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The sisterless-b function of the *Drosophila* gene *scute* is restricted to the stage when the X:A ratio determines the activity of *Sex-lethal*

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Summary

The gene *scute* (*sc*) has a dual function: the scute function which is involved in neurogenesis and the sisterless-b function which is involved in generating the X:A signal that determines the state of activity of *Sxl*, a gene that controls sex determination and dosage compensation. We show here that the lethal phase of sc^- females is embryonic and caused by the lack of *Sxl* function. We also analyze the time in development when *sc* and *Sxl* interact by means of (a) determining the thermosensitive phase (TSP) of the interaction between Sxl and *sc* and (b) a chimeric gene in which *sc* is under the control of a heat-shock promoter (*HSSC-3*). Pulses of *sc* expression from the *HSSC-3* activate *Sxl* only at a very specific and early stage in development, which coincides with the TSP of the interaction between *sc* and *Sxl*. It corresponds to the syncytial blastoderm stage and coincides with the time when the X:A signal regulates *Sxl*. At this stage sc undergoes a homogeneous transient expression in wild-type flies. We conclude that the sc expression at the syncytial blastoderm is responsible for its *sisterless-b* function. Since *sc* expression from the *HSSC-3* fully suppresses the *sisterless-b* phenotype, we further conclude that the *sisterless-b* function is exclusively provided by the *sc* protein. Finally, we have analyzed, by *in situ* hybridization, the effect of *sc* and *sis-a* mutations on the embryonic transcription of *Sxl*. Our results support the view that the control of *Sxl* by the X:A signal occurs at the transcriptional level.

Key words: *Drosophila*, sex determination, *scute*, *sisterless-b*.

Introduction

In *Drosophila melanogaster* 2X;2A individuals (X, X chromosome; A, haploid autosomal set) are female and XY;2A individuals are male. The Y chromosome does not play any role in sex determination. This process occurs by the sex-specific expression of a group of genes hierarchically organized (reviewed in Nöthiger and Steinmann-Zwicky, 1985; Baker, 1989; Hodgkin, 1989; Steinmann-Zwicky *et al.* 1990). The regulation of these genes takes place during all development and in adult life by alternative splicing of their transcripts (Bell *et al.* 1988; Boggs *et al.* 1987; Burtis and Baker, 1989). Dosage compensation (hypertranscription of the male X chromosome) is a process linked to sex determination (reviewed in Lucchesi and Manning, 1987). By means of this process, the products of the X-linked compensated genes are present at the same levels in females and males. Both sex determination and dosage compensation are triggered by a common initial signal: the ratio between the number of X chromosomes and the number of autosomal sets in each cell (X:A) (Bridges, 1925; Maroni and Plaut, 1973). The X:A ratio determines the state of activity of the gene *Sex-lethal* (*Sxl*): in females *Sxl* will be ON, while in males *Sxl* will be OFF (Cline, 1978). Activation of *Sxl* also requires the maternal *daughterless* (*da*) product (Cline, 1978). Once the state of activity of *Sxl* is defined, which occurs around the blastoderm stage, the X:A ratio is no longer relevant, and both sex determination and dosage compensation come under the control of *Sxl* (Sánchez and Nöthiger, 1983; Bachiller and Sánchez, 1991). The capacity of this gene to function as a stable genetic 'switch' is due to a positive autoregulatory function of the *Sxl* product (Cline, 1984; Bell *et al.* 1991). The regulation of the gene *Sxl* throughout most of development and in adult life occurs by alternative splicing of its primary transcripts: the male transcripts give rise to inactive truncated proteins due to the presence of a translation stop codon in an additional exon (Bell *et al.* 1988); in contrast, this exon is spliced out from the female transcripts and, consequently, functional *Sxl* proteins are produced. The gene *fl(2)d* is required for female-specific splicing of *Sxl* transcripts (Granadino *et al.* 1990). *Sxl* controls both sex determination and dosage compensation by regulating two independent sets of genes (Lucchesi and Skripsky, 1981). Failures in dosage compensation, producing hypertranscription in females or hypotranscription in males, are lethal (Cline, 1978; Lucchesi and Skripsky, 1981). Sex determination is not a vital process, and so, failures in sex determination lead to viable sex transformed pheno-

types (reviewed in Baker and Belote, 1983 and in Nöthiger and Steinmann-Zwicky, 1985). For these reasons, misexpression of *Sxl* can produce sex-specific lethality and/or sexual transformation to either males or females.

The genetic basis of the X:A signal is unknown. It is thought that it results from the interaction between X-linked elements, 'numerator elements', and autosomal elements, 'denominator elements'. So far, two numerator elements of this signal have been identified, *sisterless-a* (*sis-a*) (Cline, 1986) and a region of the *achaete–scute* complex (AS-C) that has been named sisterless-b (*sis-b*) (Cline, 1988) and which corresponds to the gene *scute* (*sc*) (Torres and Sánchez, 1989). Recently, two sets of experiments have confirmed that the gene *sc* is responsible for the sis-b function. On the one hand, it has been shown that misexpression of *sc* induces ectopic *Sxl* expression and male-specific lethality (Parkhurst *et al.* 1990). On the other hand, transformation experiments with wild-type and modified *sc* transgenes have shown that both the rescue of females lacking *sis-b* function and the lethality of males due to inappropriate *Sxl* expression, results from the presence of wild-type *sc* transgenes (Erickson and Cline, 1991). Thus, the gene *sc* has a dual function: the sc function, which is involved in neurogenesis (reviewed in Ghysen and Dambly-Chaudière, 1988), and the sis-b function which is involved in generating the X:A signal (Torres and Sánchez, 1989; Parkhurst *et al.* 1990; Erickson and Cline, 1991; this report).

The *sc* gene shows two patterns of embryonic expression: an expression during syncytial blastoderm leading to an homogeneous distribution of the *sc* transcript, and a postblastodermal expression restricted to the regions from which the precursors of the nervous system will differentiate (Romani *et al.* 1987; Cabrera *et al.* 1987). The temporal aspects of the regulation of *Sxl* by the X:A signal were not explored in the experiments reported by Parkhurst *et al.* (1990) and Erickson and Cline (1991). Here, by means of a *hsp70–sc* chimeric gene (*HSSC-3*) (Rodríguez *et al.* 1990), we have administered pulses of *sc* expression to determine the temporal specificity of its *sis-b* function. Our results show that the ectopic *sc* product is able to activate *Sxl* only at the syncytial blastoderm stage. We also present evidence of the transcriptional control of *Sxl* by the elements of the X:A signal.

Materials and methods

Culture conditions

Flies were raised on standard *Drosophila* medium under non-crowded conditions. The temperature of cultures was 25°C unless otherwise stated. For full description of markers and chromosomes used, see Lindsley and Zimm (1985, 1987, 1990). For description of the *hsp70–sc* chimeric gene construction and transformation, see Rodríguez *et al.* (1990).

Cuticular preparations

Flies were macerated in KOH 10% at 50°C and the cuticle was mounted in Faure's solution.

In situ *hybridization to whole-mount embryos*

Embryos were hybridized with the h1 genomic fragment (Bell *et al.* 1988) that detects all the *Sxl* transcripts (Salz *et al.* 1989). The non-radioactive labeling method described by Tautz and Pfeifle (1989) was used, with an additional fixation of the embryos in glutaraldehyde 0.5% for two minutes at 4°C. To quantify the staining, light absorption in the 560–620 nm range was monitored in the cellular region at the margin of the embryos, with a Vickers Microdensitometer M60A. For each preparation, values were corrected with the mean obtained for embryos in stages 7–11 (Campos-Ortega and Hartenstein, 1985) in which an unimodal distribution was found.

Crosses for the determination of the lethal phase

$Hw^{49cR5}/FM7c, y^{31d} w^a sn^{x2} v^{Of} g^4 B \times Hw^{49cR5}/v^+Yy^+$
$sc^{3-1} w f^{36a}/FM7c, y^{31d} w^a sn^x2 v^{Of} g^4 B \times sc^{10-1} f^{36a}/y^2Y67g$
$sc^{10-1} f^{36a}/FM6, y^{31d} sc^8 dm B \times sc^{10-1} f^{36a}/y^2Y67g$
$sc^{10-1} f^{36a}/FM6, y^{31d} sc^8 dm B \times sc^{3-1} w cm Sxl^{f1} ct^6 f^{36a}/Y$
$sc^{10-1} Sxl^{M1}/FM6, y^{31d} sc^8 dm B \times sc^{10-1} f^{36a}/y^2Y67g$
$sc^{10-1} f^{36a}/FM6, y^{31d} sc^8 dm B \times Df(1)N71,sis\text{-}a^-/v^+Yy^+$
$Df(1)N71, sis\text{-}a^-/FM7c, y^{31d} w^a sn^{x2} v^{Of} g^4 B \times sc^{3-1} w cm Sxl^{f1} ct^6 f^{36a}/Y$
$Df(1)N71, sis\text{-}a^-/FM7c, y^{31d} w^a sn^{x2} v^{Of} g^4 B \times y\ sis\text{-}a/Y$

Results

The lethal phase of sc *defective females is embryonic*

Females defective for *Sxl* show an embryonic lethal phase (our unpublished data). Thus, the fact that *sc* is responsible for the *sis-b* function, involved in *Sxl* activation, predicts an embryonic lethal phase for females deficient for *sc*. This prediction is fulfilled. Females carrying different defective *sc* alleles show different degrees of embryonic lethality (Fig. 1). This lethality is more severe in females containing only a single dose of Sxl^+. Viability is recovered when one of the *Sxl* copies is replaced by Sxl^{M1}, a mutation that

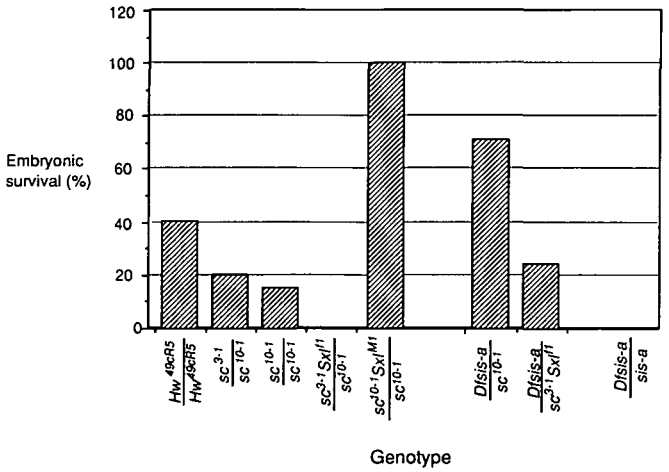

Fig. 1. Determination of the embryonic lethality in flies carrying different combinations of *sc*, *sis-a* and *Sxl* mutations. The embryonic survival of the experimental class was estimated from the overall hatching observed in each cross, where the embryonic viabilities of all the classes except the experimental one are known. The minimum number of eggs scored was 805 in each cross. See Materials and methods for full description of the crosses.

expresses the female-specific functions of *Sxl* largely independently of the X:A ratio (Cline, 1978). Females homozygous for $sc^{10\text{-}1}$, carrying Sxl^{M1}, survive to the adult stage. Significantly, these females show the same extreme sc phenotype as the $sc^{10\text{-}1}$ males. This clearly demonstrates the dual function of *sc* and the independence of its two functions: Sxl^{M1} is able to rescue the *sis-b* function but not the proneural function of *sc*. Finally, as a consequence of the involvement of *sis-a* in the X:A signal, females mutant for *sis-a*, or doubly heterozygous for *sis-a* and *sc* mutations, also show embryonic lethality (Fig. 1).

The thermosensitive phase of the lethal interaction between sc *and* Sxl *mutations occurs at early stages of embryonic development*

There are female-lethal transheterozygous synergistic interactions between *Sxl* mutations and *sis-a* and *sc* mutations (Cline, 1986, 1988; Torres and Sánchez, 1989). These interactions are thermosensitive, independently of the mutations employed, which include deficiencies of the respective genes. The permissive temperature is 18°C and the restrictive one 29°C. Similar thermosensitivity is found for the lethal phenotype of females homozygous for *sis* hypomorphic mutations (Cline, 1986; Torres and Sánchez, unpublished data). In contrast, males die if they contain three doses of either $sis\text{-}a^+$ or sc^+, or a duplication of both genes, or a duplication of either of them and a duplication of *Sxl* (Cline, 1988; Torres and Sánchez, 1989). This lethality is also thermosensitive, but 18°C is the restrictive and 29°C is the permissive temperature. These results indicate that the thermosensitivity is not due to the nature of the mutations employed; rather, it seems characteristic of the *Sxl* activation process. At 18°C activation of *Sxl* would be more efficient, making this temperature permissive for females and restrictive for males. Temperature does not affect *Sxl* regulation in wild-type flies; it only becomes a relevant factor in mutant flies in which the signal for *Sxl* activation is ambiguous.

We have determined the thermosensitive phase (TSP) for the interaction between $sc^{3\text{-}1}$ and Sxl^{f1} in females. The TSP is extremely short and occurs very early in development (Fig. 2A). It begins before the first hour of development is completed and ends around the second hour of development at 29°C. In males carrying duplications of *sis-a* and *sc*, the TSP ends at about the same time as in females (Fig. 2B). Although the interaction is strictly zygotic (Cline, 1988), we have not been able to define the beginning of the TSP. Males that escape the lethal effect of the *sis-a* and *sc* duplications show morphological alterations. The most frequent alteration is the abnormal development of the external terminalia which are absent or reduced to various degrees and show intersexual traits (Fig. 3A). Exceptionally, some of these males also show mosaic sexcombs composed of male and female bristles (Fig. 3B). These phenotypes are most likely due to the functioning of *Sxl* in the female-specific mode in some of the cells. Thus, the TSP of the lethal interaction

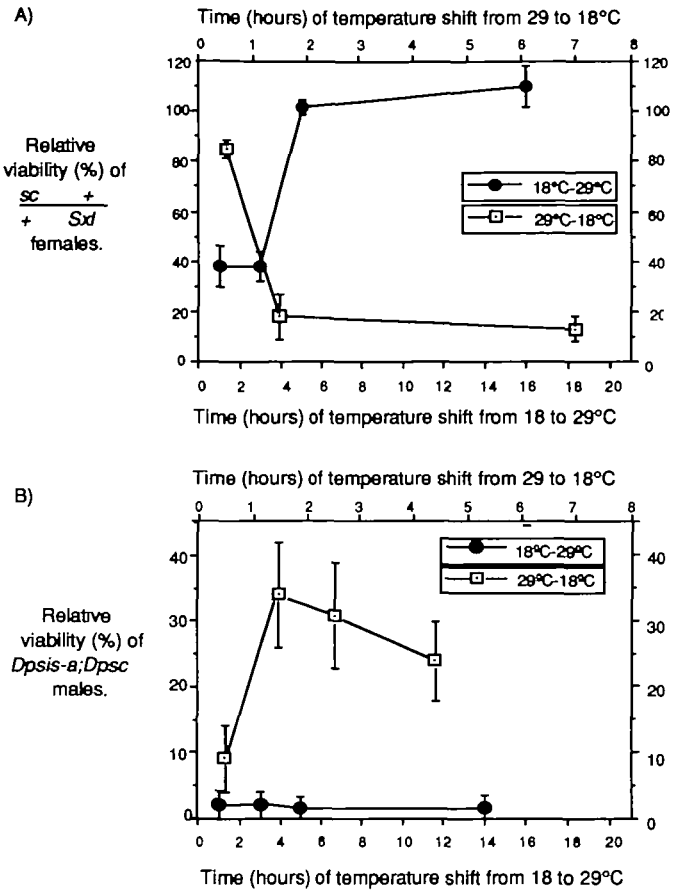

Fig. 2. Temperature-sensitive phase of $sc^{3\text{-}1}\ Sxl^+/sc^+\ Sxl^{f1}$ females (A) and $y/y^+Yv^+,\ sis\text{-}a^+;\ Dp(1;2)sc^{19},\ sc^+$ males (B). Egg laying lasted one hour at 29°C and two hours at 18°C. The abscissa gives the time from the beginning of the egg laying. Bars represent ±2×(standard error of the mean). Crosses: (A) $sc^{3\text{-}1}\ w\ f^{36a}/FM7c,\ y^{31d}\ w^a\ sn^{x2}\ v^{Of}\ g^4\ B \times cm\ Sxl^{f1}\ ct/Y$. (B) $y;Dp(1;2)sc^{19},y^+AS\text{-}C^+b\ pr\ c/In(2L+2R)Cy,Cy\ pr \times Df(1)N71,sis\text{-}a^-/v^+Yy^+,sis\text{-}a^+$. In both crosses, sibs served as controls.

between *sc* and *Sxl* coincides in males and females with the syncytial blastoderm stage, when generalized *sc* expression takes place (Romani *et al.* 1987; Cabrera *et al.* 1987).

Pulses of sc *expression rescue the sis-b phenotype in a stage specific way*

We have used pulses of *sc* expression administered at different developmental times to define precisely its temporal requirement during development to activate *Sxl*. To this end we used a *hsp70–sc* chimeric gene (*HSSC-3*) (Rodríguez *et al.* 1990). $sc^{3\text{-}1}/sc^{10\text{-}1}$ females show a strong sis-b phenotype and consequently a very low viability. We have given heat pulses to embryos from a cross in which $sc^{3\text{-}1}/sc^{10\text{-}1}$ females carrying the *HSSC-3* are produced. Expression of *sc* rescues $sc^{3\text{-}1}/sc^{10\text{-}1}$ females during the first two hours of development (Fig. 4A). This coincides with the time of the transient generalized expression of *sc* in wild-type embryos (Romani *et al.* 1987; Cabrera *et al.* 1987). The fact that there is only partial rescue is probably due to

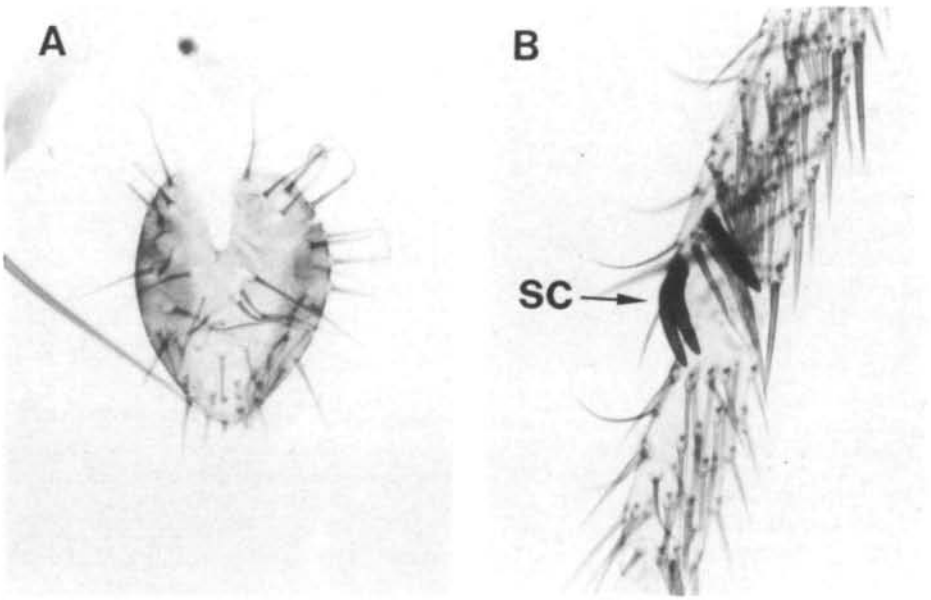

Fig. 3. Photographs (×200) showing intersexual analia (A) and intersexual mosaic sex comb (B) of *Dpsc*$^{+}$; *Dpsis-a*$^{+}$ males. In wild-type males, the analia comprise two lateral plates. The anal plates in A, however, are partially fused which is characteristic of intersexual anal plates. A sex comb in wild-type males is composed of 10–12 large blunt bristles, in a continuous array, that derive from the rotation of the last two rows of bristles in the basitarsus of females. In the leg shown in B, only the last row has rotated, and only three of the bristles in this row are large. Symbols: sc, sex comb.

asynchronization of the egg sample at the time of the heat shock. Egg retention by females would cause contamination of samples with embryos in a more advanced stage than that expected from the oviposition time. This explains why the 0–1 h class shows recovery, despite the fact that embryos should not be competent for heat-shock response until the syncytial blastoderm stage (Dura, 1981).

We have followed a different procedure to overcome the problem of the asynchronization of the samples. We carried out the same cross as in experiment-2 of Fig. 4A, in which the *HSSC-3* comes from the father, but the heat shock was not given to the embryos but to their mothers. Maternal heat shock could induce transcriptional activation from the *HSSC-3* in the egg only when the zygotic genome is already competent for transcription; i.e. at the ninth nuclear division (Anderson and Lengyel, 1980). As the *HSSC-3* comes from the paternal genome, its transcription would be caused by accumulation in the egg of activated heat-shock transcription factor (HTF) (Wu *et al.* 1987) of maternal origin. With this procedure the heat shock mediated expression of *sc* will occur at the same developmental time in all the embryos. Even 3–6 h hours after the maternal heat shock, the embryos at the syncytial blastoderm stage contain *sc* mRNA in larger amounts than the non-heat-shocked wild-type embryos at the same stage; moreover, in heat-shocked embryos, the *sc* mRNA lasts through the cellularization stage (data not shown). This indicates that *sc* expression is achieved using this procedure and, as expected, full recovery of experimental females is observed in the 3–6 h class (Fig. 4B). Thus, *sc* expression is fully able to suppress the sis-b phenotype. In the egg collections after 6 h or more from the heat shock, the degree of recovery gradually decreases, presumably due to the disappearance of the consequences of the heat shock in the mother. The time elapsed until the eggs laid are free of heat-shock effects is surprisingly long. This suggests a high perdurance of the activated HTF in the egg. In the first class (0–3 h), there is only partial recovery. This is probably due to the presence of eggs that at the moment of the heat shock were already independent of the maternal influence, but not yet competent for heat-shock response. The control cross (*HSSC-3*$^{-}$) shows some degree of recovery in the first two classes. This may be due to the maternal heat shock but not to the expression of *sc*. This result suggests that maternal heat shock affects some maternal process involved in *Sxl* early activation.

The above results show that the *sis-b* function is provided by the *sc* gene, confirming that *sc* is responsible for the sex-determining function of the AS–C. Moreover, our results eliminate the possibility that the *sis-b* function could be attributed to the existence of sequences in the gene *sc* with capacity to absorb autosomal *trans*-acting factors, since we find that the *sis-b* activity is not related to the mere presence of the *HSSC-3* but to its expression.

Pulses of sc *expression produce Sxl-dependent male lethality in a stage-specific way*

If the amount of *sc* transcripts produced during the syncytial blastoderm is making up the X:A signal that determines *Sxl* activity, *sc* expression should induce male lethality specifically at this stage due to *Sxl* activation. We have heat shocked embryos carrying the *HSSC-3* at different stages of early development. Two types of males arise from the experimental cross (see legend to Fig. 5) from which eggs were heat shocked: those with *Sxl*$^{+}$ and those with *Sxl*^{7BO}, a deficiency for the entire gene (Salz *et al.* 1987). Both types carry the *HSSC-3*. *Sxl*^{7BO} males are used as viability controls for their *Sxl*$^{+}$ brothers. In this way we should exclusively detect the lethality associated with ectopic *Sxl* expression. Overexpression of *sc* indeed promotes *Sxl*-dependent male lethality (Fig. 5) only during the first

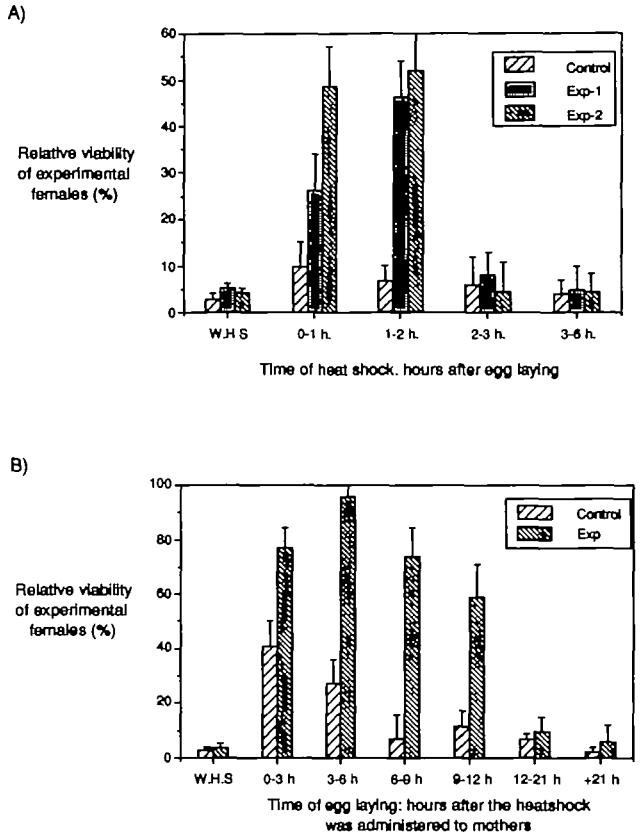

Fig. 4. Rescue of *sis-b*$^{-}$ females by pulses of sc expression. (A) Relative viability of females with the following genotypes: Exp-1 and Exp-2: $sc^{3\text{-}1}/sc^{10\text{-}1}$; *HSSC-3*/+. The *HSSC-3* comes from the mother in Exp-1 and from the father in Exp-2. Control flies: $sc^{3\text{-}1}/sc^{10\text{-}1}$. Cross to generate Exp-1 flies: $sc^{3\text{-}1}$ *w* $f^{36a}/FM7c$, w^a sn^{x2} v^{Of} g^4 *B*; *HSSC-3/HSSC-3* × $sc^{10\text{-}1}$ f^{36a}/y^2Y_{67g}. Cross to generate Exp-2 flies of (A): $sc^{10\text{-}1}$ $f^{36a}/FM6$, y^{31d} sc^8 *dm B* × $sc^{3\text{-}1}$ *w* f^{36a}/Y; *HSSC-3/HSSC-3*. Cross to generate control flies of (A) and (B): $sc^{3\text{-}1}$ *w* $f^{36a}/FM7c$, y^{31d} w^a sn^{x2} v^{Of} g^4 *B* × $sc^{10\text{-}1}$ f^{36a}/y^2Y_{67g}. Eggs were heat shocked at different developmental times (abscissa) by immersion during 30 min in water at 36°C. (B) Relative viability of females with the following genotypes: Exp: $sc^{3\text{-}1}/sc^{10\text{-}1}$; *HSSC-3*/+, Control: $sc^{3\text{-}1}/sc^{10\text{-}1}$. The cross was the same as in Exp-2 of A. Mothers were heat shocked for 30 min at 37°C and egg populations were collected at different times (abscissa) from the end of the heat-shock. Flies used as viability reference were $sc^{10\text{-}1}$ $f^{36a}/FM7c$, w^a sn^{x2} v^{Of} g^4 *B* females in Exp-1 and Control crosses and $sc^{3\text{-}1}$ *w* $f^{36a}/FM7c$, y^{31d} w^a sn^{x2} v^{Of} g^4 *B* females in Exp-2 cross. W.H.S.: Without heat shock. Bars represent ±2×(standard error of the mean).

3 h of development at 18°C. Although only partial, lethality is stronger in the 1–2 h class than in the 2–3 h class. Viability of the Sxl^{7BO} males is unaffected. We see that the developmental time at which male viability is sensitive to excess of *sc* expression coincides with the effective period in which this same expression rescues the sis-b phenotype in females and it corresponds to the syncytial blastoderm stage.

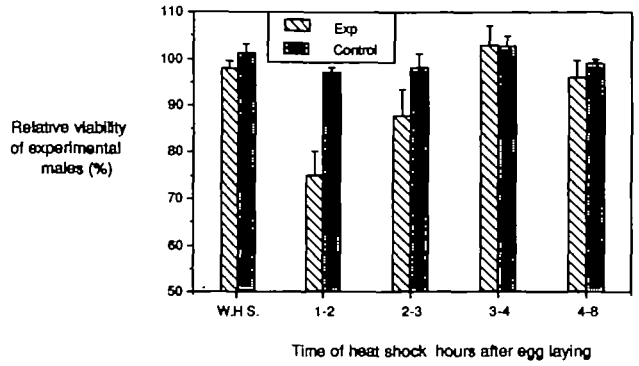

Fig. 5. Relative viability of males after pulses of sc expression at different times of development. Viability of males with the following genotypes: Exp: *y*/*Y*; *HSSC-3*/+ males. Control: *y*/*Y* males. Cross to generate Exp. males: *y*/*y cm* Sxl^{7BO} × *y*; *HSSC-3/HSSC-3*. Cross to generate control males: *y*/*y* × *y*/*Y*. The flies used as viability reference were *y cm* Sxl^{7BO}/*Y*; *HSSC-3*/+ males and *y*/*y* females, respectively. W.H.S.=Without Heat shock. The eggs were heat-shocked by immersion during 30 min in water at 36°C, at different developmental times. Bars represent 2×(standard error of the mean).

The initial expression of Sxl *in females depends on* sc *and* sis-a

The initial regulation of *Sxl* depends on the X:A signal, and seems to occur at the transcriptional level (Salz *et al.* 1989). Therefore, alteration in the X:A signal due to *sc* and *sis-a* mutations should affect this initiation process. To test this hypothesis, we have looked at the early *Sxl* transcription in a sample of embryos in which all of the females were simultaneously heterozygous for *sc* and loss-of-function *sis-a* mutations and a deficiency for *Sxl*. These females die during the embryonic stage due to their inability to activate *Sxl* (data not shown). The *Sxl* transcription has been analyzed by *in situ* hybridization of embryos with the digoxigenin-labeled h_1 probe (Bell *et al.* 1988) that detects all of the known *Sxl* transcripts. The degree of hybridization has been quantified by measuring the intensity of the staining in the individual embryos (legend to Fig. 6). As controls, we have used Oregon-R wild-type embryos. In this case, around cellular blastoderm stage, we find two types of embryos that differ in the intensity of the staining (Fig. 6) and fall into a bimodal distribution with a 1:1 ratio of the two types. This bimodal distribution most likely reflects differences between females and males, with respect to the early expression of *Sxl*. An X:A ratio of 1 in females would produce a regulatory signal that allows the initiation of *Sxl* expression, while in males an X:A ratio of 0.5 would prevent this expression. In the cross where all of the female embryos are heterozygous for *sc*, *sis-a* and *Sxl*, the embryos fall into a unimodal distribution (Fig. 6), which corresponds to the weakly stained class of wild-type embryos. This variation in the pattern of *Sxl* expression cannot be attributed to the presence of a single dose of *Sxl* in the experimental females, since *Df(Sxl)*/+ females containing two doses of both *sc* and

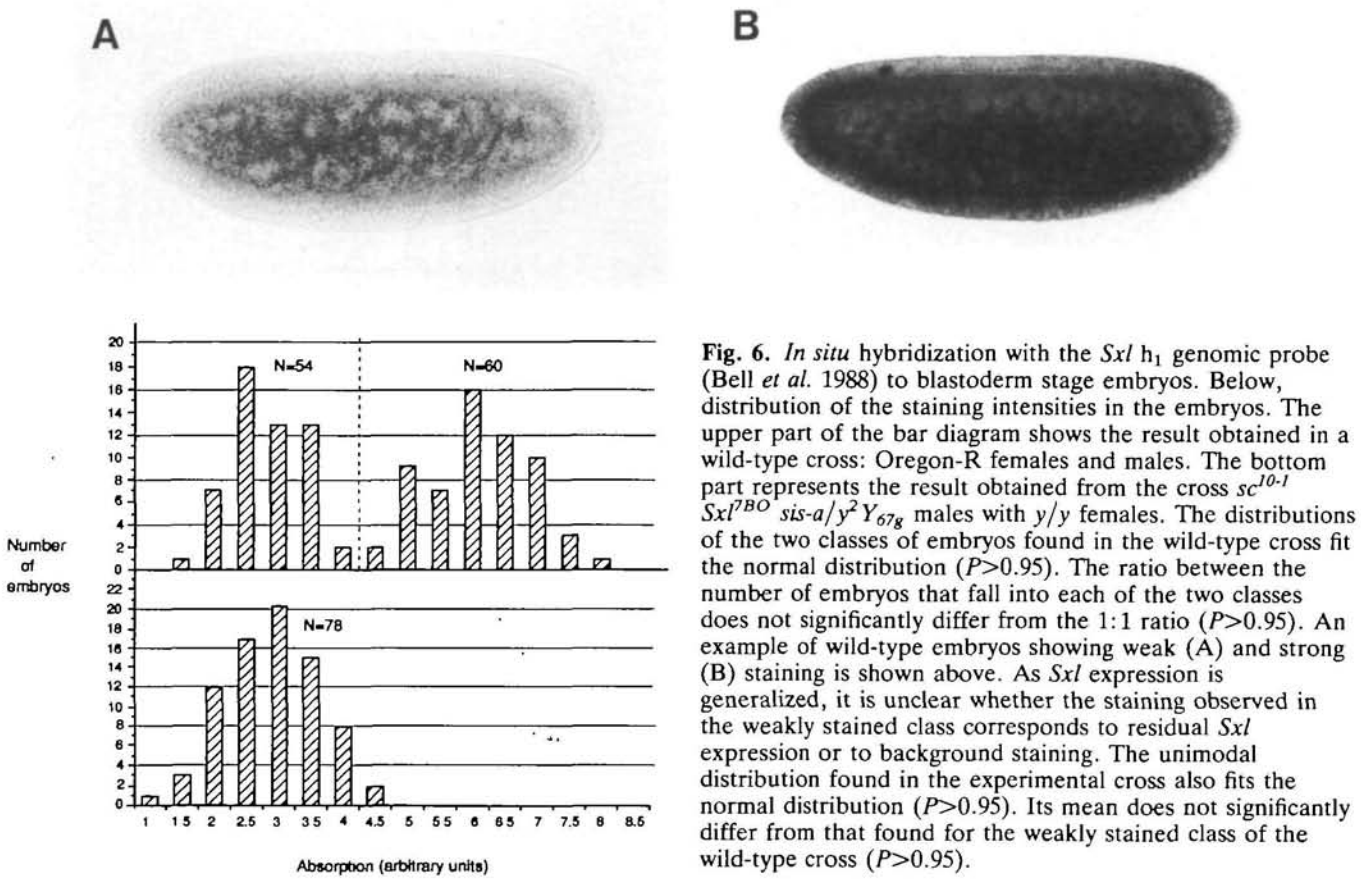

Fig. 6. *In situ* hybridization with the *Sxl* h_1 genomic probe (Bell *et al.* 1988) to blastoderm stage embryos. Below, distribution of the staining intensities in the embryos. The upper part of the bar diagram shows the result obtained in a wild-type cross: Oregon-R females and males. The bottom part represents the result obtained from the cross $sc^{10\text{-}1}$ Sxl^{7BO} *sis-a*/$y^2 Y_{67g}$ males with y/y females. The distributions of the two classes of embryos found in the wild-type cross fit the normal distribution ($P>0.95$). The ratio between the number of embryos that fall into each of the two classes does not significantly differ from the 1:1 ratio ($P>0.95$). An example of wild-type embryos showing weak (A) and strong (B) staining is shown above. As *Sxl* expression is generalized, it is unclear whether the staining observed in the weakly stained class corresponds to residual *Sxl* expression or to background staining. The unimodal distribution found in the experimental cross also fits the normal distribution ($P>0.95$). Its mean does not significantly differ from that found for the weakly stained class of the wild-type cross ($P>0.95$).

sis-a are fully viable (data not shown). Thus, the mutant condition of the females for *sc* and *sis-a* coincides with the disappearance of the strongly stained class. This indicates first, that the strongly stained class observed in the wild-type sample indeed corresponds to females and second, that the strong *Sxl* expression depends on the doses of *sc* and *sis-a*. This result supports the transcriptional control of *Sxl* by the X:A signal.

Discussion

The expression of sc *at syncytial blastoderm is responsible for its* sis-b *function*

The X:A ratio determines the state of activity of *Sxl* around the blastoderm stage; thereafter *Sxl* maintains its determined state (either ON in females or OFF in males) independently of the X:A ratio (Sánchez and Nöthiger, 1983; Bachiller and Sánchez, 1991). Thus, the function of this ratio as a genetic signal is temporally restricted to an early stage in development. Lack-of-function *Sxl* mutations produce embryonic lethality (our unpublished results). Since the X:A signal determines *Sxl* activity and the gene *sc* behaves as a numerator element of this signal, it is expected that females lacking *sc* function would show embryonic lethality. The experiments reported here confirm the prediction. The lethal phase of sc^- females is embryonic. The cause of the lethality is the lack of *Sxl* function, since this lethality is suppressed by Sxl^{M1}, a mutation that constitutively expresses the *Sxl* function (Cline, 1984). The same holds for mutations at the gene *sis-a*, thus confirming the involvement of this gene in the X:A signal.

Loss-of-function mutations at *sc* and *Sxl* interact synergistically causing female lethality, while duplications of *sc* and *Sxl* cause male lethality due to expression of *Sxl* (Cline, 1988; Torres and Sánchez, 1989). This interaction between *sc* and *Sxl* is thermosensitive. We have shown here that the TSP of this interaction is very short and spans approximately the syncytial blastoderm stage, being the same for both females and males. By means of a *hsp70–sc* chimeric gene (*HSSC-3*) (Rodríguez *et al.* 1990), we have expressed *sc* at different developmental times and checked when this expression suppresses the sis-b mutant phenotype in females, and causes lethality in males due to expression of *Sxl*. We have found that expression of *sc* activates *Sxl* only at a very specific stage in development, coinciding with the TSP delimited by the temperature-shift experiments and corresponding to the syncytial blastoderm stage. At this stage, the X:A signal determines *Sxl* activity (Sánchez and Nöthiger, 1983; Bachiller and Sánchez, 1991) and the gene *sc* undergoes an homogeneous expression (Romani *et al.* 1987; Cabrera *et al.* 1987). We conclude that the *sc* expression at the syncytial blastoderm is responsible for its sis-b function. Moreover, since the expression of *sc* from the *HSSC-3*, but not the mere presence of the

gene, fully suppresses the sis-b phenotype, the sis-b function must be provided by the *sc* protein.

Time-specificity of the X:A signal

Ectopic expression of *sc* in the heat-shock experiments only activates *Sxl* in males or in females when expressed at the time when generalized *sc* expression takes place in wild-type embryos. Moreover, *sc* expression in neurogenic regions does not induce the activation of *Sxl* in males. Consequently, the stage-specificity of *sc* expression cannot account for the stage-specificity of the *Sxl* activation process. Two hypotheses can be put forward to explain such time-specificity. First, since the X:A signal is made up of several discrete genes, such as *sis-a* and *sc* and it also needs the activity of transducer genes, such as *da*, it is possible that one or more genes are indispensable for *Sxl* activation and expressed only at the syncytial blastoderm stage. *da* cannot confer temporal specificity to the process, as it is also expressed zygotically and required, together with *sc*, in neurogenesis (Caudy *et al.* 1988*a*). However, the molecular nature and pattern of expression of *sis-a* are unknown. This gene thus remains as a candidate to be responsible for the time-specificity observed. The second hypothesis is that *Sxl* could be susceptible to activation by the X:A signal, only at the syncytial blastoderm stage; later its active or inactive states would be independent of this signal. The gene *Sxl* seems to contain two promoters: an early promoter responsible for the early *Sxl* expression, which would specifically respond to the X:A signal, and a constitutive promoter, which functions later both in males and females (Salz *et al.* 1989). The accessibility of the early promoter exclusively at the beginning of development might account for the time-specificity in receiving the X:A signal. One possibility is that the activation of the late promoter could hinder the accessibility of the more internal early promoter.

The molecular nature of the X:A signal

The X:A signal results from the interaction between X-linked (*sisterless*) and autosomal elements. *sis* elements would be genes expressed around blastoderm stage in a non-compensated way, so that female embryos would have twice the amount of *sisterless* products than male embryos. Only the molecular nature of one of the members of the X:A signal, *sc*, is known. This gene encodes a helix-loop-helix (HLH) protein (Villares and Cabrera, 1987). HLH proteins are transcriptional regulators whose activity depends on homo- or heterodimerization with other HLH proteins (Murre *et al.* 1989*a*,*b*). Association of a particular HLH protein with different members of the family produces dimers that differ in the affinity for their DNA-binding sites (Benezra *et al.* 1990; Garrell and Modolell, 1990; Ellis *et al.* 1990; Sun and Baltimore, 1991). Parkhurst *et al.* (1990) have proposed that the X:A signal is formed by X-linked *sis* products (numerator elements) which could be titrated by autosomal HLH products (denominator elements) so that an effective concentration of *sis* products would only be attained in females. The *sis* products would form heterodimers with the *da* maternal product, which is also an HLH protein (Caudy *et al.* 1988*b*), so that heterodimers between *da* and *sis* products would promote early transcription of *Sxl*. Evidence supporting the transcriptional regulation of *Sxl* by the X:A signal is presented here. The amount of *Sxl* transcripts in wild-type young embryos fall into a bimodal distribution, with an equal number of embryos in each class. This distribution would reflect the two types of embryos, female and male, with different levels of *Sxl* transcription. However, while females with a single dose of Sxl are wild-type, female embryos containing a single dose of *Sxl*, *sc* and *sis-a*, are indistinguishable from their sibling male embryos by their amount of *Sxl* transcripts. This indicates a transcriptional control of *Sxl* by the doses of *sis-a* and *sc* around the blastoderm stage. The proteins that directly bind to *Sxl* for its initial activation are unknown. The fact, however, that *sc* codes for an HLH protein and the very short time between *sc* and *Sxl* early expression suggests that the *sc* protein may be one of the factors binding directly to the *Sxl* early promoter.

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