., Brunner, C., Dübendorfer, A., Nöthiger, R. and Bopp, D. (1998) *Development* 125, 1487-1494). Our findings suggest that *Sxl* acquired its master regulatory role in sex determination during evolution of the Acalyptratae group, most probably after phylogenetic divergence of the genus *Drosophila* from other genera of this group.

Key words: *Sex-lethal*, Sex determination, *Ceratitis*, *Drosophila*, Evolution

**INTRODUCTION**

In *Drosophila melanogaster*, the primary sex-determining signal is based on the number of X chromosomes in relation to the sets of autosomes (reviewed by Cline, 1993), while in *Ceratitis capitata*, the choice between male or female development is based on the presence or absence of the Y chromosome (Lifschitz and Cladera, 1989; Willhöft and Franz, 1996). Though different sex-determining signals are used, these may act on a conserved binary switch gene that is regulated in an ON/OFF manner to implement female or male development. To test this hypothesis, we investigated whether *Ceratitis*, like *Drosophila*, uses a homologue of the *Sex-lethal* gene as a master switch to control sexual development.

The *Sxl* gene of *Drosophila melanogaster* has been thoroughly investigated at the genetic and molecular level (for details see Meise et al., 1998). In this paper, we characterize a *Ceratitis* homologue that, though well conserved in sequence, deviates in some important aspects from the gene in *Drosophila*. Most notably, this gene appears not to be controlled by the primary sex-determining signal, but instead is equally expressed in both sexes. Our results on the *C. capitata* *Sex-lethal* gene, and those of others in additional Dipteran species (Müller-Holtkamp, 1995; Sievert et al, 1997; Meise et al., 1998), provide a corpus of useful data on the poorly investigated subject of functional divergence of conserved sequences and will contribute to a more general understanding of the evolution of major regulatory pathways.

**MATERIALS AND METHODS**

**Isolation of homologous cDNA**

cDNA libraries were prepared from poly (A)+ RNA of *C. capitata* embryos and female flies in Uni-Zap™ XR using the Stratagene Zap-cDNA synthesis kit. For low-stringency conditions of hybridization, we used the following conditions: 55°C in 5× SSPE, 5× Denhardt’s solution, 0.5% SDS and salmon sperm DNA (100 μg/ml), with washes at 55°C in 2× SSC, 0.1% SDS. A 32P-labeled *Drosophila* probe was prepared from a 800 bp restriction fragment of the female-specific *Sxl* cDNA (CF1) which corresponds to the coding region of the RNA-binding domains (RRM).

**RNA analysis**

Total RNA was extracted at different developmental stages using the guanidinium/CsCl method (Sambrook et al., 1989). For northern blot analysis, RNA was fractionated on 0.8% agarose formaldehyde gel, transferred to a nylon membrane (Hybond-N, Amersham). The probe was prepared from an amplified fragment of *CcSxl* A1 cDNA using primers 423+/917− to exclude regions containing GCC/GGT-rich repeats.

For RT-PCR analysis: 1 μg of total RNA was reverse transcribed with AMV (Promega) according to the manufacturer’s directions and about 1/20th of the reaction was used for PCR reactions in 25 μl total volume (Pharmacia Taq) with the following forward and reverse primers 126+/422− (CATACGATAATGGTTAT and TCGCGATCTGCATATCCTG) and 423+/917− (CAGGATATGACAGATCGCGA and CCGAGCTGAGACATATACTG). The thermal cycle program was set to 40 cycles of 94°C denaturation, 58°C annealing and 72°C elongation for 40 seconds each step (Perkin Elmer GeneAmp 9600).

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Immunoblot analysis and whole-mount staining

Protein extracts were prepared and essentially treated as described in the accompanying paper of Meise et al. (1998). The same protocol was also used for in situ staining of overnight collections of \textit{C. capitata} embryos.

Germline transformation in \textit{Drosophila}

We prepared EcoRI/XhoI fragments of the A1 and E3 cDNAs and placed them into the unique EcoRI/XhoI sites of the pCaSpeR-hs-act (Thummel et al., 1988) vector after destroying the XhoI and XbaI sites by filling. These hsp70-CcSxl fusion constructs, \textit{CcSxlA1} and

\begin{align*}
\text{Fig. 1.} & \quad \text{Structure and sequence of \textit{CcSxl} cDNAs. (A) A schematic presentation of several aligned Sxl cDNAs isolated from \textit{Ceratitis} female adults (A1-A4) and embryonic (E1-E3) libraries. The ORF is marked by a dark gray box and the two putative RRM (RNA Binding Motifs) are labeled in a lighter shade of gray. The differences in sequence of E2 (9 bp at the 5' end) and E3 (24 bp) sequences are indicated by a black box. (B) A FASTA comparison of the DmSXL (346 aa long isofrom) and CcSXL (340 amino acid long isofrom). (C) A sequence alignment of the 24 alternatively spliced nucleotides (inserted between exon 4 and 5) in both \textit{Ceratitis} and \textit{Drosophila} Sxl transcripts and encoding 8 well-conserved amino acids. GenBank accession number of cDNA A1 is AF026145.}
\end{align*}
**RESULTS**

**Isolation of Sxl homologous sequences in C. capitata**

Using a Sxl cDNA fragment from *D. melanogaster* containing the two well-conserved RNA-binding domains (Bell et al., 1988), several overlapping cDNA clones were isolated from an embryonic library and from an adult female library prepared from *C. capitata* (Fig. 1A; Furia et al., 1993; Siden-Kiamos et al., 1993). The protein-coding region of adult cDNA A1 (Genbank accession number AF026145) of *C. capitata* corresponds to that of the female-specific cDNA cF1 of *Drosophila* (Bell et al., 1988). These sequences display 66.8% identity at the nucleotide level. Conceptual translation of both ORFs revealed a high degree of overall similarity: the 340 amino acids encoded by cDNA A1 show 69.2% identity and 78.8% similarity when aligned with the protein encoded by *Drosophila* cF1 (Fig. 1B). The highest sequence conservation is found in the region that spans both RRMs (RNA Binding Motifs; approximately 94%). The only remarkable difference in sequence is found downstream of the second RRM where the *Ceratitis* protein contains an unusually large stretch of 16 glycines not present in *Drosophila* SXL. The embryonic cDNA E2 deviates from the others in sequences upstream of the position that corresponds to the exon2/exon4 splice junction in *Drosophila* Sxl. The embryonic E3 cDNA sequence contains an additional 24 bp not present in the other cDNA sequences at the position corresponding to splice junction of exons 4/5 in *Drosophila* (Fig. 1C). The same splice variants at the junction 4/5 have been described in *Drosophila* (Samuels et al., 1991) and are well conserved in sequence (Fig. 1C). In situ hybridization to polytene chromosomes with the A1 cDNA fragment comprising the 2 RRMs detects a unique signal at the cytological site 79B on the right arm of chromosome 5 (A. Zacharopoulou, personal communication, 1992). These results demonstrate that the genome of *Ceratitis* contains an unique and structurally well-conserved gene (*CcSxl*) that corresponds to Sxl in *Drosophila*.

**CcSxl** gene produces the same transcripts and protein isoforms in XX and XY animals

Like the *Drosophila* gene (Salz et al., 1989), *CcSxl* produces a complex pattern of transcripts of which at least three different size classes can be distinguished, namely 3, 4 and 6 kb (data not shown). Differently from *Drosophila*, though, no sex-specific size classes were detected. To confirm that transcripts in males and females are structurally identical, we performed RT-PCR analysis on total RNA extracted from *Ceratitis* adult flies. Two pairs of specific primers were used for amplification. In both cases, the amplified products were found to be of the same length in males and females (Fig. 2), and subsequent sequencing of the amplified fragments did not reveal any sex-specific differences.

Antibodies against full-length SXL proteins of *Drosophila* were used to monitor expression of SXL protein in *Ceratitis*. In *Drosophila*, these anti-SXL antibodies recognize two prominent isoforms in females (38 and 36 kDa) which are absent from males (Bopp et al., 1991; see also Fig. 3). In contrast to *Drosophila*, the anti-SXL antibodies detect two prominent antigens of slightly faster mobility in both sexes of *Ceratitis* (approximately 34 and 32 kDa; Fig. 3A). No sex-specific differences in size could be noted. Although we cannot exclude that the antibodies detect some cross-reacting protein,
Ectopic expression of CcSXL in Drosophila leads to lethality of both XY and XX animals

To test if CcSXL protein, like its counterpart in Drosophila, can act as a feminizing switch when expressed in Drosophila XY animals, we constructed transgenes containing sequences of CcSxlA1 or CcSxlE3 under the control of the inducible promoter hsp70. The conceptual product of CcSxlE3 is identical to that of CcSxlA1, except for an additional 8 residues at the exon 4/5 splice junction (see Fig. 1C). These transgenes were introduced into the germline of D. melanogaster.

We first tested expression of the transgene in several stably integrated lines of CcSxlA1 and CcSxlE3. Upon repeated heat induction during development, a 32 kDa band appears in males carrying the A1 transgene, while a 34 kDa is detected in males carrying the E3 transgene (Fig. 3B). No products corresponding to the expression of endogenous SXL (38 and 36 kDa, Fig. 3A) are detectable indicating that the presence of Ceratitis proteins does not modify the pre-mRNA processing of endogenous Sxl. Immunostaining of larval tissues prepared from transgenic males confirmed that ectopically expressed Ceratitis SXL is correctly localized to the nucleus (data not shown).

Males of all lines exhibited normal external morphology and were fully viable and fertile at 25°C. One line of each construct, A1.1 and E3.1, was examined for possible phenotypic effects after heat induction. Transgenic flies were exposed to multiple rounds of heat pulses of 40 minutes at 37°C in 8 hour intervals throughout their life cycle. A slight but significant reduction in viability of both males and females carrying one copy of the transgene was observed (Table 1).

Two copies of CcSxlE3 led to a slightly higher reduction in viability, whereas the presence of two copies of CcSxlA1 had a more dramatic effect: only 14% of males and 48% of females survived the heat regimen. However, we consistently find that males are more susceptible than females to the lethal effects of overexpressing CcSxl. Furthermore, the surviving males often show posterior abnormalities. In most cases, a tissue mass extruding from the region surrounding the penis was seen and often patches of female-like pigmentation in the fifth and sixth abdominal tergites were present. The same developmental aberrations have been observed in transgenic males expressing Musca SXL protein (Meise et al., 1998).

DISCUSSION

Sex-specific expression of Sxl: a new trait in Drosophila?

In D. melanogaster, Sxl acts as binary switch that implements all aspects of sexual development. Because of its sex-specific expression in other Drosophila species, it has been proposed that Sxl is widely used as a master switch gene in sex determination in the genus Drosophila (Bopp et al., 1996; Penalva et al., 1997). However, recent reports on Sxl in more distantly related species argue against a conserved function in sex determination of other Diptera. When Sxl was examined in Ceratitis capitata (Tephritidae, this work), Chrysomya ruficollis (Calliphoridae; Müller-Holtkamp, 1995), Musca domestica (Musidae; Meise et al., 1998) and Megaselia scalaris (Phoridae; Sievert et al., 1997), the basic premise for a sex-determining switch function, namely expression of female- or male-specific transcripts, was not found. It is conceivable that the function of Sxl in these more archaic fly species is not related to sex determination. We propose that this function is ancestral to that in Drosophila where the gene was recruited to assume a sex-determining function. With respect to the species examined so far, Ceratitis is the only one belonging to the Acalyptratae group, as Drosophila does, and hence it is the most closely related in phylogenesis to this species. Our findings in Ceratitis suggest that the Sxl functional divergence must have occurred quite recently during dipteran evolution within the Acalyptratae group. Sxl’s adaptation to

Table 1. Viability rate of male and female transgenic flies when kept at 25°C or when subjected to heat pulses at 37°C

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<td>100% (198)</td>
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<tr>
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<td></td>
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<td>100% (216)</td>
<td>95% (206)</td>
<td>100% (198)</td>
</tr>
</tbody>
</table>
become a genetic switch in the *Drosophila* cascade may have resulted from changes in the use of sex-determining signals in different species of this group. We propose that, in *Drosophila*, the ON/OFF regulation of *Sxl* co-evolved with the implementation of the X:A ratio as the primary signal in sex determination.

**What is the role of Sxl in Ceratitis?**

It is still possible that one of the *CcSxl* functions is also in sex determination of *Ceratitis*, not as a discriminatory but rather as an auxiliary element in the cascade. Though expressed in both sexes, *CcSxl* may contribute to the somatic sex determination only, or mainly, in one sex. For example, one of the functions of the *rbp1* and *B52 Drosophila* genes expressed non-sex-specifically in the soma of the fly and both encoding, as *Sxl*, splicing factors seems to be female-specific and exerted on the alternative splicing of *dsx* (Heinrichs and Baker, 1997; Peng and Mount, 1995).

Ectopic expression of *CcSXL* in *Drosophila* causes a significant decrease in viability of both sexes. The same effect is also observed when *Musca* SXL protein is ectopically expressed in *Drosophila* (Meise et al., 1998). Rather than specifically acting on the known targets of *Sxl* (*Sxl*, *tra* and *msl-2*), these exogenous RNA-binding proteins appear to disrupt some basic cellular processes in *Drosophila*. Apart from a role in splicing regulation, Kelley et al. (1997) have proposed that *Sxl* also acts as a translational repressor in *Drosophila*. Previous *in vivo* binding studies showed that SXL protein accumulates at many transcriptionally active sites in the genome, most of which appear to be located on the X chromosome (Samuels et al., 1994). It has been suggested that binding of SXL to these transcripts reduces their translatability, allowing SXL to directly modulate the activity of X chromosomal genes (Kelley et al. 1997). When levels of SXL protein become exceedingly high, translation of these messages may become reduced to a level where lethality ensues irrespective of the transcriptional state of the X chromosome. This idea receives support from the observation that not only males, but also *D. melanogaster* females die when a cDNA construct of the endogenous *Sxl* gene is strongly overexpressed (D. B., unpublished results). Thus, the toxic effects of overexpressing the *Ceratitis* or *Musca* transgenes could result from a severe reduction in translation of transcripts that bind these exogenous SXL proteins. This idea leads to an interesting speculation: in *Ceratitis*, *Musca* and other nondrosophilids, *Sxl* is primarily or even exclusively used as a translational repressor in both sexes to modulate gene activity in a broad sense. In *Drosophila*, it may in addition have acquired a function as a splicing regulator for sex-specific control of a small number of target genes. *Ceratitis* and *Musca* SXL protein most notably differ from *Drosophila* SXL in the aminoterminal region. In *Drosophila*, this domain has been proposed to be important for cooperative binding of the protein to target RNA (Wang and Bell, 1994). This part may thus have served as a major target in evolution to change the molecular properties of the protein.

As the nature of *Sxl*’s function in non-drosophilid species remains speculative, the availability of a genetic transformation system in *Ceratitis capitata* makes this species a particularly suitable system for the functional analysis of this and other genes homologous to those in *Drosophila* (Loukeris et al., 1996; Zwiebel et al., 1996). Combined molecular and genetic studies will eventually reveal the extent of similarity in developmental pathways between these two distantly related dipteran species. It may also help in understanding the evolutionary mechanisms that recruit and organize genes into cascades such as the one responsible for sex determination (Hodgkin, 1992; Wilkins, 1995).

We thank Martin Meise, Rolf Näthiger and Antigoni Zacharopoulou for sharing and discussing unpublished results. We are particularly grateful to John Lucchesi and Christos Louis for their insightful comments on this manuscript and to Geoffrey Nette for critical reading of the manuscript. We thank Maria Furia, Antonio Pannuti and PierPaolo D’Avino for their suggestions. Pasquale Vito and Pasquale Delli Bovi for their contributions and Christos Delidakis for kindly providing the pCaSper vectors. We also thank Rosaria Terracciano for her excellent technical assistance. This work was supported by a grant from the National Research Council of Italy, Special Project RAISA, Subproject n° 2 and by a grant P.O.P. 94/99 delibera G. R. 6989 by Regione Campania.

**REFERENCES**


Salz, H. K., Maine, E. M., Keyes, L. N., Samuels, M. E., Cline, T. W. and


