Cloning and Characterization of the scute (sc) Gene of Drosophila subobscura

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ABSTRACT

The scute (sc) gene, a member of the achaete-scute complex of Drosophila melanogaster, has dual functions: sisterless (sis-b) function required for sex determination and dosage compensation and scute function, which is involved in neurogenesis. The sc homologue of D. subobscura was cloned. It lacks introns and encodes a single 1.7-kb transcript slightly larger than that of D. melanogaster (1.6 kb). The Sc protein of D. subobscura is slightly larger than that of D. melanogaster (382 vs. 345 amino acids). Sequence comparisons between both species show the Sc protein to have a highly conserved bHLH domain. Outside this domain, amino acid replacements are not randomly distributed. Two additional conserved domains, of 20 and 36 amino acids, are present near the C-terminal end. They may represent domains conferring specificity upon the Sc protein with respect to other proteins of the achaete-scute complex. In its 3’ untranslated region, Sc RNA contains uridine stretches, putative Sxl protein DNA-binding sites. The D. subobscura Sc protein can cooperate with other D. melanogaster bHLH proteins because D. subobscura sc supplies sis-b function when introduced into D. melanogaster transgenic flies mutant for sc.

The gene scute (sc), a member of the achaete-scute complex of Drosophila melanogaster, has a dual function: the sisterless (sis-b) function required for sex determination and dosage compensation (reviewed in PARKHUST and ISH-HOROWICZ 1992; CLINE 1993; SÁNCHEZ et al. 1994) and the scute function, which is involved in neurogenesis (reviewed in GHYSEN and DAMBLY-CHAUDIÈRE 1989; CAMPUSANO and MODOLELL 1992; CAMPOS-ORTega 1993).

In D. melanogaster, sex determination and dosage compensation (the products of the X-linked genes are present in equal amounts in females and males) are under the control of the gene Sex-lethal (Sxl). The functional state of this gene is determined by the X:A signal (ratio of X chromosomes to sets of autosomes), so that in 2X;2A females, Sxl will be ON, while in X:2A males Sxl will be OFF (CLINE 1978). One of the genes that make up the X:A signal is sc (TORRES and SÁNCHEZ 1989; PARKHUST et al. 1990; ERICKSON and CLINE 1991). Early in development, this gene shows uniform expression (ROMANI et al. 1987; CABRERA et al. 1987) coincident with the developmental stage at which the X:A signal triggers the activation of the gene Sxl (SÁNCHEZ and NÖTHLINGER 1983; BACHILLER and SÁNCHEZ 1991). This uniform expression of sc corresponds to its sis-b function (TORRES and SÁNCHEZ 1991; ERICKSON and CLINE 1993). This is expected because the gene Sxl must be active in every cell of female embryos, but inactive in every cell of male embryos, for proper adjustment of the dosage compensation process in the two sexes. The sis-b function of sc is not subjected to dosage compensation because its uniform expression precedes the activation of Sxl. Dosage compensation is established once the functional state of Sxl is determined. For this reason, the gene sc can act as a “numerator” element of the X:A signal, so that female embryos would have twice the amount of Sc product compared with male embryos.

After blastoderm, the expression of sc is restricted to groups of cells that will become the precursors of the central nervous system or the precursors of the sensory organs that form the peripheral nervous system of larvae and adults (ROMANI et al. 1987, 1989; CABRERA et al. 1987; CABRERA 1990; MARTÍN-BERMUDO et al. 1991; CUBAS et al. 1991; SKEATH and CARROLL 1991). This restricted expression of sc corresponds to its scute function and it is dosage-compensated to avoid differences between the sexes.

The gene sc lacks introns and encodes a basic helix-loop-helix (bHLH) protein (VILLARES and CABRERA 1987). Proteins with the bHLH motif are transcriptional regulators whose activity depends on homo- or heterodimerization with other HLH proteins (MURRE et al. 1988a, b). Association of a particular bHLH protein with different members of the family gives rise to dimers that differ in their affinity for DNA-binding sites (MURRE et al. 1989b; BEZELRA et al. 1990; SUN and BALTIMORE 1991). The gene daughterless (da), which is involved in Sxl activation (CLINE 1978; CRONMILLER and CLINE 1987) and in neurogenesis (CAUDY et al. 1988a), also encodes a bHLH protein (CAUDY et al. 1988b; CRONMILLER et al. 1988). It has been shown both in vitro and in vivo that Sc and Da proteins interact directly to form
transcriptionally active complexes, which constitute the common molecular basis for the two functions of the gene sc (Cabrera and Alonso 1991; Van Doren et al. 1991; Deshpande et al. 1995; Liu and Belote 1995).

This paper reports the cloning and characterization of the scute gene of D. subobscura and its comparison with scute of D. melanogaster. These two species appear to have diverged ~50 mya (Throckmorton 1975). This evolutionary period is sufficiently distant for unconstrained sequences to have diverged extensively, so that the comparative analysis of the gene sc from both species will allow the identification of putative functional elements by sequence conservation.

MATERIALS AND METHODS

Fly strains: Flies were cultured on standard food at 25°C (D. melanogaster) or 18°C (D. subobscura). For a description of the chromosomes and mutations of D. melanogaster, see Lindsley and Zimm (1992).

Cloning the sc gene of D. subobscura: The DmBar111 genomic library of D. subobscura was kindly provided by M. Aguade. This genomic library was screened with a full-length cDNA of the D. melanogaster sc gene. Hybridization was performed under stringent conditions (68°C, 5X SSC, 1% SDS). Washes were repeated twice at 65°C in 0.1X SSC, 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 the plasmids pUC18 and isolation of plasmid DNA were performed using the protocols described by Maniatis et al. (1982).

In situ hybridization: The chc strain of D. subobscura (with the standard X chromosome arrangement) and the Canton-S strain of D. melanogaster were used to prepare salivary gland polytene chromosomes slides. Female third-instar larvae were chosen from uncrowded cultures raised at 17°C. Conditions for larva dissection and polytene chromosomes squashes were as described by Secarra and Aguiade (1993). The probe was determined using the Künze-Mohl and Muller (1958) cytological map of D. subobscura, and the cytological and photographic maps of D. melanogaster of Leffvre (1976).

P-element-mediated germline transformation: The 3.5-kb EcoRI fragment containing the D. subobscura sc gene was cloned into the P-element transformation vector pWB (Klemenz et al. 1987). Germ-line transformants were obtained by standard procedures (Spradling 1986).

Transcript analysis: RNA preparation from frozen pupae, 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 prehybridized at 42°C overnight in 30 ml of 50% formamide, 5X SSC, 0.25% sodium dodecylsulfate (SDS), 5X Denhardt's solution and 0.1 mg/ml denatured salmon sperm DNA. Hybridization was carried out at 42°C for 20 hr in 10 ml of prehybridization solution containing the 32P-dCTP labeled probe. Filters were washed twice with 0.1X SSC, 0.1% SDS at 65°C.

DNA sequencing: The two 1.7- and 1.8-kb EcoRI-SalI subfragments cloned in pUC18 were sequenced from both sides by the dideoxynucleotide method (Sanger et al. 1977) using modified T7 polymerase from Pharmacia and 10 μCi of 32P-dATP as labeling precursor. To complete sequencing of both chains of sc, a series of new subclones from the 1.7- and 1.8-kb fragments was derived. Selected subclones were sequenced with the universal and reverse M13 primers (Pharmacia). From this sequence, a series of synthetic oligonucleotides were designed to fill the gaps and conflictive regions of both DNA chains. Electrophoresis was performed with a LKB-Pharmacia Macrophor chamber using 6% acrylamide and 7.5 M urea following the manufacturer's instructions. The accession number for the D. subobscura sc gene is X96479 in the EMBL Nucleotide Sequence Database.

RESULTS

Isolation of the scute homologue from D. subobscura: The genomic library of D. subobscura was screened using D. melanogaster sc cDNA as a probe under highly stringent hybridization conditions (see MATERIALS AND METHODS). Two recombinant phages were isolated and their restriction patterns showed that both were the same (λ-sc-sub). Southern blot analysis of λ-sc-sub DNA showed that a 3.5-kb EcoRI fragment had hybridized with the D. melanogaster sc cDNA probe. Because this fragment is situated at one end of the genomic insert, contiguous to the long arm of the λEMBL4 phage, it was cloned into the Bluescript KS+ plasmid. In addition, the two EcoRI-SalI fragments of 1.7 and 1.8 kb, derived from the 3.5-kb EcoRI fragment, were cloned in pUC18. Restriction and Southern blot analysis of these three subclones delimited the sc homologous region to an internal 1.8-kb fragment (see Figure 1). If the sc homologue of D. subobscura had indeed been isolated, it was taken that the complete transcription unit of this gene had been cloned. As in D. melanogaster, sc lacks introns (Villares and Cabrera 1987).

The Sc protein contains the bHLH motif of a family of transcriptional regulators (reviewed in Garrell and Campuzano 1991; Jan and Jan 1993). It is possible, therefore, that the sc homologous gene was not cloned but rather another that codes for a protein that also contains this motif. To provide supporting evidence that the λ-sc-sub clone represents the sc homologue of D. subobscura, in situ hybridization of this clone to salivary gland polytene chromosomes of D. melanogaster was performed. The results shown in Figure 2B show that this clone exclusively hybridizes in cytogenetic bands 1B1–7, which is the locus for the achaete-scute complex that contains sc. Other genes in this complex, achaete (ac) and lethal-of-scute (lsc), also contain the bHLH motif (reviewed in Garrell and Campuzano 1991). However, λ-sc-sub probably represents neither ac nor lsc homologues of D. subobscura because, under the hybridization conditions used to screen the D. subobscura genomic library, the sc gene of D. melanogaster does not hybridize with the ac or lsc genes of this species. Therefore, it cannot be expected to hybridize with either ac or lsc homologues from D. subobscura. It can be concluded that the 3.5-kb EcoRI fragment of the λ-sc-sub genomic clone contains the sc homologue from D. subobscura.
Figure 1.—Restriction map of the *D. subobscura* recombinant phage λ-sc-sub. The 3.5-kb EcoRI fragment homolog of the *D. melanogaster* sc gene is represented by the thick lines (upper map). λ, λEMBL4 phage arms. Underneath, the 3.5-kb EcoRI fragment is represented. The sequenced 1.7-kb PstI region is represented by thick lines. R, EcoRI; S, SalI; B, BamHI; P, PstI; and Sp, Sphi.

**FIGURE 2A.** In situ hybridization of the λ-sc-sub clone to salivary gland polytene chromosomes of *D. subobscura*. The *sc* locus in this species is in cytogenetic band 2B of chromosome *A*, which is the homolog of the X chromosome of *D. melanogaster*. Thus, the *sc* locus has changed from a quasitelomeric position in *D. melanogaster* to a position near the centromere in *D. subobscura*. In this species, the yellow gene (SEGARRA et al. 1995) and the ac gene (A. MUNTE, personal communication) are also located in cytogenetic band 2B of chromosome *A*. Therefore, it seems that the *achaete-scute* complex was not broken during divergence of *D. melanogaster* and *D. subobscura*. Moreover, the close linkage between yellow and the *achaete-scute* complex is maintained in both species.

**Analysis of *D. melanogaster* transgenic lines carrying the *D. subobscura sc* gene:** As an additional test to confirm that the 3.5-kb EcoRI fragment of the λ-sc-sub clone contained the *sc* gene of *D. subobscura*, P-element germline transformation was used to introduce the genomic fragment into *D. melanogaster* (see MATERIALS AND METHODS). Tests were then performed to determine whether the *D. subobscura sc* transgene (named *sc*™) was able to provide the *sis-b* function in *D. melanogaster* flies mutant for *sc*. First, the viability of females double heterozygous for *sc™/sc™* and *sis-b/sis-b* mutations were studied. *sc™/sc™* females have reduced viability because they cannot activate the gene *Sxl* due to alteration in the X:A signal (TORRES and SANCHEZ 1989; ERICKSON and CLINE 1991). Both *sc* and *sis-a* are "numerator" elements of this signal (CLINE 1986; TORRES and SANCHEZ 1989; PARKHURST et al. 1990; ERICKSON and CLINE 1991). The results in Table 1 (cross A) show that *sc™/sis-b* females have reduced viability but are fully viable when they carry the *sc™* transgene. The same results were obtained when another *sc™* transgenic line was used (data not shown). Second, the viability of males carrying simultaneous duplications for *sc* and *sis-a* were studied. These males show drastically reduced viability as a consequence of *Sxl* activation (CLINE 1988; TORRES and SANCHEZ 1989; ERICKSON and CLINE 1993). The results in Table 1 (cross B) show that males carrying two doses of *sis-a* and two doses of *sc*, the *sc* of *D. melanogaster* and the *sc™* transgene, have reduced viability. This viability, however, is recovered if the males carry a loss-of-function *Sxl* mutation instead of the *Sx™* allele. This indicates that the males die because *Sxl* is activated. These results, then, demonstrate that the *sc™* transgene

**FIGURE 2B.** In situ hybridization of the *D. subobscura* recombinant phage λ-sc-sub on polytene chromosomes of *D. subobscura* (A) and *D. melanogaster* (B). The hybridization signal is marked by the arrowhead.
TABLE 1

The scute (sc) gene of D. subobscura supplies sisb+ function in D. melanogaster

<table>
<thead>
<tr>
<th>Cross A: sc^{10.1} y^{30a}/FM6 y(sis-a,sc^{subl}), sc^+ /SM5</th>
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</thead>
<tbody>
<tr>
<td>No. of control females</td>
</tr>
<tr>
<td>Viability of experimental females</td>
</tr>
<tr>
<td>With sc^{subl}</td>
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<tr>
<td>Without sc^{subl}</td>
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</tbody>
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<table>
<thead>
<tr>
<th>Cross B: cm Sxl^+/FM6, sc^{subl}, sis-a+/CyO Y \times \text{Df}(1)N71/v^+ Y y^+, sis-a^+</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of control males</td>
</tr>
<tr>
<td>Viability of experimental males</td>
</tr>
<tr>
<td>( \frac{sc^+ Sxl^+ sis-a^+}{v^+ Y y^+, sis-a^+} )</td>
</tr>
<tr>
<td>( \frac{sc^+ Sxl^+ sis-a^+ sc^{subl}}{v^+ Y y^+, sis-a^+} ) +</td>
</tr>
<tr>
<td>( \frac{sc^+ Sxl^+ sis-a^+ sc^{subl}, sc^+}{v^+ Y y^+, sis-a^+} ) +</td>
</tr>
</tbody>
</table>

Viability of experimental females with and without the sc^{subl} transgene were compared with control females with and without the transgene, respectively.

supplies siSB+ function in D. melanogaster. This confirms that the 3.5-kb EcoRI fragment contained the gene sc of D. subobscura.

The low viability and extreme scute phenotype of sc^{10.1} mutant males were the same whether or not they carried the sc^{subl} transgene (data not shown). Moreover, this transgene does not suppress the lethality of males carrying a deficiency of scute (data not shown). Thus, the sc^{subl} transgene does not provide the scute function in D. melanogaster, though it supplies the siSB+ function (see DISCUSSION).

Transcript analysis of the sc gene from D. subobscura: In D. melanogaster, sc encodes a single transcript of 1.6 kb (CAMPUZANO et al. 1985; VILLARES and CABRERA 1987; ALONSO and CABRERA 1988). It is expressed in a generalized way in the syncytial blastoderm and in a restricted way in groups of cells in postblastoderm embryos and during most of the third-instar larval and early pupal stages (CABRERA et al. 1987; ROMANI et al. 1987). Analysis was made of the transcripts of the D. subobscura sc gene by Northern blot tests of total RNA from early pupae. Early pupae of D. melanogaster were used as controls. The probes used were either the 1.7- or 1.8-kb EcoRI-SalI fragments of D. subobscura (see Figure 1). Both probes detected a single transcript in D. subobscura of 1.7 kb, which is slightly larger than the transcript of D. melanogaster (1.6 kb; see Figure 3).

DNA sequence of the sc homologous gene of D subobscura: The complete sequence of the 1.7-kb PstI fragment corresponding to the sc gene of D. subobscura is shown in Figure 4. Comparison of this sequence with that of D. melanogaster (VILLARES and CABRERA 1987) identified the putative coding region. The first ATG of the long open reading frame was taken to be the translation start codon. Accordingly, the sequence includes the 5' flanking region (170 bp), the coding region with a unique exon (1146 bp) and the 3' flanking region (459 bp). A putative TATA box starting at position 63 was detected in the 5' flanking region. The polyadenylation signal was also identified in the 3' flanking region from position 1649 to 1654. Sequence divergence interferes with the alignment of the scute 5' and 3' flanking regions. However, it is noteworthy that in the 3' flanking region there is a stretch of 24 bp that is completely conserved in both species (Figure 4). This stretch is included in a longer sequence (47 bp) with only a transition and a single nucleotide addition/deletion. No homologous sequences to this conserved stretch were found in sequence data banks. The putative function of this sequence therefore remains unknown.

The 3' untranslated region (UTR) of the Sc transcript contains four putative Sxl-binding sites: three Us

![Figure 3](image-url)
and one U₉ stretches. They correspond to the thymidine stretches of Figure 4 (bold and underlined). In  D. melanogaster, the Sc transcript contains two U₉ and one U₉ stretches in its 5' UTR. These untranslated stretches have been also found in the 5' and 3' UTR of other transcripts from  D. melanogaster X-linked genes, which are dosage-compensated. It is believed that the Sxl protein binds to these untranslated stretches and causes an approximately twofold decrease in the translation or stabilization of these transcripts (see DISCUSSION).
The putative coding region of the \textit{sc} gene of \textit{D. subobscura} encodes for a protein with 382 amino acids. This protein has 37 residues more than that of \textit{D. melanogaster} (345 amino acids), which agrees with the slightly larger size of the \textit{D. subobscura} transcript (Figure 5). Alignment of the Scute proteins of \textit{D. subobscura} and \textit{D. melanogaster} includes 10 additions/deletions of variable sizes which account for the length difference (Figure 5). Both proteins show a 68.28\% identity. Amino acid replacements are not randomly distributed along the aligned proteins because regions with high similarity are separated by regions with strong divergence. The longest conserved region, which has 82 amino acids with only two replacements, includes the bHLH domain characteristic of DNA-binding proteins. This domain is also present in the proteins encoded by other genes of the AS-C complex, \textit{ac}, \textit{t'sc} and \textit{ase} (ALONSO and CABRERA 1988). Comparison of the bHLH domain within these proteins (Figure 6) indicates that the highest identity corresponds to the scute proteins of \textit{D. subobscura} and \textit{D. melanogaster}; this further indicates that they are homologous.

Apart from the bHLH motif, other conserved regions are present in the Scute proteins of \textit{D. subobscura} and \textit{D. melanogaster} (Figure 5). To determine whether these regions are characteristic of the Scute functional product or correspond to other domains conserved among members of the AS-C complex, the Scute proteins have been compared with those encoded by the \textit{ac}, \textit{t'sc} and \textit{ase} genes of \textit{D. melanogaster}. The conserved region DDEEILDYISM(L)WQEQ at the C-terminal end of both Scute proteins corresponds to the acidic domain of 15 amino acids with a central tyrosine described by ALONSO and CABRERA (1988) and shared by the proteins encoded by the \textit{sc}, \textit{ac} and \textit{t'sc} genes of \textit{D. melanogaster}. However, in the scute proteins of \textit{D. subobscura} and \textit{D. melanogaster}, this domain is included in a much longer conserved region of 38 amino acids with only one amino acid replacement (Figure 5). In addition, the conserved region QQQQQLQ also corresponds to a conserved domain also present with minor differences in the Achaete (VILLARES and CABRERA 1987) and Asense proteins. The other conserved regions (Figure 5) seem to be exclusive to the Scute protein.

Nucleotide alignment of the \textit{scute} coding region of \textit{D. subobscura} with that of \textit{D. melanogaster} was obtained using the corresponding protein alignment as a reference. The number of substitutions per position was estimated separately for synonymous and nonsynonymous positions according to 11 (1993). The corrected estimate for synonymous positions is K_\text{s} = 2.1196 \pm 0.4121 (mean \pm SE). That for nonsynonymous positions is K_\text{a} = 0.2814 \pm 0.0228. These estimates were also obtained considering only the nucleotide region that encodes for the bHLH protein motif (K_\text{s} = 2.3601 \pm 0.1367 and K_\text{a} = 0.0182 \pm 0.0107). The corrected estimates of synonymous and nonsynonymous substitutions per position for the \textit{scute} coding region between \textit{D. subobscura} and \textit{D. melanogaster} are high when compared with other genes sequenced in representatives of the melanogaster and obscura groups (SEGARRA and AGUADÉ 1993). The number of synonymous substitutions does not differ significantly (x^2 = 1.17, 1 d.f., P > 0.2) between the complete gene and the region encoding the bHLH domain. Therefore, the high rate of synonymous substitutions also affects the bHLH region with a high constraint against amino acid replacements, reflected by its low K_\text{s} estimate.

**DISCUSSION**

The \textit{sc} homologue of \textit{D. melanogaster} was cloned in \textit{D. subobscura}. In agreement with its role, the bHLH domain of the Sc proteins of both species is highly conserved. bHLH proteins can associate with themselves and/or other members of the family to form different complexes with distinct transcriptional specificities; the protein-protein interaction being carried out through the HLH motif and the DNA interaction through the basic region (MURRE et al. 1989a,b; DAVIS et al. 1990). This conserved domain explains the capacity of the \textit{sc}\textsuperscript{\textit{dub}} transgene to supply \textit{sis-b} function in \textit{D. subobscura}. In agreement with its role, the bHLH domain of the Sc proteins of both species is highly conserved. bHLH proteins can associate with themselves and/or other members of the family to form different complexes with distinct transcriptional specificities; the protein-protein interaction being carried out through the HLH motif and the DNA interaction through the basic region (MURRE et al. 1989a,b; DAVIS et al. 1990). This conserved domain explains the capacity of the \textit{sc}\textsuperscript{\textit{dub}} transgene to supply \textit{sis-b} function in \textit{D. subobscura}.
D. melanogaster. This is so because the D. subobscura Sc and D. melanogaster Da proteins are able to interact through their HLH domains to form functional complexes that provide the sis-b function.

Outside the bHLH domain, amino acid replacements are not randomly distributed along the protein; highly conserved domains are separated by regions of great divergence. This pattern of divergence between D. subobscura and D. melanogaster has also been described for the zerknullt gene of D. subobscura, a homeobox-containing gene of the Antennapedia complex (TEROL et al. 1995). Similar results have been found for genes controlling development in other interspecific comparisons, mainly between D. melanogaster and D. virilis (TREIER et al. 1989; HEBERLEIN and RUBIN 1990; among others). However, sequence divergence between these two species is expected to be higher because they diverged before D. subobscura and D. melanogaster (BEVERLEY and WILSON 1984). The Sc proteins of D. subobscura and D. melanogaster exhibit 68.28% identity. However, the identity reduces to 58.63% when the region including the bHLH domain is not considered. According to this level of protein identity, the other conserved regions detected in the aligned proteins may present strong constraints to variation. These constraints are doubtlessly acting on the domains shared by proteins encoded by other members of the AS-C complex, among which protein identity is much lower. However, they may also act on conserved regions exclusive of the Sc protein. Thus, these regions (MPIKTRKYTPRMAL, QLQLCLDE, CHSPTSSSNSMFDSTYE and QLQ-LKFEPEYFHQLDEEDCTP) might be functionally important sequences that confer specificity to the Scute protein with respect to other proteins of the AS-C complex.

The sc gene of D. subobscura supplies sis-b, but not sc function in D. melanogaster. This cannot be explained by the existence of two Sc proteins from sc of D. subobscura, one providing sis-b function and the other contributing to sc function, with the sc subtransgene exclusively supplying the Sis-b protein. As in D. melanogaster, sc of D. subobscura lacks introns and encodes a single transcript with a unique open reading frame. Moreover, it has been shown in D. melanogaster that all mutations known to affect the coding region of sc affect both sis-b and sc functions (TORRES and SÁNCHEZ 1989). Therefore, a unique Sc protein must be responsible for the dual function of sc. Accordingly, the two functions of this gene share a common molecular basis: the complexes between Sc and Da proteins are responsible for the two functions of sc. Because the sc subtransgene supplies sis-b function, it has also the capacity to provide sc function. The lack of sc function by the sc subtransgene is therefore explained by the different way in which sc expression is controlled in relation to its two functions. Whereas the sis-b function corresponds to a generalized expression of sc, its scute function correlates with the restricted expression of this gene in certain groups of cells, the precursors of the nervous system. It has been shown that this restricted expression is controlled by cis-acting elements that extend 4–5 kb upstream and 50 kb downstream of sc (CAMPANUZANO et al. 1985; RUTZ-GÓMEZ and MODOLELL 1987; GÓMEZ-SKARMETA et al. 1995). These cis-acting elements are not required for the generalized expression of sc as in D. melanogaster all of the mutations and/or rearrangements involving these elements affect sc but not sis-b function (CLINE 1988; TORRES and SÁNCHEZ 1989). The size of the sequences flanking the sc subtransgene in the 3.5-kb genomic fragment, used for generating the D. melanogaster transgenic lines, would not contain the cis-acting elements of D. subobscura needed for controlling the scute function of sc.

The 3' UTR region of D. subobscura Sc RNA contains four putative Sxl-binding sites: three U9 and one U6 stretches, corresponding to the thymidine stretches in the sequence shown in Figure 4. In D. melanogaster, Sc RNA contains two U9 and one U6 stretches in its 3' UTR. These U-stretches are also present in the 5' and/or 3' UTR of other RNAs from D. melanogaster X-linked genes, which are dosage-compensated. It has been proposed that the Sxl protein would bind to these U-stretches and cause an approximate twofold decrease in the translation or stabilization of these transcripts. Consequently, a similar level of X-linked transcripts that are translated is attained in both sexes, though males have one and females have two doses of these X-linked genes. This would constitute the molecular basis for the msx-independent Sxl-dependent dosage compensation process (KELLEY et al. 1995) that takes place early in development of D. melanogaster (GERGEN 1987). The fact that the Sc RNA of both species maintains these U stretches in the 3' UTR agrees with their importance in the function of the gene sc. These results support the idea of these U motifs being involved in the early dosage compensation of the gene sc and, presumably,
of other X-linked genes which contain these U motifs in their 5' and/or 3' UTR regions (this early dosage compensation being controlled by the Sxl protein through its interaction with the U stretches). This would further suggest that in D. subobscura a similar mechanism of early dosage compensation in females might be at work.

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