Production of X0 clones in XX females of *Drosophila*

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Summary

The experiments reported here are aimed at determining whether mutations deleting the function of the *Sex-lethal* (*Sxl*) gene are able to suppress the lethality of X0 clones, induced in females after the time when the state of activity of *Sxl* is irreversibly fixed by the ratio of the number of X chromosomes to sets of autosomes (X:A). This analysis was carried out by comparing the frequency of induced male clones (X0 constitution) in $Sxl^{fLS}/+$ and Sxl^+/Sxl^+ females, following irradiation at blastoderm and larval stages. The genotype used in these experiments, however, could also give rise to 2X;2A cells homozygous for Sxl^{fLS}, and such cells would also differentiate male structures. To minimize this possibility, we have constructed a genotype made up of a ring and a rod X chromosome. In such ring-rod females the production of 2X;2A clones homozygous for Sxl^{fLS} is a rather rare event, if possible at all. X0 male clones were produced in both types of females following irradiation at blastoderm stage, while X0 male clones were only observed in $Sxl^{fLS}/+$ females when irradiation took place at larval stage. In this latter case, the only X0 male clones were those that contained the Sxl^{fLS} mutation. These results support the idea of Sánchez & Nöthiger (1983) that the X:A signal irreversibly sets the state of activity of *Sxl* at blastoderm stage, and in addition show that X0 clones generated after that time are viable if they contain a Sxl^- mutation. These results are compatible with the idea of *Sxl* being the only gene that responds to the X:A signal.

1. Introduction

In *Drosophila melanogaster*, sex determination and dosage compensation (hypertranscription of the male X chromosome) are under the control of the *Sex-lethal* (*Sxl*) gene, whose state of activity is determined by the ratio of the number of X chromosomes to sets of autosomes (X:A). In 2X:2A flies, *Sxl* will be ON while in X;2A flies *Sxl* will be OFF (Cline 1978).

Sánchez & Nöthiger (1983) constructed a genotype that allowed them to remove, by mitotic recombination induced by X-rays, one of the X chromosomes from a cell at different times during development. Thus, X;2A clones were produced in 2X;2A female flies, and the response of the affected cells was assessed in sexually dimorphic regions. They employed two genotypes, one heterozygous for the Sxl^{fLS} mutant allele and the other homozygous for the Sxl^+ allele. As shown previously 2X;2A cells homozygous for Sxl^{fLS} differentiate male structures

(Sánchez & Nöthiger, 1982). They found that X0 clones can be generated at the blastoderm stage and that these clones develop male structures according to their male chromosome constitution in both genotypes. After irradiation of larval stages, male clones were only observed in Sxl^{fLS} heterozygous flies. The authors concluded that the X:A signal sets the state of activity of Sxl at the blastoderm stage and once this is achieved, the X:A signal is no longer needed, and the state of activity of *Sxl* remains set.

Due to the experimental design, it was not possible to decide unambiguously whether the male clones generated after blastoderm were really X0, Sxl^{fLS} clones or 2X;2A clones homozygous for Sxl^{fLS}. It thus remained uncertain if the mutation Sxl^{fLS} is able to suppress the lethality of the X0 clones generated after the setting time of the *Sxl* activity by the X:A signal. The experiments reported here aim at answering this question.

To remove one of the X chromosomes from a cell at different times during development, we have constructed two genotypes made up of a ring and a rod X chromosome. One is heterozygous for Sxl^{fLS} (experi-

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mental genotype) and the other is homozygous for *Sxl⁺* (control genotype). The experimental design is based on the fact that a single event of mitotic recombination between a ring and a rod X chromosome necessarily leads to a dicentric complex (Stern 1936; Walen 1964; Merriam, Nöthiger & García-Bellido 1972). This dicentric complex can be lost, thus producing two X0 daughter cells; or it can initiate a breakage-fusion-bridge-cycle which would give rise to aneuploid cells whose development is expected to be abortive (Morata & Ripoll, 1975). In such ring–rod genotypes the formation of 2X;2A clones homozygous for Sxl^{fLS} would be a rather rare event, because it would require either a double recombinational event, or a precise breakage of the dicentric complex into two near wild-type rod X chromosomes. Therefore, the ring X-rod X genotype should eliminate the drawback that characterized the previous genotype constructed by Sánchez & Nöthiger (1983).

2. Materials and methods

Flies were raised on standard *Drosophila* medium at 25 °C. For a description of the genetic markers see Lindsley & Grell (1968); for Sxl^{fLS} see Sánchez & Nöthiger (1982).

The irradiation dose at blastoderm and larval stages was 10 Gy, applied by a Philips X-ray machine at a rate of 5 Gy/min. The adult flies were kept in a mixture of ethanol:glycerol (3:1) for several days, and subsequently mounted for analysis under a compound microscope.

(i) *Identification of the sex of clones*

The sexual phenotype of the marked clones was assessed in sexually dimorphic regions of the fly, namely sex comb region on basitarsus of foreleg, 5th and 6th tergites and external derivatives of the genital disc. For a description of the morphology of these structures see Bryant (1978). Male clones in a female foreleg basitarsus were recognized by the presence of sex comb bristles. In the tergites, the male clones were defined by the presence of marked bristles associated with male pigmentation in the unpigmented area of the female 5th and 6th tergites. In the external derivatives of the genital disc, male genital elements as well as large males clones in the anal plates can easily be recognized. Small anal clones, however, could only be identified as male when they comprised the ventral part of the male anal plate where size and density of bristles clearly differ from the female pattern. Marked clones whose sex was doubtful were excluded from the analysis.

(ii) *The genotype*

The genotypes of the experimental and control females were, respectively, $f^{36a}/R(1)2$, $y\ w\ Sxl^{fLS}$; *mwh jv*/ + and $f^{36a}/R(1)2$, $y\ w\ Sxl^+$; *mwh jv*/ +. Fig. 1 gives a schematic representation of the experimental females, together with the chromosome constitution and phenotype of the induced clones, as a result of mitotic recombination between the ring and the rod X chromosomes. Mitotic recombination in the third
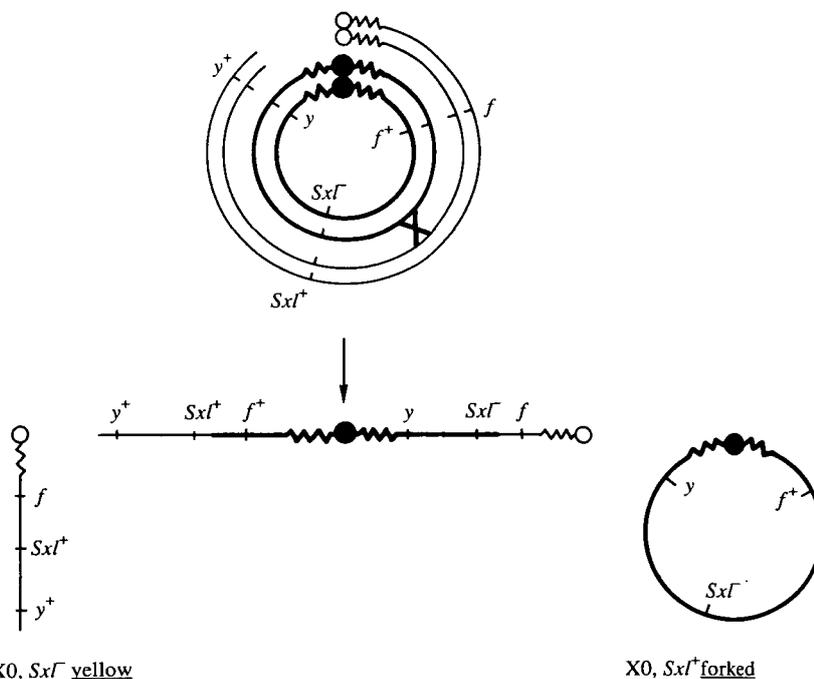
Fig. 1. Schematic representation of the experimental genotype and consequences in daughter cells after mitotic recombination. Thin line, rod-X chromosome; heavy line, ring-X chromosome; wavy line, heterochromatin. *Sxl⁻* stands for the mutant allele Sxl^{fLS}. Ring and rod X chromosomes, and the location of the markers, are not drawn to scale.

chromosome gives rise to *mwh-jv* clones that have been used as an internal control (not shown in Fig. 1).

In Fig. 1 only one single event of mitotic recombination between homologous chromatids is considered, and only in one chromosomal interval. The result is the formation of a dicentric complex (Stern 1936; Walen 1964; Merriam *et al.* 1972). If this complex is lost, two X0 cells will be formed, one marked with forked and carrying the *Sxl⁺* allele, the other with yellow and carrying the *Sxl^{fLS}* mutant allele. The genotype of the X0 daughter cells is independent of where mitotic recombination took place. If the dicentric complex breaks and initiates a breakage-fusion-bridge cycle, aneuploid cells will be generated with different chromosome constitutions, depending on where the breakage took place.

Mitotic recombination between the sister strands of the ring X-chromosome can also generate a dicentric complex. Another formal source of X0 clones could be the distortion in the plane of replication of the ring X-chromosome, with the same results as if mitotic recombination had taken place between the two sister strands of the ring X. In both cases, the X0 clones would carry the rod-X marked with forked and carrying *Sxl⁺*.

All the events described above can also occur in the control genotype except that the X0 clones always contain the *Sxl⁺* allele.

3. Results and discussion

Table 1 presents the data on frequencies and sizes of the different male clones that were obtained, and Fig. 2 shows four examples.

(i) *Experimental genotype* (Sxl^{rLS})

Following irradiation at the blastoderm stage, we found male clones that were either *yellow* or *forked*. The frequency of the *yellow* male clones was higher than that of the *forked* male clones. No *yellow* male//*forked* female twins were found. When irradiation took place at the larval stage, we found *yellow* male clones in all sexually dimorphic regions, but no *forked* male clones were obtained; and only in the tergites 14 *yellow* male//*forked* female twins were observed. The size of the clones decreased between blastoderm and larval irradiations. The origin of the *yellow* male//*forked* female twins has to be related with the formation of the dicentric X-chromosome, followed by its breakage and the segregation of the two fragments to the daughter cells. These will be aneuploid and the degree of aneuploidy will determine their viability. The *yellow* partner of the twin can be hemizygous for *Sxl^{fLS}* and consequently will differentiate male structures. These twins would appear in the tergites following irradiation at the larval stage, because tergites are a more permissive environment for aneuploid cells (Ripoll, 1980).

(ii) *Control genotype* (Sxl⁺)

Following irradiation at the blastoderm stage, we found male clones that were either *yellow* or *forked*. No *yellow* male//*forked* female twins were found. When irradiation took place at the larval stage, no *yellow* male clones were observed, while 3 *forked* male clones were obtained in the tergites. We also found in these structures 2 *yellow* male//*forked* female twins.

As an internal control of mitotic recombination we have analysed the frequency of *mwh* clones in the wings. The frequency of these clones was similar in the experimental and control genotypes: 1·0 and 0·6, respectively, at blastoderm irradiation; and 3·7 and 4·1, respectively, at larval irradiation.

The analysis of 270 experimental females and 230 control females that have not been irradiated showed that the spontaneous frequency of male clones was zero for both genotypes.

The main observation is the difference in the results obtained with the control (*Sxl⁺*) and the experimental (*Sxl^{fLS}*) genotypes. In both genotypes, *yellow* and *forked* male clones were found following irradiation at blastoderm stage. However, when irradiation took place at the larval stage, male clones were only observed in the experimental genotype and in this case only *yellow* male clones were found. These results agree with those reported by Sánchez & Nöthiger (1983) that X0 male clones can be produced at the blastoderm stage, but not later. This is the reason for finding *forked* male clones at blastoderm irradiation but not at larval irradiation. With respect to the *yellow* male clones, the question then is whether these clones generated after larval irradiation represent X0 male clones that survive because of the presence of the *Sxl^{fLS}* mutation, or whether they are viable because they are 2X clones homozygous for *Sxl^{fLS}*. If this were the case, one should expect *yellow* female clones in the control genotype, where they are expected to be perfectly viable. However, they were not found. This is in agreement with the expectation mentioned earlier that it is rather unlikely that the ring-rod X genotype used here would be able to generate 2X clones. Thus, we conclude that the *yellow* male clones must have the X0 constitution and that they are viable because they contain the *Sxl^{fLS}* mutation.

In the blastoderm irradiation of the experimental genotype, the X0 clones containing the *Sxl^{fLS}* mutation (*yellow* clones) appeared more frequently than the X0 clones containing the *Sxl⁺* allele (*forked* clones). This can be explained by the variation in the ages of the embryos at the time of irradiation resulting from the two hour egg laying period. The X0 clones generated in embryos younger than the setting time of *Sxl* will survive and differentiate male structures, whether they contain the *Sxl^{fLS}* or the *Sxl⁺* allele. However, the X0 clones generated in embryos older than the setting time of *Sxl* will only survive if they contain the *Sxl^{fLS}* allele.

Table 1. *Number (n), frequency (%) and size ($\overline{X} \pm$ s.e.) of male clones in female flies*

Experimental genotype (Sxf^{fLS})

Age at irradiation	Sexual dimorphic regions	Number of analysed structures	yellow ♂ n	%	$\overline{X} \pm$ s.e.	forked ♂ n	%	$\overline{X} \pm$ s.e.	yellow ♂//forked ♀ n	%	$\overline{X} \pm$ s.e.
1·5–3·5 h	Basitarsus	1326	5	0·4	2·8±1·1	0			0		
	5th and 6th tergites	610	11	1·8	8·1±1·3	2	0·3	7·5±4·5	0		
	Analia	570	1	0·2	24	1	0·2	7	0		
	Genitalia	570	3	0·5	14·0±7·0	0			0		
24–48 h	Basitarsus	1484	3	0·2	1·7±0·7	0			0		
	5th and 6th tergites	808	20	2·5	6·5±0·9	0			14	1·7	5·6±0·6 / 4·4±0·6
	Analia	830	4	0·5	18·0±6·0	0			0		
	Genitalia	830	4	0·5	9·0±1·5				0		

Control genotype (Sxf^+)

Age at irradiation	Sexual dimorphic regions	Number of analysed structures	yellow ♂ n	%	$\overline{X} \pm$ s.e.	forked ♂ n	%	$\overline{X} \pm$ s.e.	yellow ♂//forked ♀ n	%	$\overline{X} \pm$ s.e.
1·5–3·5 h	Basitarsus	1106	1	0·1	3	0			0		
	5th and 6th tergites	629	4	0·6	15·4±0·3	18	2·9	11·1±2·2	0		
	Analia	574	0			0			0		
	Genitalia	574	0			1	0·2	35	0		
24–28 h	Basitarsus	763	0			0			0		
	5th and 6th tergites	505	0			3	0·6	6±2·5	2	0·4	6·0±0·0 / 2·5±0·0
	Analia	476	0			0			0		
	Genitalia	476	0			0			0		

The size of the male clones in the basitarsus refers to the number of sex comb bristles. Since the size of the male genital clones is difficult to quantify, data on clone size are only given if the clones contained clasper, lateral plate and/or genital arch structures. In the 'clone size' column of the *yellow//forked* twins, the numerator refers to the size of the *yellow* partner and the denominator to the size of the *forked* partner. The *forked* phenotype of a clone in the female genitalia was recognized in the long bristle of the vaginal plates and/or in the bristles of the 8th tergite.
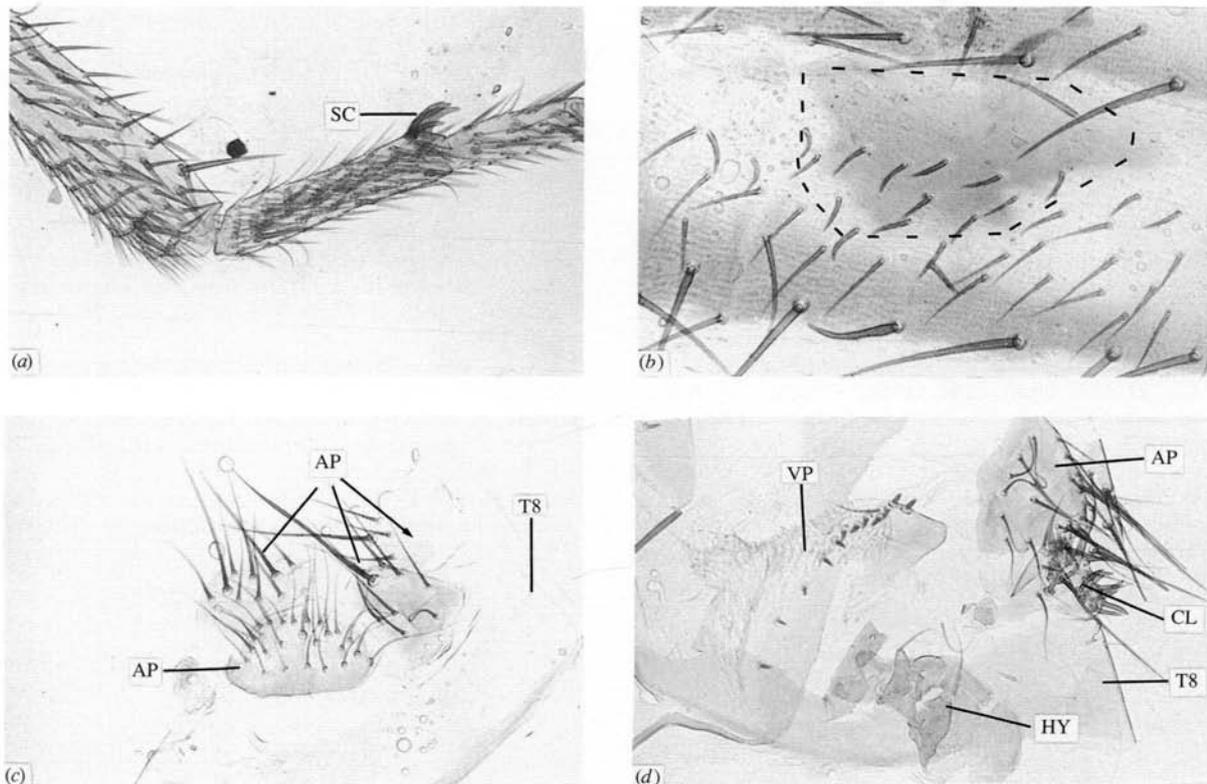
Fig. 2. Photographs ($\times$ 200) showing male clones in the experimental (*a*, *c*, *d*) and control (*b*) females following irradiation at the blastoderm stage. In all cases there was a complete correspondence between the mutant markers and the male phenotype. This shows that the expression of the genotypic sex is cell autonomous. The sex comb bristles exhibited normal morphology and in some cases even were rotated. As a rule, the sex comb bristles replaced the corresponding female bristles in the region of the 7th and 8th transversal rows of the basitarsus. The male genital clones were accompanied by a complete set of female genitalia. Male clones in the anal plates were correlated with the absence of the corresponding female part. Symbols: sex comb (SC), anal plate (AP), vaginal plate (VP), 8th tergite (T8), clasper (CL), hypandrium (HY). The male clone in the 5th tergite is surrounded by a dashed line.

Thus, once the state of activity of *Sxl* is determined, an event that occurs at the blastoderm stage, both sex determination and dosage compensation come under the control of *Sxl*. Accordingly, X0 clones generated after the blastoderm stage would die because the *Sxl*[+] product continues to be made, which results in a fatal deficit of the X chromosomal gene products in such X0 clones. If, on the other hand, the X0 clones contain the *Sxl^{fLS}* mutation, the rate of transcription of that single X chromosome increases, allowing those X0 clones to survive and to form male structures. The fact that these X0, *Sxl^{fLS}* clones grow normally, even in a *Minute*[+] background, strongly suggests that the transcriptional increase of the X chromosome practically approaches the level found in normal males. Otherwise, these X0 clones should express a *Minute* phenotype, due to the reduced level in the expression of the X-linked *Minute* genes, and consequently, they should either be competed out by the surrounding *Minute*^{+} cells, or hardly grow giving rise to small clones. In this context, the results of a clonal analysis of the male-specific-lethal (*msl*) mutations become relevant. Mutations in *msl* specifically reduce the transcriptional level of the X chromosome to 65 % of the value found in normal males (Belote & Lucchesi, 1980). When *msl* homozygous clones were generated during larval growth of *msl*/ + males, they were lethal if the background was *Minute*[+] (Bachiller & Sánchez, 1989), or hardly grew if the background was *Minute* (Belote, 1983).

We conclude that X0 clones generated in females, after the setting time of *Sxl* activity by the X:A ratio signal, can only survive if they contain a mutation deleting the *Sxl* function. This is compatible with the idea of *Sxl* being the only gene that responds to the X:A signal.

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