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The non-dosage compensated *LSP1-α* gene of *Drosophila melanogaster* lies immediately downstream of the dosage compensated *L12* gene

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Summary. The X-linked gene *LSP1-α* of *Drosophila melanogaster,* expressed in the third larval instar, does not exhibit dosage compensation at its normal locus but does compensate when it is relocated to ectopic sites on the X chromosome. A transcription unit designated *L12*, which is active in the second larval instar and capable of encoding a putative protein of 28.5 kDa, lies immediately downstream from *LSP1-α*. We have determined that *L12* is dosage compensated by measuring the steady-state level of its transcript in male and female larvae. The difference in response of these two adjacent genes should be taken into consideration when models of the mechanism of dosage compensation are formulated.

Key words: Dosage compensation – *LSP1-α* – *L12* – Drosophila

Introduction

The difference in the dosage of X-linked genes present in males and females of *Drosophila melanogaster* is compensated by an increase in the transcriptional activity of most of these genes in males resulting in comparable levels of gene product in the two sexes (reviewed in Lucchesi and Manning 1987). X-linked genes transposed or transduced to autosomal sites often remain fully or partially compensated while autosomal genes relocated to the X chromosome often gain some level of compensation. These observations suggest that dosage compensation is mediated by compensatory elements distributed along the X chromosome.

Although the X-linked gene *LSP1-α* does not exhibit dosage compensation (Roberts and Evans-Roberts 1979; Brock and Roberts 1982), we have shown that it is capable of compensation when it is relocated to ectopic sites on the X chromosome (Ghosh et al. 1989). This observation suggests that *LSP1-α* normally resides in a chromosomal domain where compensatory elements are not present or where, for some reason, they have no influence on its transcriptional activity. Therefore it seemed of interest to determine whether the genes immediately adjacent to *LSP1-α* exhibit dosage compensation. Here we report the existence of a gene, *L12*, located immediately upstream of *LSP1-α*. Measurements of steady-state RNA in males and females indicate that, in contrast to *LSP1-α*, *L12* is fully compensated.

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Materials and methods

Collection of timed developmental stages. Adults from an Oregon-R strain were maintained at room temperature in population cages under a 10 h light/14 h dark cycle. Eggs were collected on trays of standard cornmeal-molasses-agar medium overlaid with a yeast paste. Embryos from 0–24 h of age, in 3 h increments, first (22–46 h), second (40–70 h) and third (70–120 h) instar larvae, pupae (120–220 h) and adults were obtained by allowing collected eggs to develop at 25° C. To mass isolate second instar larvae of one particular sex, the genetic scheme of Lyttle (1989) and Walker et al. (1989) was employed as described in Polito et al. (1990).

Analytical procedures. DNA was extracted from adult flies as described in Manning et al. (1975). Plasmid and cosmid DNA was purified as described in Maniatis et al. (1982). Agarose gel electrophoresis of DNA, Southern transfer and filter hybridization were performed according to Fouts et al. (1981) and Beard et al. (1988). DNA restriction fragments were radiolabelled with (α-^{32}P)dNTP using a nick-translation kit from Bethesda Research Laboratories (Gaithersburg, Md). Synthetic oligonucleotides were endlabelled with (γ-^{32}P)ATP using T4 polynucleotide kinase Boehringer-Mannheim, Indianapolis, Ind) according to Maniatis et al. (1982). All restriction enzymes were purchased from Boehringer-Mannheim and used as recommended.

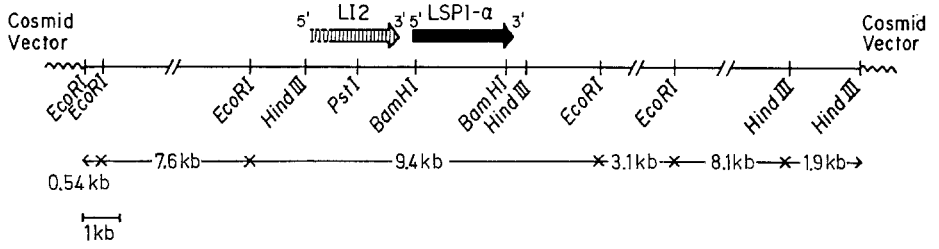

Fig. 1. Schematic diagram of the LSP1-αCos3 clone showing the restriction sites of interest. The *solid line* represents *Drosophila* genomic DNA; the *solid arrow* represents the transcribed region of the *LSP*1-α gene; the *cross-hatched arrow* denotes the transcribed region of the *L12* gene

Total RNA was extracted by the hot phenol/chloroform method (Jowett 1988) and poly(A)$^+$RNA was isolated as described in Levy and Manning (1981). Northern blot analysis was performed by fractionation of the RNA on denaturing 1.1% agarose gels containing formaldehyde followed by transfer to Hybond N membranes (Amersham, Arlington Heights, Ill) and hybridization with radiolabelled DNA probes (Maniatis et al. 1982). For quantitation, appropriate regions of the membrane were excised using an exposed film as template and the radioactivity determined by liquid scintillation counting (Ghosh et al. 1989).

A cDNA library was constructed in phage λgt10, using poly(A$^+$)RNA from second instar larvae, by methods described in Maniatis et al. (1982). The library was screened with the 1.4 kb *Hin*dIII-*Pst*I restriction fragment from the pELBA plasmid of Ghosh et al. (1989). The inserts present in positive phages were subcloned into plasmid BSSK (Stratagene, LaJolla, Calif), and partial restriction maps were established for each insert.

A cosmid library was constructed in the Cosper transformation vector of Pirrotta (1988). The library was screened with a *Hin*dIII fragment containing the *LSP1-α* gene (Ghosh et al. 1989).

Sequencing of cDNA fragments that encode *L12*, inserted into the BSSK vector, was performed by the dideoxynucleotide chain termination method of Sanger et al. (1977). Oligonucleotides were synthesized in the Department of Molecular Biology and Biochemistry, UC-Irvine. The cDNA sequence was edited and analyzed using the IBI Pustell program.

Results and discussion

Based on the restriction endonuclease cleavage pattern, one cosmid clone (LSP1-αCos3) was found to contain the entire *LSP1-α* gene (Fig. 1). Hybridization of this cosmid to Northern blots of RNAs extracted from different developmental stages detected a 2.8 kb transcript in third instar larvae which, on the basis of size and developmental specificity, is likely to be the *LSP1-α* mRNA. In addition, a 1.4 kb transcript, prevalent in second instar larvae, was detected (Fig. 2). Probing of duplicate blots with a 2.0 kb *Eco*RI cDNA fragment containing a portion of the Drosophila glucose-6-phosphate dehydrogenase gene (Zw^+) indicated to us that transcripts synthesized in relatively low abundance would have been detected by our approach in all of the developmental stages tested (Ganguly et al. 1985).

The transcription unit encoding the 1.4 kb RNA was mapped by probing Southern blots of restriction

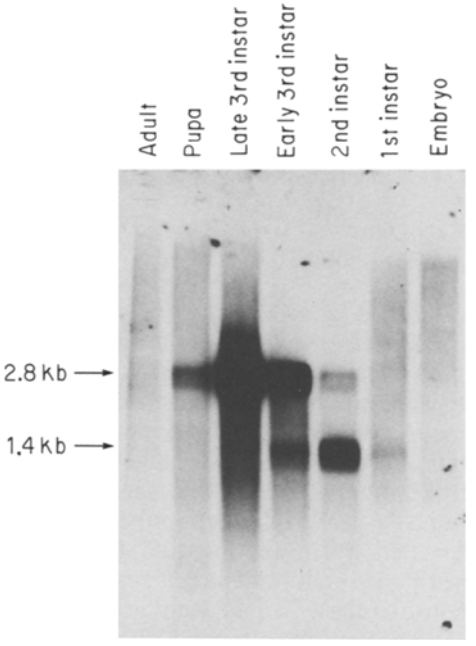

Fig. 2. Developmental Northern blot of *Drosophila* RNA hybridized with LSPI-αCos3 DNA. Poly(A)$^+$RNA (7 μg per lane) isolated from each of the developmental stages indicated was fractionated by electrophoresis, blotted to a nylon filter and hybridized with ^{32}P-labeled LSP1-αCos3 DNA (specific activity = 1×10^9 cpm/μg)

endonuclease digests of LSP1-αCos3 with ^{32}P-labelled poly(A$^+$)RNA isolated from second instar larvae. This transcription unit, designated *L12*, lies within the 1.4 kb *Hin*dIII-*Pst*I and 1.6 *Pst*I-*Bam*HI fragments immediately 5′ to the *LSP1-α* gene (Fig. 1). Using strand-specific transcripts of the 1.4 kb *Hin*dIII-*Pst*I fragment, the orientation of transcription of *L12* was found to be the same as that of the *LSP1-α* gene.

Since no transcription unit was detected downstream of *LSP1-α* in the LSP1-αCos3 insert, a new cosmid (1.9Cos1) was selected by screening the library with the 1.9 kb *Hin*dIII fragment from the distal end of the insert. No sequences from the 1.9Cos1 insert were found to be represented in poly(A)$^+$RNA from the various developmental stages tested. This observation suggests that there are no transcriptional units within approximately 44 kb of DNA 3′ to *LSP1-α*. Clearly, we cannot rule out the existence of genes encoding very rare RNAs such as those found in low abundance in specific tissues.

A cDNA library from second instar larvae was screened with the 1.4 kb *Hin*dIII-*Pst*I genomic fragment containing the 5′ end of *L12* and three positive clones were identified. The complete nucleotide sequence of the

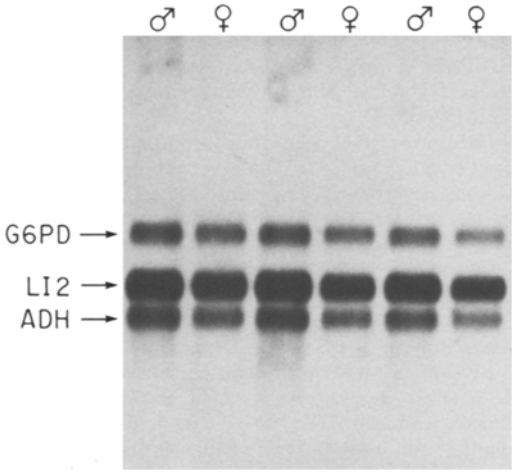

Fig. 3. Determination of the relative steady-state level of *L12* RNA in male and female second instar larvae of *Drosophila*. A northern blot containing either male or female poly(A)$^+$RNA (7 μg per lane) isolated from second instar larvae was hybridized with ^{32}P-labeled *L12* DNA, ADH DNA and G6PD DNA. The areas of the blot containing the hybridizing bands were excised and the radioactivity determined. The results are shown in Table 1

Table 1. Analysis of steady-state levels of *L12* transcripts in males and females

Ratios of cpm hybridized in males			Ratios of cpm hybridized in females		
L12 / G6PD	*L12* / ADH	G6PD / ADH	*L12* / G6PD	*L12* / ADH	G6PD / ADH
2.62 ±0.07	1.78 ±0.04	0.68 ±0.03	2.52 ±0.17	1.87 ±0.04	0.76 ±0.02

Male/female ratio		
L12 / G6PD	*L12* / ADH	G6PD / ADH
1.03	0.95	0.89

Areas showing hybridization in the Northern blot in Fig. 3 were excised and their radioactivities were determined. An area of membrane of equivalent size was excised from a region in which no hybridization was observed, and assayed to determine background radioactivity. ^{32}P-labeled probes used were: *L12*, 1.4 kb *Hind*III-*Pst*I fragment of LSP1-αCos 3; ADH, *Sac*I fragment; G6PD, *Eco*RI fragment from λDmC20. Three independent pairs of male and female RNA samples were measured. The means of these measurements and their standard deviations are presented

longest cDNA insert (1265 bp) was determined (data to be published elsewhere). This sequence, representing approximately 90% of the sequences present in the 1.4 kb *L12* transcript, reveals a single long open reading frame with a codon usage in excellent agreement with that of several Drosophila genes (O'Connell and Rosbash 1984; Marsh et al. 1986; Fouts et al. 1988). The consensus polyadenylation sequence in the *L12* cDNA is located 871 bp 5′ to the transcriptional start site of the *LSP1-α* gene (Jowett 1985).

To determine whether *L12* exhibits dosage compensation, Northern blots of poly(A$^+$)RNA isolated from male or female second instar larvae were probed with a ^{32}P-labelled 1.4 kb *Hind*III-*Pst*I fragment from the LSP1-αCos3 genomic insert to detect the *L12* transcript, as well as with an 11.8 kb *Sac*I fragment of the *Adh*$^+$ gene encoding alcohol dehydrogenase (Goldberg 1980), and a 2.0 kb EcoRI fragment from λDmC20 containing a portion of the *Zw*$^+$ gene insert to detect the G6PD message (Ganguly et al. 1985). The latter two transcripts are expected to occur in equal amounts in males and females since *Adh*$^+$ is an autosomal gene and *Zw*$^+$ is X-linked and is known to be fully dosage compensated. These transcripts serve as internal controls to allow for correction of the differences in the relative amount of mRNA loaded onto the gels or transferred to the Northern blots (Fig. 3). Male to female ratios of the *L12* transcript, normalized with either the alcohol dehydrogenase or the G6PD transcripts, are approximately equal to 1.0 (Table 1). Therefore, it is reasonable to conclude that the *L12* gene is dosage compensated.

A number of rationalizations can be provided to explain why two adjacent genes may differ with respect to dosage compensation. For example, the elements which are responsible for the compensation of the *L12* gene may not influence *LSP1-α* because their effect is narrowly localized and does not extend even to closely adjacent genes. Alternatively, these regulatory elements may exhibit developmental specificity and function only when the gene is transcribed (in the case of *L12* and *LSP1-α* maximal gene activity occurs at distinctly different stages of larval development). A third possibility is that sequences are present which shield the *LSP1-α* gene from the long-ranging effects of the regulatory elements. While additional experiments are needed to test these possibilities, it is important to recognize that closely adjacent genes can differ with respect to dosage compensation.

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