The EMBO Journal vol.8 no.10 pp.3079-3086, 1989

The *scute* (T4) gene acts as a numerator element of the X:A signal that determines the state of activity of *Sex-lethal* in *Drosophila*

Miguel Torres and Lucas Sánchez

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

Communicated by J.Modolell

The ratio of X chromosomes to sets of autosomes (X:A) is the primary genetic signal that determines sex and dosage compensation in *Drosophila*. The gene *Sex-lethal* (*Sxl*) receives this signal and is responsible for the execution of the alternative developmental programmes of males and females. We have found that the *scute* (T4) gene, which is involved in neurogenesis, also plays a role in the activation of *Sxl*. The following results suggest that *scute* (T4) may be a numerator element of the X:A signal: *scute* (T4) mutations show female-specific lethality. There are female-specific lethal synergistic interactions between *sis-a*, a previously described numerator element, and mutants for T4. The female lethality is suppressed by Sxl^{M1}, a constitutive allele which expresses an active *Sxl* product independently of the X:A ratio. The Hw^{685} mutation, which overexpresses T4, is lethal to males with a duplication of *sis-a*. This lethality is suppressed by either Sxl^{fl}, or the T4 point mutation sc^{10-1}. There are female-specific lethal interactions between sc^{10-1} and *daughterless* (*da*), a gene needed maternally for *Sxl* to become active. The sc^{10-1} mutation masculinizes triploid intersexes.

Key words: Drosophila/scute/*Sex-lethal*

Introduction

In *Drosophila melanogaster*, the ratio of the X chromosomes to sets of autosomes (X:A) is the primary genetic signal that triggers dosage compensation (hypertranscription of the male X chromosome) and sex-determination, by defining the state of activity of *Sex-lethal* (*Sxl*): in 2X;2A flies *Sxl* will be ON while in X;2A flies *Sxl* will be OFF (Cline, 1978). Activation of *Sxl* requires the maternal *daughterless* (*da*) product (Cline, 1978). Recently, it has been claimed that the maternal as well as the zygotic activity of the X-linked gene *sans-fille* (*snf*) [*liz* in Steinmann-Zwicky's (1988) terminology] is also needed for *Sxl* activation; however, this gene is not a component of the X:A ratio (Cline, 1988; Oliver *et al.*, 1988; Steinmann-Zwicky, 1988). Once the state of activity of *Sxl* is defined, which occurs around blastoderm stage (Sánchez and Nöthiger, 1983), the X:A ratio is no longer needed as a genetic signal. Both sex determination (Cline, 1979; Sánchez and Nöthiger, 1982) and dosage compensation (Lucchesi and Skripsky, 1981) come under the control of *Sxl*. It is thought that *Sxl* operates through two independent sets of genes. One set are the sex-determination genes, which control the sexual developmental pathway (Nöthiger and Steinmann-Zwicky, 1985). The other set are the *male-specific lethal* genes (*msl*) whose function in males determines the hypertranscription of its single X chromosome (Lucchesi and Manning, 1987).

The genetic basis of the X:A signal is unknown. It is thought that it results from the interactions between X-linked elements, 'numerator elements', and autosomal-linked elements, 'denominator elements'. So far, two numerator elements of that signal have been identified, *sisterless-a* (*sis-a*) (Cline, 1986) and a region of the *achaete–scute* complex (AS-C) that has been named *sis-b* (Cline, 1988; this report). To find numerator elements of the X:A signal, we have identified a set of regions on the X chromosome that interact with *Sxl* by determining X-chromosome deficiencies that cause female-specific lethality when heterozygous with the null allele Sxl^{fl}. Afterwards, a detailed genetic analysis has been carried out for each of the selected regions to verify which ones behave as expected for components of the X:A signal. A numerator element should display several properties. Reduction of its zygotic doses should kill females as a consequence of a failure to activate *Sxl*, while an increase in its zygotic doses should kill males because *Sxl* is inappropriately activated. The female lethality is expected to be suppressed by the constitutive Sxl^{M1} mutation (Cline, 1978), while the male lethality is expected to be suppressed by loss-of-function mutations at the *Sxl* locus. Mutations at *da* and at any numerator elements are expected to display female-specific lethal synergistic interactions; such interactions should also take place between mutants in different numerator elements. This female-specific lethality should again be suppressed by Sxl^{M1}. It is also expected that a variation of the zygotic doses of numerator elements should alter the sexual phenotype of triploid intersexes (2X;3A); an increase should feminize while a reduction should masculinize these animals.

Here we report the detailed analysis of an X-chromosomal region found to interact with *Sxl*. It is included within the AS-C which is located at the tip of the X chromosome (1B1-4), and which plays an important role in the development of the embryonic central nervous system and the peripheral nervous system of larvae and adult flies (García-Bellido, 1979; Dambly-Chaudière and Ghysen, 1987; Jiménez and Campos-Ortega, 1987). Genetic analysis has allowed its subdivision into four functional regions. Distally to proximally these are: *achaete* (*ac*), *scute* (*sc*)α, *lethal of scute* (*l'sc*) and *scute* (*sc*)β (García-Bellido, 1979). The existence of a fifth region, proximal to *sc*β and named *sc*γ has been proposed (Dambly-Chaudière and Ghysen, 1987; Jiménez and Campos-Ortega, 1987). The cloning of the AS-C DNA has revealed four genes coding for transcripts which share extensive sequence homologies and which are considered to correspond to the *ac*, *sc*, *l'sc* and *asense* (*sc*γ) functions. They have been named T5, T4, T3, and T1a or T8 respectively (Campuzano *et al.*, 1985; Villares and Cabrera, 1987; Alonso and Cabrera, 1988; Balcells *et al.*,

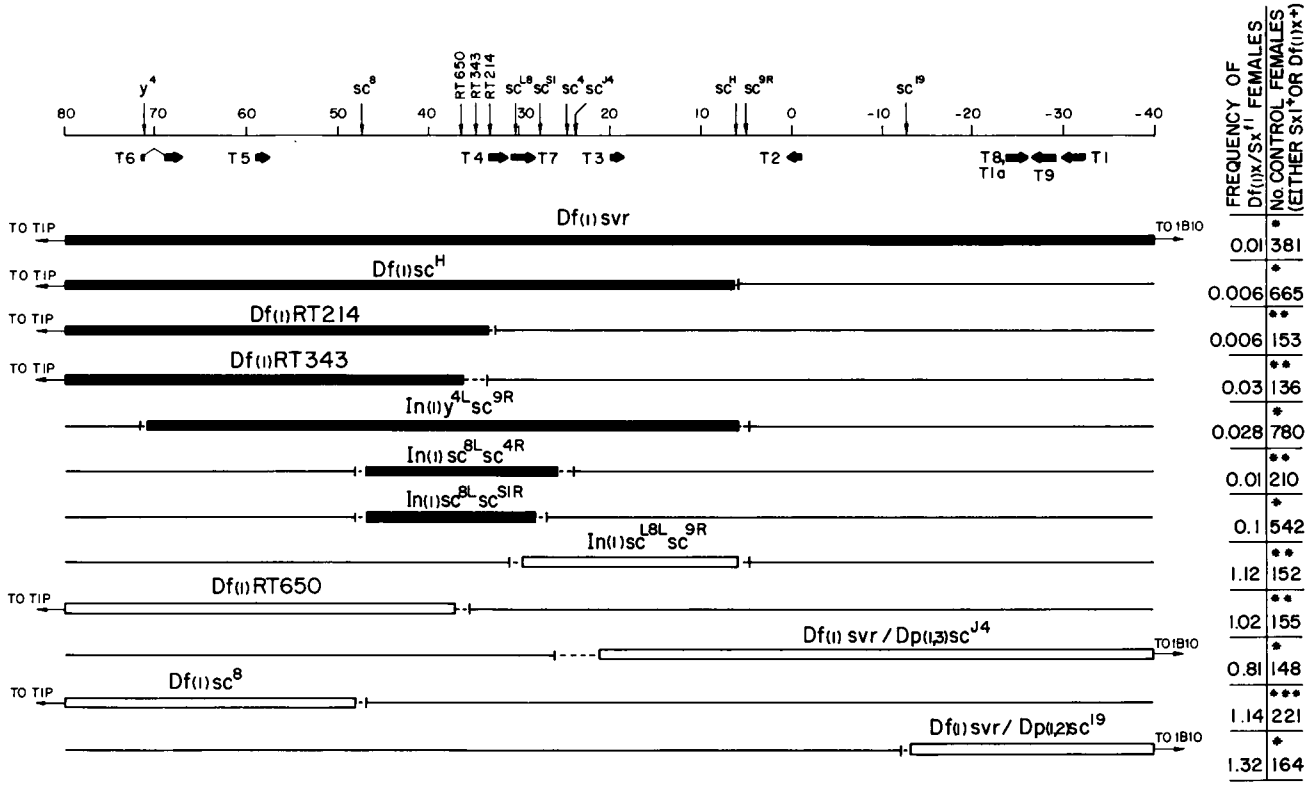

Fig. 1. Chromosome rearrangements involving different AS-C regions tested for lethal interaction with Sxl^{f1}. Black bars represent deficiencies showing lethal interaction and open bars those that do not. Continuous lines represent wild-type chromosomal material and discontinuous lines the ambiguity in the delimitation of the deficiencies according to the molecular data. The upper part of the figure shows the DNA map of the AS-C with the location of breakpoints as well as DNA regions coding for the different transcripts (black arrows) (Campuzano *et al.*, 1985; Cabrera *et al.*, 1987; Ruiz-Gómez and Modolell, 1987; Alonso and Cabrera, 1988). On the right side of the figure is given the viability of the experimental females: *Df(AS-C)/Sxl*^{f1} relative to control flies whose actual number is shown. For the different crosses, control flies were: * Sxl^{f1} / + females, ** *Df(AS-C)* + / + Sxl^{f1} ; *Dp(1;3)sn*^{13a1}, Sxl^{+} / + females, *** Sxl^{f1}/*Y* males. The *FM7* and *FM6* balancer chromosomes carry the sc^{8} mutation associated to the *In(1)sc*^{8}; both balancers, however, are wild type for the female-lethal interaction described here, so that the sc^{8} mutation of *FM7* and *FM6* will be represented by '+' whenever the markers of either balancer are specified.

Table I. Sxl^{M1} suppresses the female-lethal interaction of *Df(AS-C)* with *Sxl* or *Df(sis-a)*

		Cross: A	Cross: B	Cross: C
Genotype of experimental females		AS-C^{-} Sxl^{x} + / + Sxl^{f1} +	AS-C^{-} Sxl^{x} + / + + sis-a^{-}	$sc^{\alpha-}$ + + / + Sxl^{x} sis-a^{-}
Viability (number of control flies)	$Sxl^{x}=Sxl^{+}$	2% (381)	7% (407)	3.6% (111)
	$Sxl^{x}=Sxl^{M1}$	130% (309)	87% (365)	117% (181)

Full genotypes of crosses:

A. *Df(1)sc*^{19}, *AS-C*$^{-}$ w^{c} Sxl^{M1} / *FM7* or *Df(1)svr, AS-C*$^{-}$ *spl* f^{36a} / *FM6* females with *cm* Sxl^{f1} *ct* / *Y* males.

B. *Df(1)sc*^{19}, *AS-C*$^{-}$ w^{c} Sxl^{M1} / *FM7* females with *Df(1)N71, sis-a*$^{-}$ / *Y*; *Dp(1;2)v*^{65b}, *sis-a*$^{+}$ / *In(2L+2R)Cy, Cy pr* males or *Df(1)svr, AS-C*$^{-}$ *spl* f^{36a} / *FM6* females with *Df(1)N71, sis-a*$^{-}$ / $v^{+}Yy^{+}$, *sis-a*$^{+}$ males.

C. *Df(1)N71, y cho cv* Sxl^{M1} *sis-a*$^{-}$ / *FM6* or *Df(1)N71, sis-a*$^{-}$ / *FM6* females with *In(1)sc*^{8L4R}, $sc^{\alpha-}$ *cv; Dp(1;2)sc*^{19}, y^{+} *AS-C*$^{+}$ *Br pr* / *In(2L+2R)Cy, Cy pr* males.

In all cases control females carry the X balancer and the paternal X chromosome. Females carrying the *In(2L+2R)Cy* chromosome are the control flies in crosses involving this balancer.

1988). Cline (1988) assigned *sis-b* functions to a 20 kb subregion of the AS-C centered around position 40 on the molecular map of the complex (Campuzano *et al.*, 1985). In this work, we show that the *scute* α (T4) gene product is responsible for the *sis-b* function. Our results show a dual function for the T4 gene, which is the second gene, together

Table II. Suppression of the female-lethal interaction between Sxl^{f1} and *Df(sis-a)* or *Df(AS-C)* by *Dp(AS-C)* or *Dp (sis-a)* respectively

	Cross A			Cross B		
	Genotypes			Genotypes		
Experimental females, viability	AS-C$^-$ + / + Sxl^{f1} +	With *Dp(sis-a)*	77%	+ + / sis-a$^-$ + Sxl^{f1} +	With *Dp(AS-C)*	58%
		Without *Dp(sis-a)*	14%		Without *Dp(AS-C)*	2%
Control females number obtained	+ + / + Sxl^{f1} +	With *Dp(sis-a)*	167	+ + / + Sxl^{f1} +	With *Dp(AS-C)*	343
		Without *Dp(sis-a)*	187		Without *Dp(AS-C)*	314

Full genotype of crosses (females × males):

A. *Df(1)sc*^{19}, *AS-C*$^-$ f^{36a} / *FM7* × *cm* Sxl^{f1} *ct* / *Y*; *Dp(1;2)v*^{65b}, *sis-a*$^+$ / *In(2L+2R)Cy, Cy pr.*
B. *Df(1)N71, sis-a*$^-$ / *FM6* × *cm* Sxl^{f1} *ct* / *Y*; *Dp(1;2)sc*^{19}, y^+ AS-C$^+$ *b pr c* /*In(2L+2R)Cy, Cy pr.*

Experimental females with the *Dp(1;2)v*^{65b} or *Dp(1;2)sc*^{19} chromosomes were compared with control females with the same duplication, and those without it with non-duplicated control females.

with *da* (Caudy *et al.*, 1988a; Dambly-Chaudière *et al.*, 1988), involved in the activation of *Sxl* and in neurogenesis.

Results

Mapping the subregion of the achaete–scute complex that interacts with Sxl^{f1}

Females doubly heterozygous for both Sxl^{f1} and *Df(1)svr* have drastically reduced viability; ≤10% of these females survive compared with Sxl^{f1}/+ or *Df(1)svr*/+ females, which are fully viable. *Df(1)svr* is a terminal deficiency with the breakpoint at the chromosomal band 1B10. Different deficiencies were tested to determine the chromosomal region(s) or gene(s) included in *Df(1)svr* responsible for the interaction with Sxl^{f1}. The results are shown in Figure 1. Deletion of material proximal to *In(1)sc*^{L8} or distal to *Df(1)RT650* breakpoints did not substantially reduce the viability of females heterozygous for Sxl^{f1}. (The question remains if simultaneous deletion of both sequences could show the lethal interaction.) In contrast, all the tested deficiencies deleting sequences within those breakpoints show lethal interaction with Sxl^{f1}. The variation observed for the viability values ranges from 0.6 to 10% and is similar to the one found among a large number of *Df(1)svr* recombinant X chromosomes (data not shown). This variability, which has also been reported by Cline (1988), seems to be due to differences in genetic background of the stocks used in these experiments. Thus, we map the lethal interaction between Sxl^{f1} and deficiencies of the AS-C to the 8.3 kb fragment between *In(1)sc*^{L8} and *Df(1)RT650* breakpoints.

Whenever a lethal effect has been found, surviving females show morphological abnormalities and lack of cuticular structures mainly in the legs and eyes, as well as sexual transformation in tergites and sternites. A similar phenotype has been reported for daughters that escape the lethal maternal effect of females homozygous for *da*. Thus, the interpretation put forward by Cline (1980), assuming that in surviving females some of the cells are unable to activate *Sxl* and either die, causing deformities and lack of structures, or differentiate male structures instead of female ones, may also apply to the lethal interaction between AS-C deficiencies and Sxl^{f1}.

Constitutive expression of Sxl suppresses the female-lethal interaction between Df(AS-C) and Sxl^{f1} *or Df(sis-a)*

If the AS-C sequences delimited here are involved in *Sxl* activation, the replacement of one Sxl^+ copy by the constitutive allele Sxl^{M1} should restore female viability. This is in fact the case for females doubly heterozygous for Sxl^{f1} and *Df(AS-C)* (Table I, column A). Sxl^{M1} also suppresses the dominant female-lethal synergism between *Df(AS-C)* and *Df(sis-a)* (Table I, column B). Females doubly heterozygous for *Df(1)sc*^{8L4R} and *Df(sis-a)* have strongly reduced viability. This indicates that the AS-C subregion identified to have *sis-b* function, because of its interaction with Sxl^{f1}, is probably the same that interacts with *Df(sis-a)*, as it is included in the *Df(1)sc*^{8L4R}. These females are also rescued by the presence of the Sxl^{M1} mutation (Table I, column C).

To define further the relationship between *sis-a* and *sis-b* we have examined the ability of a duplication of one of these elements to substitute for the deficiency of the other in their interaction with Sxl^{f1}. *Dp(sis-a)* is unable to fully rescue a deficiency for *sis-b* and *vice versa* (Table II). *Dp(sis-b)* rescues more efficiently (×29) the lethality due to *Df(sis-a)* than *Dp(sis-a)* does with *Df(sis-b)* (×5.5). This indicates that their interaction is not strictly additive.

Mutations at the scute (T4) gene cause female-specific lethality and interact synergistically with Sxl^{f1} *or Df(sis-a)*

Our analysis shows that the *sis-b* function must reside in the AS-C subregion between positions 28.8 and 37.1 on the molecular map. Two transcripts are produced from this region, T4 and T7. The T4 transcript appears to be responsible for the *scute* function (Campuzano *et al.*, 1985; Villares and Cabrera, 1987), while the T7 transcript does not seem to be involved in neurogenesis (Alonso and Cabrera, 1988). This last transcript probably does not affect the *sis-b* function since *In(1)sc*^{L8L}*sc*^{9R} seems to disrupt this transcription unit without causing a lethal interaction with Sxl^{f1} (see Figure 1). The T4 transcript remains unaffected in all those deficiencies that do not show an interaction with *sis-b*. In contrast, all the deficiencies deleting the T4 gene or the sequence close to its transcription start site show the

Table III. Complementation analysis of *AS-C* mutations showing female-specific lethality

		Male viability	Female viability, complementation with:						
			$sc^{10\text{-}1}$	Hw^{49cR5}	$sc^{3\text{-}1}$	*Df(1)RT650*	*Df(1)RT343*	ac^3	Hw^{49c}
Allele tested	$sc^{3\text{-}1}$	90% (158)	<0.4% (249)	3% (367)	7% (158)	95% (359)	<0.2% (482)	102% (130)	87% (425)
	Hw^{49cR5}	67% (367)	<0.2% (496)	1.6% (334)					
	$sc^{10\text{-}1}$	44% (249)	<0.2% (436)						

The actual numbers of control flies used as reference are given in parenthesis.

Full genotypes of crosses:

sc^{3-1} *w* f^{36a} / *Y* males were crossed respectively with the following females to generate female viability data in the first row: $sc^{10\text{-}1}$ f^{36a} / *FM6* ‖ Hw^{49cR5} / *FM7* ‖ $sc^{3\text{-}1}$ *w* f^{36a} / *FM7* ‖ *Df(1)RT650, y / FM6* ‖ *Df(1)RT343, y / FM6* ‖ *In(1)ac*^{3}, ac^3 w^a ‖ Hw^{49c} / *Df(1)sc*^{19}, *y AS-C*$^-$. The data of male viability were obtained in the first three crosses. Controls were $sc^{3\text{-}1}$ *w* f^{36a} / *Balancer* females for the first five crosses and *In(1)ac*^{3} or Hw^{49c} males in the last two crosses.

Hw^{49cR5}/sc^8Y males were crossed respectively with $sc^{10\text{-}1}f^{36a}$ / *FM6*, and Hw^{49cR5} / *FM7* females to obtain viability in the second row. Controls were Hw^{49cR5} / *FM7* females. For female's viability in third row we crossed $sc^{10\text{-}1}f^{36a}$ / *FM6* females with $sc^{10\text{-}1}f^{36a}$ / y^2Y67g males.

lethal interaction with Sxl^{f1}. Thus, we have analysed two mutations affecting T4, $sc^{10\text{-}1}$ and Hw^{49cR5} . The $sc^{10\text{-}1}$ mutation was obtained from the *In(1)ac*^{3} chromosome, which causes an extreme achaete phenotype, is associated with an inversion near the T5 gene, and causes a decrease in the amount of T5 RNA (Campuzano *et al.*, 1985). $sc^{10\text{-}1}$ has, in addition, a point mutation which places a stop codon within the T4 gene (Villares and Cabrera, 1987) and induces an extreme scute phenotype (García-Bellido, 1979). Hw^{49cR5} is a revertant of the strong Hw^{49c} mutation; both overexpress T4 and T5 (Balcells *et al.*, 1988). The Hw^{49cR5} mutation, however, has a strong scute phenotype as a consequence of a deletion of 8 bp in the T4 gene causing a frameshift early in its coding region (Balcells *et al.*, 1988).

Mutations at the AS-C affecting *sis-b* function should show a female-specific lethal phenotype. $sc^{3\text{-}1}$ fulfils this criterion (García-Bellido, 1979; Cline, 1988). This mutation was isolated as a revertant of the strong sc^3 allele and is not associated wtih DNA aberrations (Campuzano *et al.*, 1985). We have also tested this mutation for the *sis-b* function.

The results in Table III show that $sc^{3\text{-}1}$, Hw^{49cR5} and $sc^{10\text{-}1}$ affect male viability according to the strength of their scute phenotype. This is consistent with the genetic results, showing that deficiencies of the AS-C comprising the T4 gene display appreciable levels of male lethality not due to *lethal of scute* (García-Bellido, 1979). Females, however, show a much stronger lethality when carrying different combinations of these three alleles. Both chromosomes, *In(1)ac*^{3} and Hw^{49c}, from which the T4-specific mutations were obtained, do not display lethal phenotypes, neither in combination with $sc^{3\text{-}1}$ (Table III) nor in males or homozygous females (data not shown). $sc^{3\text{-}1}$ complements *Df(1)RT650*, which is $sis\text{-}b^+$, while it does not complement *Df(1)RT343* which is defective for *sis-b* function (Table III).

The results in Table IV show that females doubly heterozygous for any of the three *scute* mutations ($sc^{3\text{-}1}$, $sc^{10\text{-}1}$ and Hw^{49cR5}) and Sxl^{f1} or *Df(sis-a)* have reduced viability. In contrast, the *In(1)ac*^{3} and Hw^{49c} chromosomes do not interact with Sxl^{f1}.

We conclude that the female-specific lethality of $sc^{10\text{-}1}$ and Hw^{49cR5} , as well as their female-lethal synergistic interaction with Sxl^{f1} and *Df(sis-a)*, are due to an altered T4 product in the tested *scute* mutations. This indicates that the *scute* (T4) gene is responsible for the *sis-b* function of the AS-C. From the behaviour of $sc^{3\text{-}1}$ we conclude that it must be mutant for the T4 gene, because it displays a *sis-b* phenotype.

Table IV. Female-lethal synergism between Sxl^{f1} and *Df(sis-a)* with *AS-C* mutations

		AS-C mutation tested				
		$sc^{3\text{-}1}$	Hw^{49cR5}	$sc^{10\text{-}1}$	ac^3	Hw^{49c}
Interaction with:	Sxl^{f1}	31% (242)	68% (573)	18% (723)	98% (290)	109% (110)
	Df(sis-a)	5% (440)	17% (302)	<0.3% (334)	–	–

The percentage refers to the viability of experimental females relative to control females, whose actual number is given in parenthesis. Full genotype of crosses:

For the interaction with Sxl^{f1} we crossed *cm* Sxl^{f1} *ct / Y* males with the following females: $sc^{3\text{-}1}$ *w* f^{36a} / *FM7* ‖ Hw^{49cR5} / *FM7* ‖ $sc^{10\text{-}1}$ f^{36a} / *FM6* ‖ *In(1)ac*^{3}, ac^3 w^a ‖ Hw^{49c} / *Df(1)sc*^{19}, *AS-C*$^-$. In the first three crosses *cm* Sxl^{f1} *ct / Balancer* females were used as controls, in the last two crosses the control flies were *In(1)ac*^{3} and Hw^{49c} males, respectively.

For the interaction with *Df(sis-a)*, we crossed *Df(1)N71, sis-a*$^-$ / v^+Yy^+ males with the following females: $sc^{3\text{-}1}$ *w* f^{36a} / FM7 ‖ Hw^{49cR5} / *FM7* ‖ $sc^{10\text{-}1}$ f^{36a} / *FM6*. Controls were *Df(1)N71, sis-a*$^-$ / *Balancer* females.

Sxl^{M1} *suppresses the female-specific lethality caused by scute mutations that alter the T4 gene*

Results in Table V show that Sxl^{M1} totally suppresses the lethality of $sc^{3\text{-}1}/sc^{3\text{-}1}$, $Hw^{49cR5}/sc^{3\text{-}1}$ and $sc^{10\text{-}1}/sc^{3\text{-}1}$ females (for comparison see Table III); however, it does not suppress their scute phenotype. This demonstrates that the female-specific lethality associated with T4 mutations is due to a failure in activating *Sxl* and not to an effect on neural development. This is also supported by the suppression by Sxl^{M1} of the female-lethal interactions between the *Df(sis-a)* and either $sc^{3\text{-}1}$, Hw^{49cR5} or $sc^{10\text{-}1}$ (for comparison see Table IV). The fact that some of the experimental genotypes are not fully viable cannot be ascribed to an inability of

Table V. The female-specific lethality caused by *scute* mutations affecting the T4 gene is suppressed by Sxl^{M1}

Female genotype	Viability (no. control females)	Female genotype	Viability (no. control females)	Female genotype	Viability (no. control females)
(1) sc^{3-1} Sxl^{M1} + / + Sxl^{f1} +	97% (528)	(4) sc^{3-1} Sxl^{M1} + / sc^{3-1} + +	92% (138)	(7) sc^{3-1} + + / + Sxl^{M1} $sis\text{-}a^-$	84% (120)
(2) sc^{3-1} + + / sc^{3-1} Sxl^{f1} +	<0.6% (167)	(5) Hw^{49cR5} + + / sc^{3-1} Sxl^{M1} +	97% (145)	(8) Hw^{49cR5} + + / + Sxl^{M1} $sis\text{-}a^-$	75% (241)
(3) sc^{3-1} Sxl^{f1} + / sc^{3-1} Sxl^{M1} +	50% (456)	(6) sc^{10-1} + + / sc^{3-1} Sxl^{M1} +	117% (113)	(9) sc^{10-1} + + / + Sxl^{M1} $sis\text{-}d^-$	92% (179)

Viability of experimental females of genotype 3 was 75% of their control sisters, but these females do not represent the optimal value of viability. For this reason, this value has been corrected with the viability obtained for these females in other crosses to obtain an optimized reference (see text).

Genotypes 1 and 3–6 were generated by crossing sc^{3-1} *w* Sxl^{M1} / *FM7* females with the following males respectively: *cm* Sxl^{f1} *ct* / *Y* ‖ sc^{3-1} *w cm* Sxl^{f1} *ct* f^{36a} / *Y* ‖ sc^{3-1} *w* f^{36a} / *Y* ‖ Hw^{49cR5} / $sc^{8}Y$ ‖ sc^{10-1} f^{36a} / y^2Y67g. Controls were females carrying *FM7* and the parental X chromosome.

Genotypes 7–9 were generated by crossing *Df(1)N71, y cho cv* Sxl^{M1} $sis\text{-}a^-$ / *FM6* females with the following males respectively: sc^{3-1} *w* f^{36a} / *Y* ‖ Hw^{49cR5} / sc^{8} *Y* ‖ sc^{10-1} f^{36a} / y^2Y67g. Controls were females carrying *FM6* and the parental X chromosome.

Genotype 2 was generated in the following cross: sc^{3-1} *wf*^{36a}/*FM7* females with sc^{3-1}*w cm* Sxl^{f1} *ctf*^{36a}/y males.

Table VI. The male-specific lethal interaction of *Dp(AS-C)* and Hw^{685} with *Dp(sis-a*$^+$*)* is suppressed by Sxl^- or sc^{10-1} mutations

	Genotype of males					
	1		2		3	
	AS-C$^+$ Sxl^x *sis-a*$^+$; Y	*Dp(AS-C)* / *Dp(sis-a*$^+$*)*	sc^x Sxl^+ *sis-a*$^+$; v^+Yy^+, *sis-a*$^+$	*Dp(AS-C)* / +	Hw^{685} Sxl^x *sis-a*$^+$ / v^+Yy^+, *sis-a*$^+$	
	$Sxl^x = Sxl^+$	$Sxl^x = Sxl^-$	$sc^x = sc^+$	$sc^x = sc^{10-1}$	$Sxl^x = Sxl^+$	$Sxl^X = Sxl^{f1}$
Viability (no. control flies obtained)	5% (192)	63% (192)	10% (155)	73% (155)	18% (136)	85% (116)

All crosses were made at 18°C. Full genotype of crosses (females × males):

To generate genotypes 1: *y / Df(1)Sxl-7B0, y cm* Sxl^-; *Dp(1;2)*sc^{19}, y^+ *AS-C*$^+$ *b pr c* / + × *Df(1)N71, sis-a*$^-$ / *Y; Dp(1;2)*v^{65b}, *sis-a*$^+$ / *In(2L+2R)Cy,Cy pr*. Controls were *y/Y; In(2L+2R)Cy,Cy pr*/+ males.

To generate genotypes 2: *y /* sc^{10-1} f^{36a}; *Dp(1;2)*sc^{19}, y^+ *AS-C*$^+$ *b pr c / In(2L+2R)Cy,Cy pr* × *Df(1)N71, sis-a*$^-$ / v^+Yy^+, *sis-a*$^+$. Controls were *y* / v^+Yy^+ ; *In(2L+2R)Cy,Cy pr*/ + males.

To generate genotypes 3: Hw^{685}, *y / FM6* × *Df(1)N71, sis-a*$^-$ / v^+Yy^+ and Hw^{685}, *y cm* Sxl^{f1} *ct / FM6* × *Df(1)N71, sis-a*$^-$ / v^+Yy^+. Controls were *FM6* / v^+Yy^+ males.

The v^+Yy^+ chromosome is duplicated for the T5 gene but not for T4 and carries *Dp(sis-a*$^+$*)*.

Sxl^{M1} to provide enough *Sxl* functions, as neither aberrations in legs and eyes nor sexual transformation were found in those surviving females.

While sc^{3-1} Sxl^{M1} / + Sxl^{f1} females are viable, only 50% of sc^{3-1} Sxl^{M1} /sc^{3-1} Sxl^{f1} females are recovered. In the cross to generate the latter females we assumed that control females (sc^{3-1} Sxl^{f1} / + +) have their viability reduced in the same magnitude as in other crosses (Table IV). Although this experimental limitation may induce some error in estimating the viability, it is clear that Sxl^{M1} is not able to fully suppress the lethality of sc^{3-1} Sxl^{M1} /sc^{3-1} Sxl^{f1} females. In support of this statement is the fact that surviving females show frequent alterations in legs and eyes, besides masculinization of tergites and sternites. Thus, one Sxl^{M1} allele alone does not provide enough wild-type *Sxl* functions for full survival of sc^{3-1} homozygous females. This indicates that the constitutive Sxl^{M1} mutation retains some degree of regulation by the X:A ratio. A similar conclusion was reached by Steinmann-Zwicky (1988) in her analysis of *liz*.

The male-specific lethality of Dp(AS-C) in combination with Dp(sis-a) is mimicked by Hw^{685}*, a mutation that overexpresses T4, and is suppressed by either* Sxl^{f1} *or* sc^{10-1}

To test one of the expectations mentioned in the Introduction for an X:A numerator element, we assayed the viability of males carrying simultaneous duplications for *sis-a* and *sis-b*, knowing that each one alone is not lethal to males. Table VI shows that most of these males die. This lethality is largely rescued either when Sxl^+ is replaced by Sxl^{f1} or when one AS-C$^+$ copy is replaced by the sc^{10-1} mutation. The remaining lethality is probably due to an unspecific effect of the aneuploidy caused by both duplications. The Hw^{685} mutation overexpresses T4 at the late larval and early pupal stages (Balcells *et al.*, 1988), long after the activity of *Sxl* has become independent of the X:A signal (Sánchez and Nöthiger, 1983). If T4 overexpression also occurs in earlier stages of development we should expect that Hw^{685} mimics the effect of a duplication for T4. Suggestive of this early overexpression is the observation that females affected by

the lethal synergistic interaction with Sxl^{f1} described above are partially rescued when their AS-C^+ copy is replaced by the Hw^{685} mutation (data not shown). Thus, we have analysed the viability of males that carry this mutation together with a duplication for *sis-a*. Hw^{685}/v^+Yy^+, $Dip(sis\text{-}a^+)$ males have a reduced viability (Table VI) which is increased when Sxl^+ is replaced by Sxl^{f1}. It can be argued that the male lethality is not caused by Hw^{685} itself, but by something else on the X chromosome; so we crossed $Hw^{685}/cm\ Sxl^{f1}\ ct$ females, in which free recombination is allowed between the two X chromosomes, to $FM6/v^+Yy^+$, $Dp(sis\text{-}a^+)$ males. All the male progeny carry the $Dp(sis\text{-}a^+)$ in the Y chromosome. The number obtained for each genotype class was: 148 and 260 males for the parental classes Hw^{685}/v^+Yy^+ and $cm\ Sxl^{f1}ct/v^+Yy^+$ respectively; and 61 and 64 males for the recombinant classes $Hw^{685}cm\ Sxl^{f1}ct/v^+Yy^+$ and $+/v^+Yy^+$ respectively. Parental males carrying Hw^{685} are reduced compared with those carrying Sxl^{f1}; however, recombinant classes show the same viability and appear in numbers expected from the map position of markers. This shows that the lethality is exclusively associated with the Hw^{685} mutation and only in the presence of a Sxl^+ copy. All these results indicate that the described male-specific lethality is a consequence of the activation of *Sxl* due to an excess of T4 activity.

Negative results were obtained with the stronger allele Hw^{49c} (data not shown). This mutation is associated with a chromosomal inversion that places the *scute* (T4) and *achaete* (T5) genes in the middle of the 2B5 ecdysone-induced puff, with very little or no loss of DNA of either the AS-C or the 2B5 region (Balcells *et al.*, 1988). It has been suggested that this new location of the AS-C in the Hw^{49c} mutant is the cause of the overexpression of T4 and T5 at late larval and early pupal stages (Balcells *et al.*, 1988), when the maximum peak of ecdysone takes place. The lowest level of ecdysone is detected around the blastoderm stage (Garen *et al.*, 1977). If T4 and T5 are indeed under the control of ecdysone in the Hw^{49c} mutant, their overexpression would not take place at the time when the X:A signal is effective, but later. This would have no effect on *Sxl* activation and would explain our negative results.

The $sc^{10\text{-}1}$ mutation causes masculinization of triploid intersexes

Triploid intersexes are animals with a chromosomal constitution of 2X;3A. They have poor viability and those that survive exhibit a mosaic sexual phenotype (Bridges, 1921). Increasing the dose of a numerator element of the X:A ratio should feminize triploid intersexes, while decreasing it should masculinize them. This is actually what Cline (1988) found, when he changed the dose of elements of the AS-C region in triploid intersexes. If the T4 transcript is responsible for the *sis-b* function of the AS-C, we expect that the $sc^{10\text{-}1}$ mutation, which produces an abnormal T4 product, causes a masculinization of triploid intersexes. To generate the intersexes we crossed $sc^{10\text{-}1}\ f^{36a}/FM6$ females to $y^2/Y;C(2L)RM,dp;C(2R)RM,px;C(3L)RM,h;C(3R)RM,+$ males. The sexual phenotype was studied in the external terminalia of the flies, because these show the most pronounced sexual dimorphism. As expected, the majority of the $FM6/y^2$ triploid intersexes were mosaically composed of female and male tissues (37 specimens), and only a few had either pure female (six specimens) or pure male (six specimens), though incomplete tissues. In contrast, all the $sc^{10\text{-}1}/y^2$ triploid intersexes (61 specimens) have male terminalia only and, in all cases, these were indistinguishable from normal male terminalia. Furthermore these flies also had a normal male abdomen, with fully pigmented 5th and 6th tergites and no 7th tergite which characterizes female flies. These results confirm that the T4 gene provides the *sis-b* function of the AS-C.

Table VII. Female-specific dominant synergism between $sc^{10\text{-}1}$ and *da*

T[a]	Females: $sc^{10\text{-}1}$ / + ; *SM1*, da^+ / da^+	
	Viability relative to brothers da/da^+ (no. control flies)	Viability relative to sisters $sc^{10\text{-}1}/+;da/da^+$ (no. control flies)
25°C	87% (178)	110% (140)
29°C	51% (245)	102% (123)

Full genotype of cross (females × males):*da* / *SM1* × $sc^{10\text{-}1}\ f^{36a}$ / y^2Y67g.

Female-specific dominant synergism between $sc^{10\text{-}1}$ and *da*

Mutations at *sis-a* and *da* display female-specific dominant synergism, each enhancing the other's sex-specific lethal effect (Cline, 1986). As a further test for the role of T4 transcript, we analysed whether $sc^{10\text{-}1}$ interacts with *da*. We measured the viability of females heterozygous for $sc^{10\text{-}1}$ and deriving from mothers heterozygous for *da*. The results in Table VII show that, indeed, these females have reduced viability which is more pronounced at 29 than at 25°C. This temperature-dependence is expected for the lethal effects involving the *da* maternal product (Cline, 1978). As for *sis*-a, the female-specific lethal synergism between $sc^{10\text{-}1}$ and *da* depends on maternal, not on zygotic, function of *da*, as demonstrated by the fact that da/da^+ females are as viable as their da^+/da^+ sisters (see column to the right of Table VII). These results provide additional evidence that the T4 gene corresponds to the *sis-b* function.

Discussion

Our results and those recently reported by Cline (1988) show the existence of a function in the AS-C that acts as a numerator element of the X:A signal, which determines the state of activity of *Sxl*. This function has been named *sis-b* (Cline, 1988). Contrary to Cline's proposal that *sis*-b may not be a conventional gene, the main conclusion from our genetic data is that the *scα* gene of the AS-C, which codes for the T4 transcript, is responsible for both the *sc* and the *sis-b* functions.

The dual function of the T4 gene

Since the X:A ratio determines the state of activity of *Sxl* around the blastoderm stage (Sánchez and Nöthiger, 1983), expression of the genes that form the X:A ratio has to occur at or before this stage. It has recently been demonstrated that the T4 gene undergoes a preblastodermal transient expression (Romani *et al.*, 1987; Villares and Cabrera, 1987), leading to an homogeneous distribution of T4 RNA that coincides in time with the first zygotic activation of many

genes (Anderson and Lengyel, 1980; Weir and Kornberg, 1985; Edgar and Schubiger, 1986). This time of occurrence and distribution fits well with the expectation for the T4 gene being an element of the X:A signal. This is compatible with the results of Gergen (1987), who showed that dosage compensation is already in operation at the blastoderm stage, and that this dosage compensation reflects the zygotic sex-differential expression of the *Sxl* gene in response to the X:A signal.

If both, the *sis-b* and the *scute*, functions are carried out by a single T4 product, its activity in either *Sxl* activation or neurogenesis would depend on the developmental stage at which the gene is expressed. Alternatively, the different functions could involve two different T4 products, each one expressed at different developmental times. At present there is no evidence supporting the existence of two different T4 products, although this possibility is not completely excluded.

In this context, the particular behaviour of $sc^{3\text{-}1}$ is worth mentioning. While $sc^{3\text{-}1}$ strongly affects the *sis-b* function it has only a very weak scute phenotype, observable only in combination with *Df(AS-C)* or strong *scute* mutations (García-Bellido, 1979). If two T4 products were made, $sc^{3\text{-}1}$ could affect differently the two proteins. In the case of a single T4 product, $sc^{3\text{-}1}$ could mostly affect only one of two possible protein domains, each responsible for *scute* and *sis-b* functions respectively. A further possibility is that $sc^{3\text{-}1}$ would alter regulatory sequences of the T4 gene, and that low amounts of a normal T4 transcript would be sufficient for *scute*, but not for *sis-b* functions. Still another possibility would be that $sc^{3\text{-}1}$ mostly affects regulatory sequences involved in the preblastoderm expression of the T4 gene rather than those involved in the late, neurogenic expression of the gene. Regulatory sequences needed for *sis-b* functions should lie within an interval of 3.3 kb defined by the breakpoints of *Df(1)RT650* and *Df(1)RT343*, since the first is $sis\text{-}b^{+}$ and the second is $sis\text{-}b^{-}$. A molecular analysis of $sc^{3\text{-}1}$ is in progress (S.Campuzano, unpublished) to distinguish between these different possibilities.

Recently, it has been reported that the zygotic da^{+} product is involved, together with the AS-C, in committing cells into the neurogenic pathway (Caudy *et al.*, 1988a; Dambly-Chaudière *et al.*, 1988). The lethal synergistic effect, however, that we have observed between *da* and $sc^{10\text{-}1}$ is exclusively due to the maternal *da* function which is involved in *Sxl* activation (Cline, 1978). Two different *da* transcripts have been detected, a maternal one (3.2 kb) and a zygotic one (3.7 kb) (Caudy *et al.*, 1988b). Thus, it may well be possible that these two transcripts correspond to the two functions of the *da* gene.

A functional relationship exists between the maternal *da* product and the T4 gene to activate *Sxl*. There are two possibilities: either the T4 gene requires maternal *da* product to be expressed; or *da* product could be acting in parallel with, or downstream of, T4 and the other elements of the X:A signal. It is tempting to speculate that a similar functional relationship between *da* zygotic function and the T4 gene is at the basis of their effect in neurogenesis.

Is the T5 transcript able to substitute for the T4 transcript in the sis-b function?

Although the T4 and T5 genes are responsible for the *scute* and *achaete* functions respectively, there is evidence indicating that each gene can assume some of the other's functions (Dambly-Chaudière and Ghysen, 1987; Jiménez and Campos-Ortega, 1987; Balcells *et al.*, 1988). Our results suggest that T5 can partially substitute for the *sis-b* activity of T4. $sc^{10\text{-}1}$, a double mutant affecting both T4 and T5 genes, is more defective for *sis-b* function than Hw^{49cR5}, a *sc* mutation that overexpresses a normal T5 transcript (Campuzano *et al.*, 1985; Balcells *et al.*, 1988). This difference might explain the different behaviour of $sc^{10\text{-}1}$ and Hw^{49cR5} with respect to the *sis-b* function. In the presence of wild-type levels of T4 transcript, the reduction of T5 product does not seem to affect female viability. This, together with the extensive homology between T4 and T5 (Villares and Cabrera, 1987), suggests a certain ability of T5 to assume the *sis-b* function of T4 when this is reduced. The possibility remains that T5 has minor female-specific functions in wild-type females.

The nature of the X:A signal

According to the criteria outlined in the Introduction, the *scute* (T4) gene qualifies as a numerator element forming the X:A signal. At least for this element the model proposed by Chandra (1985) which assumes that the numerator elements of the X:A signal are non-coding sequences able to bind with high affinity a *Sxl* repressor of autosomal origin, must be questioned. Similarly, the suggestion of Waring and Pollack (1987), that a moderately repeated sequence found almost exclusively on the X chromosome might be 'counted' to establish the X:A ratio numerator, must also be questioned. So far, only two numerator elements have been identified, the T4 gene (*sis-b* activity) and *sis-a*. Hemizygosity for any of these genes has similar effects on Sxl^{f1} heterozygous females. There is, however, an asymmetric relationship in the ability of a duplication of either element to suppress the lethal effect of the other. Moreover, mutations at *da* and the T4 gene, or the *sis-a* gene, display female-specific synergistic lethality that is stronger for the T4 gene than for *sis-a* (M.Torres and L.Sánchez, unpublished). These data indicate that these numerator elements do not play strictly equivalent roles in producing the X:A signal.

Although more elements that make up this signal have to be identified and characterized at the molecular level, the results with the T4 gene suggest that a conventional genetic system might be at its basis. Generalizing our findings for *sis-b*, *sisterless* elements would be genes expressed around the blastoderm stage in a non-compensated way, so that XX embryos would have twice the amount of *sisterless* products than XY embryos. The amount of these products constitutes what is called the numerator of the X:A ratio. Their interaction with autosomal denominator elements would form the X:A signal. Recent molecular data show that T4 and *da* products present a DNA-binding and protein-dimerization motif also found in transcriptional activators such as *GCN4* (Alonso and Cabrera, 1988; Caudy *et al.*, 1988b; Murre *et al.*, 1989). Based on these properties of the T4 product, the interactions with the autosomal elements could either be product−product or gene−product interactions. This would result in different signals in males versus females which are then translated into different activity states of *Sxl* in the two sexes.

Materials and methods

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 chromosomes and genetic markers see Lindsley and Grell (1968) and Lindsley and Zimm (1982, 1985, 1987). The *Df(1)RTs* were obtained and localized on the X chromosome by Mason *et al.* (1984, 1986). Ruíz-Gomez and Modolell (1987) mapped their breakpoints at the molecular level. Some of the stocks used came from the Drosophila Stock Centers at Umea (Sweden) and Bowling-Green (USA). To analyse the cuticular structures of the adults, flies were macerated with 10% KOH at 50°C for 15 min, washed in H_2O and mounted in Faure's solution.

Acknowledgements

We are grateful to J.Modolell, S.Campuzano, B.Granadino, A.San Juan and E.Torroja for helpful discussions and comments on the manuscript and to M.C.Partearroyo for typing it. This work was supported by the Comisión Asesora de Investigación Científica y Técnica and the Consejo Superior de Investigaciones Científicas. M.T. is recipient of a predoctoral fellowship from the Ministerio de Educación y Ciencia.

References

Alonso,M.C. and Cabrera,C.V. (1988) *EMBO J.*, **7**, 2585–2591.
Anderson,K.V. and Lengyel,J.A. (1980) *Cell*, **21**, 717–727.
Balcells,Ll., Modolell,J. and Ruiz-Gómez,M. (1988) *EMBO J.*, **7**, 3899–3906.
Bridges,C.B. (1921) *Science*, **54**, 252–254.
Cabrera,C.V, Mártinez-Arias,A. and Bate,M. (1987) *Cell*, **50**, 425–433.
Campuzano,S., Carramolino,L., Cabrera,C.V,. Ruiz-Gómez,M., Villares,R. Boronat,A. and Modolell,J. (1985) *Cell*, **40**, 327–338.
Campuzano,S., Balcells,Ll., Villares,R., Carramolino,L., García-Alonso,S. and Modolell,J. (1986) *Cell*, **44**, 303–312.
Caudy,M., Grell,E., Dambly-Chaudière,C, Ghysen,A., Jan,L.Y. and Jan,Y.N. (1988a) *Genes Dev.*, **2**, 843–852.
Caudy,M., Vaessin,H., Brand,M., Turner,R., Jan,L.Y. and Jan,Y.N. (1988b) *Cell*, **55**, 1061–1067.
Chandra,H.S. (1985) *Proc. Natl. Acad. Sci. USA*, **82**, 1165–1169.
Cline,T.W. (1978) *Genetics*, **90**, 683–698.
Cline,T.W. (1979) *Genetics*, **91**, S22.
Cline,T.W. (1980) *Genetics*, **96**, 903–926.
Cline,T.W. (1986) *Genetics*, **113**, 641–663.
Cline,T.W. (1988) *Genetics*, **119**, 829–862.
Dambly-Chaudière,C. and Ghysen,A. (1987) *Genes Dev.*, **1**, 197–306.
Dambly-Chaudière,C., Ghysen,A., Jan,L.Y. and Jan,Y.N. (1988) *Roux's Arch. Dev. Biol.*, **197**, 419–423.
Edgar,B.A. and Schubiger,G. (1986) *Cell*, **44**, 871–877.
García-Bellido,A. (1979) *Genetics*, **91**, 491–520.
Garen,A., Kauvar,L. and Lepesant,J.A. (1977) *Proc. Natl. Acad. Sci. USA*, **74**, 5099–5103.
Gergen,P. (1987) *Genetics*, **117**, 477–485.
Jiménez,F. and Campos-Ortega,J.A. (1987) *J. Neurogenet.*, **4**, 179–200.
Lindsley,D.L. and Grell,E.H. (1968) *Carnegie Institute of Washington Publication*, No. 627.
Lindsley,D.L. and Zimm,G. (1982) *Dros. Info. Service*, **57**.
Lindsley,D.L. and Zimm,G. (1985) *Dros. Info. Service*, **62**.
Lindsley,D.L. and Zimm,G. (1987) *Dros. Info. Service*, **65**.
Lucchesi,J.C. and Manning,J.E. (1987) *Adv. Genet.*, **24**, 371–429.
Lucchesi,J.C. and Skripsky,T. (1981) *Chromosoma*, **82**, 217–227.
Mason,J.M., Strobel,E. and Green,M.M .(1984) *Proc. Natl. Acad. Sci. USA*, **81**, 6090–6094.
Mason,J.M,. Voelker,R.A., Rosen,D., Campos,A.R., White,K. and Lim,J.K. (1986) *Dros. Info. Service*, **63**, 164–165.
Murre,C., McCaw,P.S. and Baltimore,D. (1989) *Cell*, **56**, 777–783.
Nöthiger,R. and Steinmann-Zwicky,M. (1985) *Trend Genet.*, **1**, 209–215.
Oliver,B., Perrimon,N. and Mahowald,A.P. (1988) *Genetics*, **120**, 159–171.
Romani,S., Campuzano,S. and Modolell,J. (1987) *EMBO J.*, **6**, 2085–2092.
Ruiz-Gómez,M. and Modolell,J. (1987) *Genes Dev.*, **1**, 1238–1246.
Sánchez,L. and Nöthiger,R. (1982) *Roux's Arch. Dev. Biol.*, **191**, 211–214.
Sánchez,L. and Nöthiger,R. (1983) *EMBO J.*, **2**, 485–491.
Steinmann-Zwicky,M. (1988) *EMBO J.*, **7**, 3889–3898.
Villares,R. and Cabrera,C.V. (1987) *Cell*, **50**, 415–424.
Waring,G.L. and Pollack,J.A. (1987) *Proc. Natl. Acad. Sci. USA*, **84**, 2843–2847.
Weir,M.P. and Kornberg,T. (1985) *Nature*, **318**, 433–439.

Received on March 22, 1989; revised on June 28, 1989