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The segmentation gene *runt* is needed to activate *Sex-lethal*, a gene that controls sex determination and dosage compensation in *Drosophila*

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Summary

In *Drosophila*, sex is determined by the relative number of X chromosomes to autosomal sets (X:A ratio). The amount of products from several X-linked genes, called *sisterless* elements, is used to indicate to *Sex-lethal* the relative number of X chromosomes present in the cell. In response to the X:A signal, *Sex-lethal* is activated in females but remains inactive in males, being responsible for the control of both sex determination and dosage compensation. Here we find that the X-linked segmentation gene *runt* plays a role in this process. Reduced function of *runt* results in female-specific lethality and sexual transformation of XX animals that are heterozygous for *Sxl* or *sis* loss-of-function mutations. These interactions are suppressed by Sxl^{M1}, a mutation that constitutively expresses female *Sex-lethal* functions, and occur at the time when the X:A signal determines *Sex-lethal* activity. Moreover, the presence of a loss-of-function *runt* mutation masculinizes triploid intersexes. On the other hand, *runt* duplications cause a reduction in male viability by ectopic activation of *Sex-lethal*. We conclude that *runt* is needed for the initial step of *Sex-lethal* activation, but does not have a major role as an X-counting element.

1. Introduction

In *Drosophila melanogaster* 2X;2A individuals (X, X chromosome; A, autosomal set) are females and XY;2A individuals (Y, Y chromosome) are males. The Y chromosome does not play any role in sex determination. Sex determination occurs by the sex-specific expression of a group of genes that are hierarchically organized (reviewed in Baker & Belote, 1983; Nöthiger & Steinmann-Zwicky, 1985; Steinmann-Zwicky *et al.* 1990). Sex-specific regulation of these genes takes place throughout development by alternative splicing of their transcriptional products (reviewed in Baker, 1989; Hodgkin, 1989). Sex determination is linked to dosage compensation (hypertranscription of the male X chromosome) (Lucchesi & Skripsky, 1981; Cline, 1983; Gergen, 1987). Dosage compensation ensures that the products of the X-linked compensated genes are present at the same levels in females and males. Both processes, 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 & Plaut, 1973). The X:A ratio determines the state of activity of *Sex-lethal* (*Sxl*): in females *Sxl* will be ON, whereas in males it will be OFF (Cline, 1978). Activation of *Sxl* also requires the maternal *daughterless* (*da*) product (Cline, 1978). The activity of *Sxl* becomes locked in the female-specific or male-specific mode around the blastoderm stage (Sánchez & Nöthiger, 1983; Bachiller & Sánchez, 1991) and from this moment *Sxl* stably maintains the determined state by an autoregulatory process (Cline, 1984). *Sxl* controls both sex determination and dosage compensation by regulating independent sets of genes for each process (reviewed in Baker & Belote, 1983; Lucchesi & Manning, 1987). Failures in dosage compensation, either by hypertranscription in females or hypotranscription in males, are lethal (Lucchesi & Skripsky, 1981). In contrast, sex determination is not a vital process, and so, failures in sex determination lead to sex-transformed phenotypes (reviewed in Baker & Belote, 1983; Steinmann-Zwicky *et al.* 1990). For these reasons, misexpression of *Sxl* can produce sex-specific lethality and/or sexual transformation either in males or females.

The gene *Sxl* produces two temporally distinct sets of transcripts (Salz *et al.* 1989). The early set is composed of three transcripts found only around blastoderm stage. These transcripts are specifically produced in females as a response of *Sxl* to the X:A

signal (Salz *et al.* 1989; Torres & Sánchez, 1991). The late set is formed by three male-specific and three female-specific transcripts which appear slightly later in embryonic development and persist throughout development. Male-specific transcripts differ from the female-specific transcripts by the inclusion of a specific exon that places a stop codon in the coding region and, therefore, gives rise to truncated, presumably non-functional, proteins. In females this exon is spliced out and functional protein is produced (Bell *et al.* 1988; Bopp *et al.* 1991). The female-specific protein is needed for female-specific splicing of the transcripts of *transformer* (Sosnowsky *et al.* 1989; Inoue *et al.* 1990), the next gene in the sex determination hierarchy. In addition, *Sxl* product is needed for female-specific splicing of *Sxl* transcripts (Bell *et al.* 1991), generating a positive autoregulatory loop that provides a molecular basis for *Sxl* functioning as a stable genetic switch. The gene *fl(2)d* is required for female-specific splicing of *Sxl* transcripts and is thought to play a role in the positive autoregulatory loop of *Sxl* (Granadino *et al.* 1990).

sisterless elements are X-linked loci that determine *Sxl* activity in a dosage-dependent way (Cline, 1988; Torres & Sánchez, 1989). For this reason, they are considered to be components of the X:A signal. 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 & Sánchez, 1989, 1991; Parkhurst *et al.* 1990; Erickson & Cline, 1991). Two of the genes needed to activate *Sxl*, *sc* and *da*, encode helix–loop–helix (HLH) proteins (Villares & Cabrera, 1987; Caudy *et al.* 1988). 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 their affinity for DNA-binding sites (Murre *et al.* 1989*b*; Benezra *et al.* 1990; Sun & Baltimore, 1991). Parkhurst *et al.* (1990) have proposed that the X:A signal is formed by X-linked sisterless products (numerator elements) which are titrated by autosomal HLH products (denominator elements), so that an effective concentration of sisterless products would only be attained in females.

The isolation of genes involved in determining the mode of expression of *Sxl* could, in principle, be approached by selection of sex-specific lethal mutations. However, such genes may display pleiotropic phenotypes affecting, besides *Sxl* regulation, some other sex-non-specific vital function. In this case, the identification of these genes by isolation of sex-specific mutations is very difficult. A good example is the gene *sc*, well known since the 1930s for its implication in sensory organ development in the adult and thereafter intensively analyzed both genetically (García Bellido, 1979) and molecularly (Campuzano *et al.* 1985). Its sex-determining function, however, has only recently been found (Torres & Sánchez, 1989). Despite the extensive mutational analysis of the X-chromosomes and of the AS-C in particular, there is only one sex-specific lethal *sc* mutation available, sc^{3-1} (García Bellido, 1979; Cline, 1988; Torres & Sánchez, 1989). A second approach has proved to be effective in revealing such genes: the study of synergistic lethal interactions in animals that are transheterozygous for *Sxl* mutations and deficiencies for different regions of the X chromosome (Cline, 1988; Oliver *et al.* 1988; Steinmann-Zwicky, 1988; Torres & Sánchez, 1989). Following this procedure, we have identified a proximal X chromosome region that interacts with *Sxl*. After detailed analysis, we find that the gene *runt*(*run*) is responsible for this interaction. This gene belongs to the group of 'pair-rule' genes, involved in the subdivision of the embryo into a segmented pattern that underlies the general organization of the embryo (Gergen & Butler, 1987). The common feature of the pair-rule genes is that they are expressed in seven to eight stripes during the cellularization of the blastoderm (Ingham, 1988). Our results show that *run* activity is required, before its role in segmentation, for the initial step of *Sxl* activation. Similar conclusions have been reported by Duffy & Gergen (1991).

2. Materials and methods

(i) *Culture conditions*

Flies were raised on standard *Drosophila* medium. The temperature of cultures was 25 °C unless otherwise stated. For full description of markers and chromosomes used see Lindsley & Zimm (1985, 1987, 1990).

(ii) *Cuticular preparations*

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

(iii) *Crosses*

Df(1)N71, sis-a^{-}*/v*$^{+}$*Yy*$^{+}$ males were crossed to the following females: *Df(1)16–3–22/FM6,* ‖ *Df(1)-run*^{1112}, *y* f^{36a}*/FM6* ‖ *Df(1)16–3–35/Binsn* ‖ *Df(1)B57/FM6* ‖ *In(1)sc*^{8} *Df(1)mal*^{10}, sc^{8} *B/In(1)dl*^{49}, $sn^{\times 2}$ v^{Of} mal^{2} ‖ *Df(1)LB6/FM6* ‖ *Df(1)A118/FM6* ‖ *Df(1)A53/FM6* ‖ *Df(1)Q539/FM6.* Controls were Balancer females.

y sis-a/Y males were crossed to *Df(1)run*^{1112}, *y* f^{36a}*/FM6/y*$^{+}$*Ymal*^{171} females. Experimental females were *Df(1)run, y* f^{36a}*/Df(1)N71, sis-a*$^{-}$*/y*$^{+}$*Ymal*^{171} females. Control flies were *FM6/Y* males. Both, experimental and control flies come from the same chromosome segregation event.

Df(1)N71, sis-a^{-}*/FM6* or *Df(1)N71, y cho cv* Sxl^{M1} *sis-a*$^{-}$*/FM6* females were crossed to the

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following males: $run^{AA33}/y^+ Ymal^{106} \| y\ w\ f^{36a}\ run^{YP17}/-$ $y^+ Ymal^{106} \| y\ w\ f^{36a}\ run^{XK52}/y^+ Ymal^{106} \| y\ w\ f^{36a}$ $run^{YD24}/y^+ Ymal^{106} \| w\ run^{YC28}/y^+ Ymal^{106} \| y\ w\ f^{36a}$ $run^{YC47}/y^+ Ymal^{106} \| w\ run^{YE96}/Ymal^{106} \| y\ run^{XA06}/y^+$ $Ymal^{106} \| y\ w\ f^{36a}\ run^{XD106}/y^+ Ymal^{106} \| Df(1)run^{1112}$, $y\ f^{36a}/y^+ Ymal^{106}$. Control flies were FM6 females.

y cm $Sxl^{7BO}/y/y^+ Ymal^+$, run^+ *x* $y/y^2 Y_{67g}$. Cross to generate males carrying duplications for both *sis-a* and *run*. *y cm* $Sxl^{7BO}/y/y^+ Ymal^+$, run^+ *x y; Dpv*^{65b}, *sis-a*$^+$*/SM5*

cm Sxl^{7BO}; *Dp(1;3)*sn^{13a1}, Sxl^+*/TM3* males were crossed to *y w* $f^{36a}\ run^{XD106}/FM7 \| Df(1)runt^{1112}\ y\ f^{36a}$ $run^-/FM7$ and $Sxl^{M1}\ f^{36a}\ run^{XD106}/FM7$ females. Controls were *FM7* females carrying the *Dp(1;3)*sn^{13a1}, Sxl^+ duplication. In both crosses, daughters of the genotype $Sxl^{7BO}/FM7$ show no viability reduction (data not shown), therefore there is no deleterious dominant effect of Sxl^{7BO}.

y sis-a/FM6 females were crossed to *y w* f^{36a} $run^{XD106}/y^+ Ymal^+$ males. Controls were Balancer females. Since both, control and experimental females, carry the same *run* mutations, their possible dominant effect on the viability of females was corrected.

y w $f^{36a}\ run^{YP17}/y^+ Ymal^{106}$, run^+ and $Df(1)run^{1112}$, *y* $f^{36a}\ run^-/y^+ Ymal^{106}\ run^+$ females were crossed to *y sis-a/Y* males. Controls were the *y w* f^{36a} $run^{YP17}/y^+ Ymal^{106}$ males. Appropriate control crosses showed that, in the absence of lethal effects, females with and without the duplication occur at the same frequency in the progeny from females carrying the $y^+ Ymal^{106}$ chromosome.

Df(1)svr, spl $f^{36a}/FM6 \| sc^{10-1}\ f^{36a}/FM6$ and sc^{10-1} $Sxl^{M1}/FM7$ females were crossed to *y w* $f^{36a}\ run^{XD106}/$ $y^+ Ymal^{106}$ and *Df(1)* run^{1112}, *y* $f^{36a}\ run^-/y^+ Ymal^{106}$ males. Controls were Balancer females. Since both, control and experimental females, carry the same *run* mutations, their possible dominant effect on the viability of females was corrected.

$Df(1)run^{1112}$, *y* $f^{36a}\ run^-/y^+ Ymal^{106}$ and *Df(1)16-3-22, y* $f^{36a}\ run^-/y^+ Ymal^{106}$ males were crossed to *y; d* $da^2/CyO \| y; d\ fy^2\ da^{11B31}\ b\ pr\ c/CyO$ and $da^{X136}\ b\ pr$ *cn wx bw/CyO* females.

3. Results

(i) run *mutations interact synergistically with mutations at* Sxl *and the genes that determine its activity*

Females doubly heterozygous for both Sxl^{f1} and *Df(1)HF396*, a deletion for the proximal X chromosome including *run*, have drastically reduced viability; less than 10% of these females survive compared to their $Sxl^{f1}/+$ siblings (10 flies of the experimental genotype versus 121 of the control ones). A lethal interaction also occurs between deficiencies for the proximal X chromosome and either *sis-a* or different *sc* loss-of-function mutations (see below). To localize the proximal X chromosome region responsible for this interaction, we have analyzed the viability of females doubly heterozygous for both *Df(sis-a)* and different deficiencies for the proximal X chromosome region (Fig. 1). The base of the X chromosome is one of the most extensively studied and mutationally saturated regions of the *Drosophila* genome. Deficiencies have been described that divide the region into a set of complementation groups. The lethal interaction with *sis-a* appears associated with the deficiency for a region in which only the *run* complementation group has been defined (Perrimon *et al.* 1989). This strongly suggests that *run* is responsible for the interaction observed. To test this possibility, we analyzed a set of loss-of-function *run* alleles induced by EMS (Gergen & Wieschaus, 1986) (Fig. 2). These alleles have been classified according to the strength of their runt phenotype. Since EMS mostly induces point mutations, it is unlikely that these mutations affect other genes besides *run*. Moreover, the chromosomes carrying the mutations had been originally cleaned up by recombination so that the only lethal mutations they carry are the *run* ones (Gergen, personal communication). Two amorphic mutations show interaction with *Df(sis-a)*, similar in intensity to the interaction shown by deficiencies that completely remove the region, as for example $Df(1)run^{1112}$. The weak hypomorphs produce very weak or no lethal interaction. Intermediate penetrance of lethality is found for the intermediate and strong hypomorphs. Within these groups, however, no strict correlation is found between runt phenotype and strength of the lethal interaction; some of the strong hypomorphs show less interaction than the intermediate ones. Specially informative is the temperature-sensitive allele run^{YP17}. At 18 °C, this allele behaves in segmentation as a weak hypomorph, while at 29 °C it behaves as a strong hypomorph (Gergen & Wieschaus, 1986). The same behaviour is found in its interaction with the *Df(sis-a)*: the run^{YP17} mutation shows much less lethality in its interaction with the *Df(sis-a)* at 18 than at 25 °C (Fig. 2). No differences have been observed between 25 and 29 °C (data not shown). This thermosensitivity is specific to the run^{YP17} allele, since other *run* mutations do not show thermosensitivity in their interaction with the *sis-a* deficiency (data not shown). In addition, the data in Fig. 2 come from crosses in which the *run* mutations are paternally inherited. This eliminates the possibility that maternal *run* dosage is responsible for the interaction.

There are also female-lethal synergistic interactions between *run* mutations and mutations in *sis-a*, *sc* and *Sxl* (Table 1). Moreover, females that escape the lethal interactions are masculinized (Fig. 3). In addition, they show lack of cuticular structures, a phenotype that has been frequently found in flies that misregulate *Sxl* and which is thought to be the consequence of abnormal dosage compensation. These phenotypes and the observed lethality are suppressed

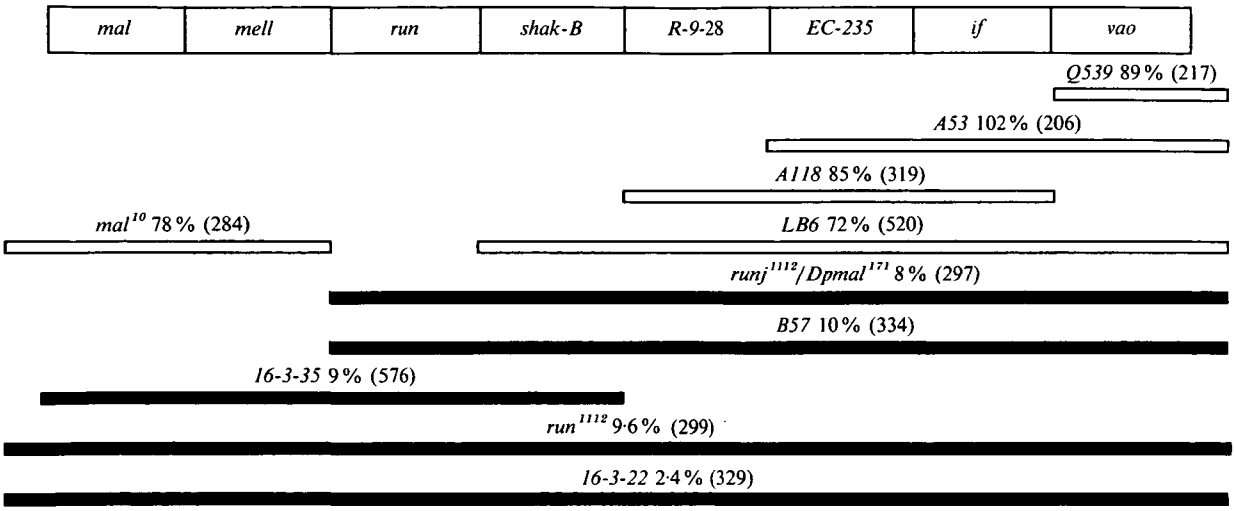

Fig. 1. Genetic mapping of the proximal region of the X-chromosome responsible for the lethal interaction. Females doubly heterozygous for a *sis-a* deficiency and different deficiencies for the proximal X chromosome were produced and their viability determined. Filled bars represent deficiencies that display lethal phenotype in combination with *Df(1)N71, sis-a*$^{-}$ and empty bars those that do not. The genetic map of the region is represented on the upper part of the figure. The name of the deficiency and the viability of experimental females, as percentage relative to control females, are indicated. The number of control females (see Material and methods) obtained appears in parentheses.

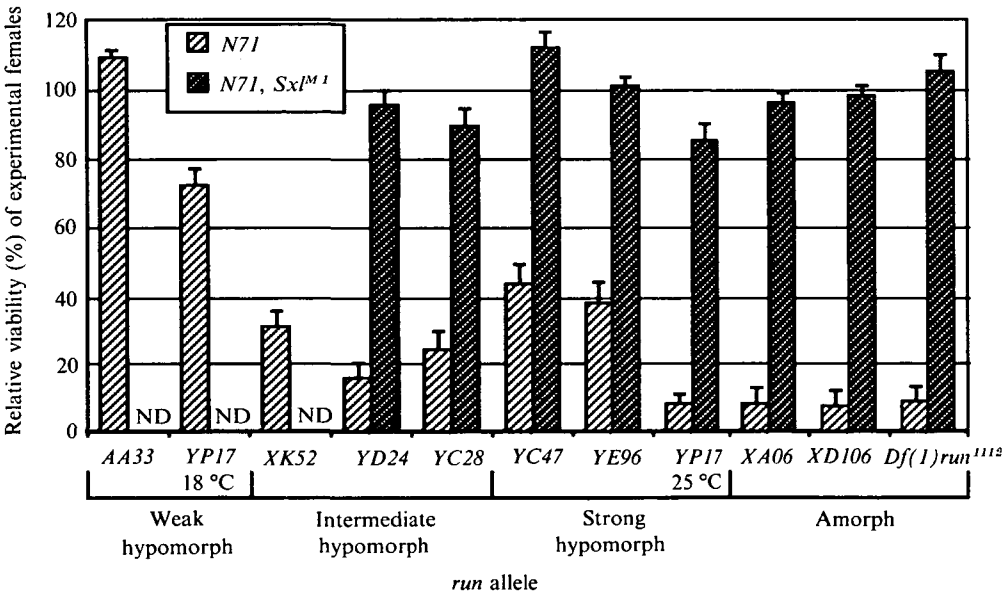

Fig. 2. Interaction of different *run* alleles with *sis-a*. The figure represents the viability ($\pm 2 \times$ S.E.M.) of females doubly heterozygous for *Df(1)N71, sis-a*$^{-}$ or *Df(1)N71, sis-a*$^{-}$*Sxl*^{M1} and the *run* allele that appears on the abscissa. Alleles are ordered according to the strength of their run phenotype, as described in Gergen & Wieschaus (1986). The strength of the mutations increases from left to right. The cross with the *run*^{YP17} allele was made at two different temperatures, 18 and 25 °C, as specified in the figure. Since control females carry the different *run* mutations (see Materials and methods), their possible dominant effect on the viability of females is corrected.

by *Sxl* or *run* duplications, or by *Sxl*^{M1} (Table 1, Fig. 2) a mutation that constitutively expresses the *Sxl* function (Cline, 1978).

Gergen & Wieschaus (1986) described a *run* dosage-dependent phenotype in segmentation: females hemizygous for *run* showed weak run phenotypes, while males carrying *run* duplications showed an anti-run phenotype. These phenotypes were sensitive to the genetic background. Besides, as a consequence of *run* being dosage compensated, loss-of-function mutations at *Sxl* and *da*, which produce inappropriate X chromosome hypertranscription (Lucchesi & Skripsky, 1981), ameliorate the segmentation defects caused by *run* hypomorphic mutations in females (Gergen, 1987). Considering these observations, it could be argued that the female lethal interaction described is not directly due to the *run* mutations, but to the presence of modifiers in the stocks in which *run*

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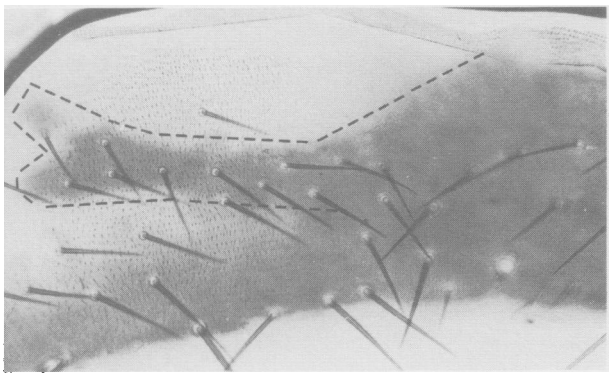

Fig. 3. Masculinization of females that escape the *run–sis-a* interaction. Fifth and sixth tergites in wild-type males are completely pigmented, while in wild-type females the anterior part remains unpigmented. The photograph (×200) shows male tissue (marked by a dashed line) on the fifth tergite of a *Df(1)N71, sis-a*$^{-}$*/run*^{XD106} female.

mutations are maintained in heterozygosis. These modifiers would have been positively selected to favour a low *Sxl* activity, that would ameliorate the dominant effects of the *run* lesion. The presence of the modifiers would affect neither sex determination nor dosage compensation in wildtype conditions, but would be apparent in the presence of a single copy of *Sxl* or its regulators, producing the lethal interaction. However, several observations demonstrate that this is not the case. First, in most of the stocks used, the *run* mutation was maintained in males with a *run*$^{+}$ duplication, and therefore, there was no possibility of a selection effect. Second, for the *run*^{YD24} mutation the interaction with the *sis-a* deficiency was tested with two different stocks, one in which the mutation was maintained in males and a second one in which it was maintained in females. The viability of the *run*^{YD24}*/ Df(sis-a)* females was 16 ± 5% in the first case (Fig. 2) and 11 ± 4% in a similar cross in the second case (data not shown). Third, the suppression by a *run*$^{+}$ duplication of the lethal interaction between *sis-a* and *run* mutations (Table 1) would not take place if the interaction was due to modifiers at any genomic location. And fourth, the results with the temperature-sensitive allele *run*^{YP17} show that the interaction is indeed due to the different levels of *run* activity in the same background conditions. Thus, the above results show that *Sxl* was not properly activated in females heterozygous for both *run* and *sis* mutations; therefore, we conclude that *run* is needed for the correct expression of *Sxl*.

(ii) *There is a weak female-specific dominant synergism between* run *and* da *mutations*

Mutations at *da* and at *Sxl* (Cline, 1978), or *sis-a* (Cline, 1986), or *sc* (Cline, 1988; Torres & Sánchez, 1989), display a female-specific dominant synergism which is in good agreement with the role of the maternal *da* product in the initial step of *Sxl* activation (Cline, 1984). The interaction of *run* with the elements of the X:A signal, *sis-a* and *sc*, as well as with *Sxl*, implicate this gene in the initial step of *Sxl* activation. Therefore, we have also analyzed the interaction between *run* and the maternal *da* product. Females heterozygous for *run* deficiencies show reduced viability when coming from *da*^{X136}/+ mothers, independently of their zygotic genotype for *da* (Table 2). However, the synergistic interaction is less strong than the observed between *da* and *sis-a* (Cline, 1986), or *da* and *sc* (Cline, 1988; Torres & Sánchez, 1989). In the case of the other two *da* mutations tested, a synergistic lethal interaction is only observed between *da*^{11B31} and *Df(1)run*^{1112} (Table 2). Despite the fact that the three *da* mutations used are amorph (Cronmiller & Cline, 1986, 1987; Caudy *et al.* 1988*b*), they show different behaviour in their interaction with *run* mutations. This may be due to variations in the genetic backgrounds of these stocks, something that has been previously reported for genotypes affecting *Sxl* activation (Cline, 1988).

(iii) *The* run^{YP17} *mutation causes masculinization of triploid intersexes*

Triploid intersexes are individuals with a chromosomal constitution of 2X;3A. Due to the ambiguous X:A ratio of 0·67 they exhibit a mosaic sexual phenotype (Bridges, 1921). Interfering with the ac-

Table 1. *Transheterozygous synergistic lethal interaction between* run *and* Sxl, sc *or* sis-a *mutations*

run allele	*Sxl*^{7B0}			*sis-a*		*Df(1)svr, sc*$^{-}$	*sc*$^{10-1}$	
	With *Dp(Sxl*$^{+}$*)*	With *Sxl*^{M1}		With *Dp(run*$^{+}$*)*			With *Sxl*^{M1}	
run^{YP17}	—	—	—	22%[a] (210)[b]	110% (210)	—	—	—
run^{XD106}	3% (358)	75% (358)	87% (237)	25% (149)	—	1% (219)	0·4% (459)	122% (210)
run^{1112}	2% (378)	126% (378)	—	10% (121)	108% (121)	3% (374)	22% (209)	120% (180)

[a] Viability of females heterozygous for the mutations specified in the entries with respect to controls.
[b] Number of control flies.

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Table 2. *Transheterozygous synergistic lethal interactions between* Df(run) *and the maternal* da *product*

Zygotic genotype	Maternal genotype		
	da^2/da^+	da^{11B31}/da^+	da^{X136}/da^+
$Df(1)16\text{-}3\text{-}22, run^-/run^+$	103%[a] (269)[b]	110% (295)	56% (553)
$Df(1)run^{1112}, run^-/run^+$	138% (475)	67% (172)	40% (412)

[a] Viability of da^+/da^+ females. This viability did not significatively differ ($P > 0{\cdot}05$) from the viability of their da^+/da sisters, showing that the interaction is strictly maternal.
[b] Number of control flies, which were males of the genotype da^+/da^+.

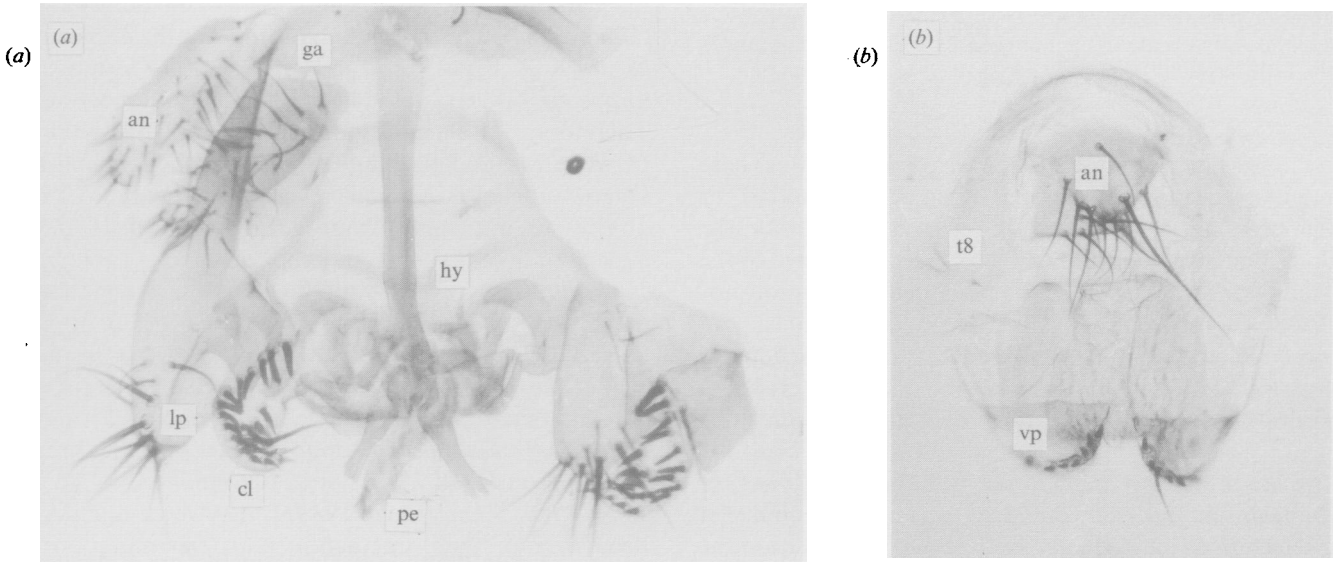

Fig. 4. Masculinization of triploid intersexes by the run^{YP17} mutation. Photographs (×200) show the terminalia of 2X;3A flies carrying the run^{YP17} mutation with only one (*a*) or with two run^+ copies (*b*). The terminalia in (*a*) show all the typical male structures, indistinguishable from those of wild-type males, and the absence of female tissue. The terminalia in (*b*) show all the typical female structures but slightly reduced, with fewer thorn bristles in the vaginal plates and in the eighth tergite and analia than are usually found in wild-type females. Symbols: analia (an), genital arch (ga), clasper (cl), lateral plate (lp), penis apparatus (pe), hypandrium (hy), vaginal plates (vp), 8th tergite (t8). Cross: $y\ w\ f^{36a}\ run^{YP17}/FM7 \times y^2/Y;\ C(2L)RM,\ dp;\ C(2R)RM,\ px;\ C(3L)RM,\ h;\ C(3R)RM,\ +$.

tivation of *Sxl* causes masculinization of triploid intersexes (Cline, 1983, 1988; Torres & Sánchez, 1989). If *run* is involved in the initial step of *Sxl* activation, loss-of-function *run* mutations are expected to masculinize these individuals. We have produced triploid intersexes heterozygous for run^{YP17} (see footnote to Fig. 4 for a full description of the cross) and studied their sexual phenotype in the external terminalia, which show the most pronounced sexual dimorphism. The control intersexes (13 specimens) carried two run^+ copies and were exclusively composed of female tissues, whose inventory was reduced in most cases: part of vaginal plates, 8th tergite and/or anal plates were absent. In contrast, among the 15 experimental triploid intersexes, 8 contained a complete inventory of male tissues indistinguishable from wild-type structures and no female tissue, 6 of them were mosaically composed of female and male tissues, and only 1 individual was exclusively composed of a reduced set of female tissues. These results confirm that *run* is involved in the initial step of *Sxl* activation.

(iv) *The lethal interaction caused by* run *is restricted to the developmental stage when the X:A signal activates* Sxl

Sxl activity is determined by the X:A signal around the blastoderm stage (Sánchez & Nöthiger, 1983; Bachiller & Sánchez, 1991). In good agreement with these results, the activity of *sc* is specifically required around the syncytial blastoderm stage to determine *Sxl* activity (Torres & Sánchez, 1991). We have used the temperature-sensitive phenotype of run^{YP17} to explore the time in development when the *run* activity is needed for *Sxl* activation. For this purpose, we have determined the temperature-sensitive period (TSP) of females doubly heterozygous for run^{YP17} and *Df(sis-a)*. The TSP is extremely short and occurs very early in

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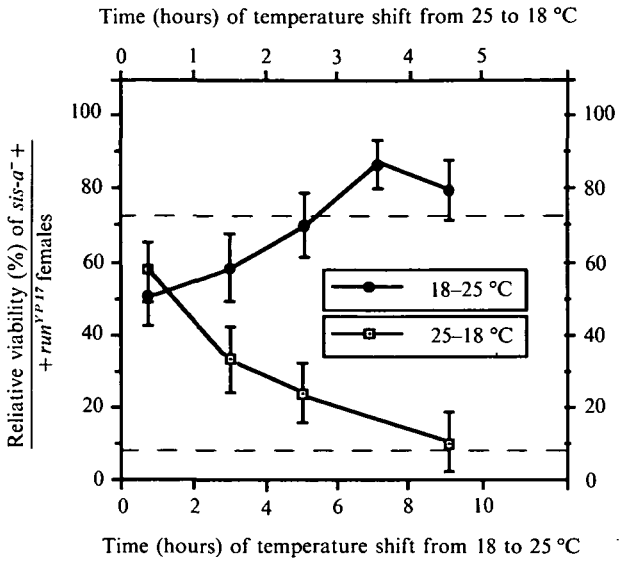
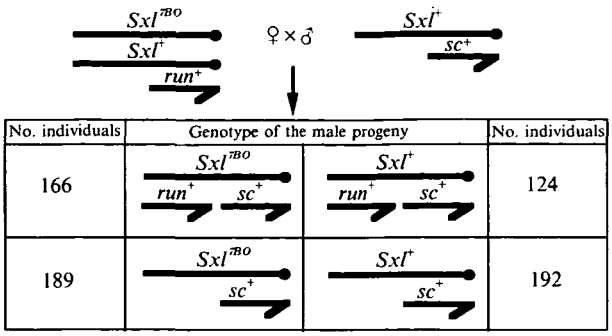


Fig. 5. Time for requirement of *run* activity. Egg laying lasted for 1 hr at 25 °C, except for the last point for which it lasted for 3 h, and 2 h at 18 °C. The culture vials were shifted from the restrictive to the permissive temperature and vice versa, at different times after egg laying as specified in the abscissa. Points are located at the average time elapsed from the egg laying at the moment of the temperature shift. Vertical bars represent $\pm 2 \times$ S.E.M. The upper and lower horizontal dashed lines represent the viability of experimental females when raised throughout development at 18 or 25 °C, respectively. Cross: *Df(1)N7, sis-a⁻/FM6 × y w f^36a run^YP17/y⁺ Ymal^106*. Control flies were FM6 females.

development (Fig. 5). It begins between 1 and 2 h of development and ends around the third hour of development at 25 °C. The period delimited spans the syncytial blastoderm stage and at least part of the cellularization stage. This result shows that *run* activity is needed during the initial step of *Sxl* activation by the X:A signal.

(v) run *duplications show limited ability to induce* Sxl*-dependent male-specific lethality*

To further characterize the role of *run* in *Sxl* activation, we have tested the ability of *run* duplications to induce *Sxl* activation. Simultaneous duplication of *sis-a* and *sc* causes male lethality due to *Sxl* ectopic activation (Cline, 1988; Torres and Sánchez, 1989). If *run*, like *sis-a* and *sc*, is one of the elements counted to establish the numerator of the X:A signal, its duplication should induce male lethality together with *sis-a* or *sc* duplications. This test differentiates between a gene merely needed for *Sxl* activation and a gene whose dose is measured to determine *Sxl* activity. We have produced males with an extra copy of both *run* and *sc* that carry either a *Sxl*⁺ copy or a deficiency for this gene. To ensure that the phenotypic differences between the two types of males are due to the presence or absence of the *Sxl*⁺ copy and not to modifiers, both classes of males arise from the same cross and no balancer chromosomes are used (Fig. 6). In this cross,



No. individuals	Genotype of the male progeny		No. individuals
166	*Sxl*^{mBO} *run*⁺ *sc*⁺	*Sxl*⁺ *run*⁺ *sc*⁺	124
189	*Sxl*^{mBO} *sc*⁺	*Sxl*⁺ *sc*⁺	192

Fig. 6. Viability of males containing duplications for both *run* and *sc*. The sex chromosomes and the relevant genotypes are shown. See Materials and methods for a full description of the cross and the genetic markers used to distinguish the genotypic classes. In this cross we cannot distinguish males *Sxl*⁺ *run*⁺ *sc*⁺ from males *Sxl*⁺ *run*⁺. These later males would arise from a non-disjunctional event in the mothers. However, we know from similar crosses, in which every progeny is phenotypically distinguishable, that the frequency of this event is negligible (less than 2%).

the *sc* duplication did not produce *Sxl*-dependent male lethality in the males without the *run* duplication. However, the presence of a *run* duplication in addition to the *sc* duplication, causes male lethality. This lethality is significantly rescued by the substitution of the wild-type *Sxl*⁺ copy by a *Sxl* deficiency. Since the *run* duplication by itself does not induce *Sxl*-dependent male lethality (data not shown), the result obtained must be due to interaction between the duplications that include *run* and *sc*. The fact that the lethality is dependent on the presence of a wildtype *Sxl* copy strongly suggests that it is due to *Sxl* ectopic activation. However, the lethal interaction between these two duplications seems not to be exclusively due to *Sxl* activity as in the absence of *Sxl* there is a remaining lethality (Fig. 6). The intensity of the *Sxl*-dependent lethal effect is very low compared with the lethal effect produced by *sis-a* and *sc* duplications (Cline, 1988; Torres & Sánchez, 1989). In addition, in a similar experiment (see materials and methods for description of the cross) involving the *run* and *sis-a* duplications we have not observed male lethality (data not shown). Thus, in this case, the *run* duplication is not able to induce *Sxl* activity. These results reveal that *run* extra doses have a limited ability to activate *Sxl*, which shows no correlation with the strong negative effect that the deficiencies of *run* activity have in *Sxl* activation. These observations and those reported above suggest that *run* is needed to activate *Sxl* but does not have as major a role as an X-counting element of the X:A signal such as *sis-a* and *sc*.

4. Discussion

In a search for genes involved in *Sxl* regulation, we have identified a proximal region in the X chromosome which, in hemizygous condition, causes lethality when females contain a single dose of *Sxl*, or of either of the

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two previously identified regulators of this gene, *sis-a* and *sc*. A detailed analysis of this region reveals that the female lethality is in fact associated with the presence of mutations at the *run* locus. *run* is one of the 'pair-rule' genes involved in the specification of the segmented pattern that underlies the general organization of the embryo (Gergen & Butler, 1987).

The female lethality observed in the interactions described cannot be attributed to a role of *Sxl* in segmentation since *Sxl* is not a vital gene for males (Salz *et al.* 1987). Rather, it seems to be caused by alterations of the dosage compensation process as a consequence of impaired *Sxl* activity. Our results support this hypothesis: first, females that escape from the lethal interactions are masculinized and show lack of cuticular structures, a phenotype characteristic of females with failures in *Sxl* functioning (Cline, 1976), and second, this phenotype and the female lethality are suppressed by Sxl^{M1}, a mutation that constitutively expresses *Sxl* functions. This further indicates that *Sxl* is not properly activated in females doubly heterozygous for *run* and *Sxl* mutations, suggesting an involvement of *run* in *Sxl* activation. Additional evidence in this direction comes from the fact that female-lethal synergistic interactions also take place between *run* mutations and mutations at the elements of the X:A signal, *sis-a* and *sc*, or some mutations in *da*, a gene required for the proper transduction of the signal. Thus, the role of *run* appears to be highly related to the determination of *Sxl* activity by the X:A signal. This is confirmed by the masculinization of triploid intersexes in response to the reduction of *run* doses. Moreover, the developmental stage at which *run* interacts with *sis-a* coincides with the time when *Sxl* activity is determined by the X:A signal. However, *run* duplications have a very low ability to activate *Sxl* in males, compared with duplications at *sis-a* or *sc* (Cline, 1988; Torres & Sánchez, 1989). This suggests that the role of *run* may be more similar to that of *da*, which is needed for *Sxl* activation, but whose doses do not determine its activity. We suggest that *run* is needed for the initial step of *Sxl* activation by the X:A signal, but has not as a major role as a counting element of the X:A signal as *sc* and *sis-a* do. The intensity of the interaction between *da* and *run* is low, suggesting that they do not directly interact to activate *Sxl*.

The *run* protein is expressed at the cellular blastoderm stage in the typical pair-rule stripe pattern (Kania *et al.* 1990). On the other hand, we have shown that *run* sex-determining functions are needed before the cellular blastoderm stage, that is, prior to the function of *run* in segmentation. The early activation of *Sxl* by the X:A signal is common to all the somatic cells of the embryo (Bopp *et al.* 1991). Therefore, if the mechanism of *Sxl* activation has the same basis in all the blastodermal cells, the products of the genes involved in this process should be present in all the cells at the time when the X:A signal is assessed; this is, for example, the case of *sc* (Romani *et al.* 1987; Cabrera *et al.* 1987). However, results obtained by Duffy & Gergen (1991), show that the *Sxl* expression in *run* mutant female embryos is abolished specifically in the broad domain in which *run* is expressed during the precellularization blastoderm stages (Gergen & Butler, 1988). Therefore, the initial activation of *Sxl* seems to require different gene activities in different regions of the embryo.

The activity of *run* involved in segmentation exhibits dosage compensation (Gergen, 1987). Thus, there is a double regulatory relationship between *Sxl* and *run*. First, when dosage compensation has not yet occurred, *run* expression is needed to determine *Sxl* activity. Later, the activity state of *Sxl* by means of its effects on dosage compensation, will determine the transcriptional level of *run*. This behaviour is also shown by *sc*, whose proneural expression is also dosage compensated, while, necessarily, its *sis-b* function is not. Indeed, this behaviour may be true for any gene that functions as an X-counting element and has, in addition, some sex-non-specific function later in development. As an X-counting element, such a gene has to show a dosage-dependent phenotype that will be apparent before *Sxl* activity is determined, whereas its later sex-non-specific function has to be dosage compensated to avoid differences between the two sexes.

The *sc* and *da* proteins contain HLH motifs characteristic of transcriptional regulators (Villares & Cabrera, 1987; Caudy *et al.* 1988) whose activities depend on the dimerization with other HLH proteins (Murre *et al.* 1989*a*, *b*; Benezra *et al.* 1990; Sun & Baltimore, 1991). The mode of action of *sc* and *sis-a* seems to be the transcriptional activation of *Sxl* specifically around the blastoderm stage (Torres & Sánchez, 1991). This would produce an output of *Sxl* product that, by positive autoregulation on the late sex-non-specific transcripts, would lead to the correct expression of *Sxl* in all the cells throughout development (Salz *et al.* 1989; Bopp *et al.* 1991). Our results with the allelic series and the run^{YP17} allele show that the *run* product involved in *Sxl* activation is the same, or is highly related, to the one needed in segmentation. Since there is no evidence for multiple *run* products (Gergen & Butler, 1988), it is possible that the same product carries out the two functions. *run* encodes a nuclear protein which does not contain any of the known DNA-binding motifs nor any of the protein–protein recognition motifs for transcriptional regulators (Kania *et al.* 1990). Although *run* may not be a DNA binding protein, it nevertheless affects the transcription of other genes such as *even-skipped* or *hairy* (Ingham & Gergen, 1988; Kania *et al.* 1990). Thus, *run* may be needed for the early *Sxl* transcriptional activation by modulating the activity of any of the elements that make up the X:A signal. In this context, it is worth mentioning that the *run* protein contains a putative ATP-binding site (Kania *et al.*

1990) and that the *sc* protein is putatively susceptible of being phosphorylated (Villares & Cabrera, 1987). Moreover, the *run* protein is also expressed extensively in the developing central and peripheral nervous system (Kania *et al.* 1990). Thus, it is possible that the function of *run* in both processes, *Sxl* activation and neurogenesis, has to do with the modulation of the *sc* protein activity by phosphorylation. The involvement of *run* in sex determination, segmentation and neurogenesis is another example of the utilization of regulators of gene expression in quite different processes, a strategy that seems to be widely used during development.

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