Genet. Res., Camb. (1995), **65**, *pp.* 167–174 *With 5 text-figures* Copyright © 1995 Cambridge University Press

IR hybrid dysgenesis increases the frequency of recombination in *Drosophila melanogaster*

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(*Received 26 September 1994 and in revised form 16 February 1995*)

Summary

The I factor is a LINE-like transposable element responsible for the I-R system of hybrid dysgenesis in *Drosophila melanogaster*. Inducer strains of this species contain several I factors whereas reactive strains do not. I factors are stable in inducer strains, but transpose at high frequency in the germ-line of females, known as SF females, produced by crossing reactive females and inducer males. Various abnormalities occur in SF females, most of which result from this high rate of transposition. We report here that recombination is increased in the germ-line of these females. This is a new characteristic of the I-R system of hybrid dysgenesis that might also be associated with transposition of the I factor.

1. Introduction

The IR system of hybrid dysgenesis in *Drosophila melanogaster* is a particular syndrome (Picard & L'Heritier, 1971) occurring in females resulting from crosses between appropriate strains. *Drosophila melanogaster* is divided into two types of strain according to this system of hybrid dysgenesis: inducer strains which contain about ten copies of active I factors localized in euchromatic regions of chromosomes, and reactive strains which are devoid of functional I elements (Bucheton *et al.* 1984). Nevertheless, both kinds of strains contain defective pericentromeric I elements which seem to be unable to transpose (Crozatier *et al.* 1988; Vaury *et al.* 1990) (for reviews: see Finnegan, 1989; Bucheton, 1990).

Complete and functional I factors are 5·4 kb long and have two large open reading frames (ORFs) showing similarities with the *gag* and *pol* genes of retroviruses (Fawcett *et al.* 1986; Abad *et al.* 1989). They transpose by reverse transcription of an RNA intermediate (Jensen & Heidmann, 1991; Pélisson *et al.* 1991). A full-length RNA that is presumably the transposition intermediate is synthesized specifically during transposition (Chaboissier *et al.* 1990). It is produced under the control of an internal RNA polymerase II promoter (McLean *et al.* 1993). Expression of I factors occurs between stages two to ten of oogenesis (Tatout *et al.* 1994).

I factors are stable in inducer stocks but transpose at high frequency in the germline of 'SF' female progeny, resulting from crosses between inducer males and reactive females (Picard, 1976). They also transpose in the germ-line of RSF females, that are obtained by crossing inducer females and reactive males, but about five times less than in the germ-line of SF females. Transposition does not occur in males.

SF females show a characteristic type of sterility, i.e. they lay a normal number of eggs, the development of which is arrested at the earliest stages of embryogenesis after 3–4 cleavage divisions (Lavige, 1986). High rates of mutations and chromosomal rearrangements affecting the SF female germ-line have been described (Picard *et al.* 1978; Pélisson & Bregliano, 1981; Proust & Prudhommeau, 1982; Prudhommeau & Proust, 1990; Proust *et al.* 1992). These mutations are mainly due to insertion of I elements (Pélisson, 1981; Bucheton *et al.* 1984; Sang *et al.* 1984; Busseau *et al.* 1989*a*). Chromosomal rearrangements appear to result from recombination events occurring mainly between integrating I elements (Busseau *et al.* 1989*b*; Proust *et al.* 1992).

The extent of the genetic abnormalities occurring in the germ-line of SF females correlates with the

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https://doi.org/10.1017/S0016672300033255 Published online by Cambridge University Press

frequency of transposition of I factors, which mainly depends on a particular state characteristic of the reactive strains known as reactivity. The reactive mothers in a dysgenic cross can range from strong to weak according to the rate of unhatched eggs laid by their SF daughters. The hatching percentage of the eggs laid by SF females coming from strong reactive females is very low (approximately 0%), whereas this percentage is high when SF females come from weak reactive mothers (nearly normal) (Bucheton *et al.* 1976; Bucheton & Picard, 1978).

The study of the transposition mechanism and of its control is difficult because quite a few elements can potentially transpose during IR hybrid dysgenesis. More detailed studies require tagged I elements as those previously designed (Jensen & Heidmann, 1991; Pélisson *et al.* 1991; Jensen *et al.* 1994). In order to make a marked element, the transposition of which could be studied by direct observation of the phenotype of flies, we replaced most of the I factor sequence by the *yellow* gene. We were not able to detect any transposition event of this marked element. However, its detailed study provided results indicating that the frequency of recombination is increased in the germ-line of dysgenic females.

2. Materials and methods

(i) *Stocks of* Drosophila melanogaster

Strains were grown on standard *Drosophila* food (Gans *et al.* 1975) and incubated at 20 or 23 °C, according to the experiments. All strains used in these experiments were M in the PM system of hybrid dysgenesis (see Engels, 1989). The genetic symbols are those used by Lindsley & Zimm (1992).

(ii) *Reactive strains (R)*

Binscy is a strongly reactive stock, homozygous for the inversion complex $In(1)sc^{S1L}sc^{8R}+dl49$, marked by the y^{c4} and *B* mutations. This chromosome is abbreviated *Binscy* in the text.

Cy/Pm; DcxF/H-(R) is strongly reactive and is *In (2L+2R) Cy/Pm*; *In (3LR) DcxF/H*. Its second balancer chromosome is called *Cy* in the text.

(iii) *Inducer strains (I)*

Cy/Pm; H/Sb-(I) is an inducer stock and is *In (2L+2R) Cy/Pm*; *H/Sb*. It has been fully described by Picard (1976). For simplification, the *In (2L+2R) Cy* chromosome is called *Cy* in the text.

(iv) *Construction of the pIyC1 plasmid*

pIyC1 (Fig. 1) was constructed by replacing the 4·6 kb *Asu* II–*Asp* 718 I factor fragment of plasmid pI407

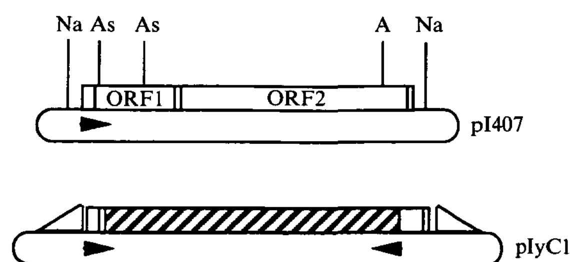

Fig. 1. Structure of the marked I element *Iy1-2*. Open and striped boxes correspond to I factor and *yellow* sequences respectively. Arrowheads indicate transcriptional orientations and white triangles correspond to the P element inverted repeats. Restriction enzyme abbreviations are A.: *Asp* 718; As.: *Asu* II; Na.: *Nae* I. The *Asu* II-*Asp* 718 fragment of the I factor was replaced by the *yellow* gene to get a 5·6 kb *Iy1-2* element (see text).

(Bucheton *et al.* 1984), by a 4·9 kb *Sal* I DNA fragment containing the *yellow* gene cloned in plasmid Dint c20 (Geyer and Corces, 1987). The *Asu* II and *Asp* 718 sites are located at positions 289 and 4849 respectively in the I factor sequence published by Fawcett *et al.* (1986).

The *Nae* I fragment containing the tagged I element was introduced into the *Sma* I restriction site of the Carnegie 1 transformation vector (Rubin & Spradling, 1983). The *yellow* reporter gene and I factor transcriptional orientations are opposite in this plasmid. This fragment of *yellow* restores a wild-type phenotype in a y^{c4} mutant background. The tagged I element contained in pIyC1 was called *Iy1-2*.

(v) *Construction of transgenic lines*

P element transformation was performed according to Spradling & Rubin (1982) using the helper plasmid puchspΔ2·3. Microinjections for pIyC1 were done in G0 embryos of the *Binscy* reactive stock. Transgenic lines were established from [y^+] G1 flies.

Two transgenic lines (*S2H* and *E1H*) containing the *Iy1-2* element on the second chromosome were used in the experiments reported here.

(vi) *Southern blot and* in situ *hybridization experiments*

Southern blot and *in situ* hybridization experiments to salivary gland chromosomes of larvae were carried out as described by Maniatis *et al.* (1992) and Ashburner (1989) respectively. Probes were marked with either ^{32}P or biotin according to the procedures described by these authors.

3. Results

(i) *The* Iy1-2 *element is stable in reactive strains*

Most of the coding sequences have been removed in the *Iy1-2* element (Fig. 1), suggesting that it would be

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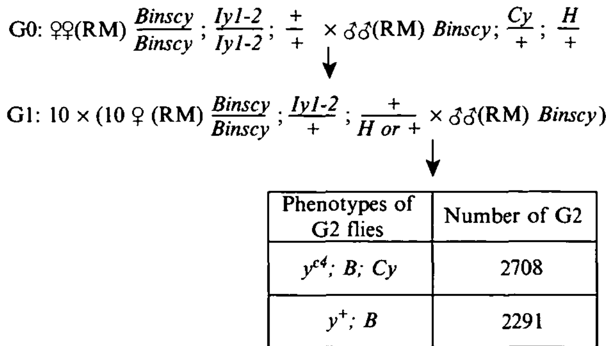


Phenotypes of G2 flies	Number of G2
y^{c4}; *B*; *Cy*	2708
y^{+}; *B*	2291

Fig. 2. Stability of *Iy1-2* in reactive strains. The letters in parentheses correspond to the category of strains according to the IR and PM systems of hybrid dysgenesis. *Iy1-2*:*Iy1-2* element. The *Iy1-2* element was located on the second chromosome. The *Binscy*; *Cy*/+; *H*/+ males used in G0 were obtained by crossing females of the *Binscy* and *Cy*/*Pm*; *DcxF*/*H*-(*R*) reactive stocks.

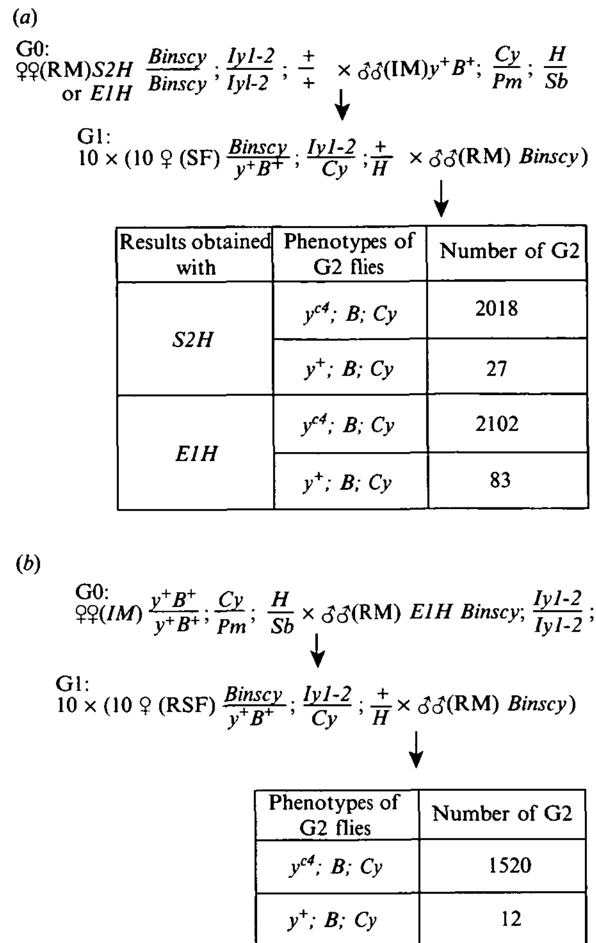
unable to transpose if not complemented by functional I factors which are not present in a reactive background.

The inability of *Iy1-2* to transpose autonomously was checked by crossing transgenic females homozygous for an *Iy1-2* insert with reactive males carrying a balancer second chromosome (Fig. 2). Transposition was studied in the germ-line of the *Cy*/*Iy1-2* female progeny. Transposition to a new chromosome should result in the production of descendants showing a [y^{+}; *Cy*] phenotype. 2708 G2 [*Cy*] flies were examined. All of them exhibited a [*y*] phenotype, suggesting that the *Iy1-2* element is stable in a reactive background.

(ii) *Transgenic lines containing the* Iy1-2 *element allow detection of genetical events increased during IR hybrid dysgenesis*

Two experiments described in Fig. 3*a* were done in order to introduce the *Iy1-2* element of transgenic lines *S2H* and *E1H* in the presence of actively transposing I factors, into the germ-line of SF females. [*B*; *Cy*] G2 flies were selected. They carry the *Binscy* and *Cy* balancer chromosomes. 2045 and 2185 flies with this phenotype were observed in experiments involving transgenic lines *S2H* and *E1H* respectively. Twenty-seven and 83 flies respectively showed a wild-type body colour instead of the *yellow* phenotype characteristic of the *Binscy* balancer (Fig. 3*a*). This corresponds respectively to 1·3 and 3·8% G2 flies that might contain putatively transposed *Iy1-2* elements.

In order to study the behaviour of the *Iy1-2* elements in RSF females, reciprocal crosses were done with transgenic line *E1H* as a paternal strain (Fig. 3*b*), and [*B*; *Cy*] G2 flies were selected. 1532 individuals were observed, and only 12 were [y^{+}] (0·8%). Chi square analysis indicates that this value is significantly lower than that obtained with isogenic SF females ($p = 10^{-4}$).



Results obtained with	Phenotypes of G2 flies	Number of G2
S2H	y^{c4}; *B*; *Cy*	2018
	y^{+}; *B*; *Cy*	27
E1H	y^{c4}; *B*; *Cy*	2102
	y^{+}; *B*; *Cy*	83

Phenotypes of G2 flies	Number of G2
y^{c4}; *B*; *Cy*	1520
y^{+}; *B*; *Cy*	12

Fig. 3. The letters in parentheses correspond to the category of strains according to the IR and PM systems of hybrid dysgenesis. *Iy1-2*:*Iy1-2* element. The experiments described in (*a*) have been done using the transgenic lines *S2H* or *E1H* and the *Cy*/*Pm*; *H*/*Sb*-(*I*) strain. The reciprocal crosses presented in (*b*) were done with the line *E1H*. The [y^{c4}] phenotype is due to the y^{c4} allele of the *Binscy* chromosome.

Sixteen and 34 independent lines exhibiting a [y^{+}] phenotype were established from G2 flies of Expt 3*a* (SF), made with transformed lines *S2H* and *E1H* respectively, and seven others from Expt 3*b* (RSF) for further studies.

(iii) *The* Iy1-2 *element does not transpose*

The *Iy1-2* element has been introduced into the genome by P element mediated transformation. Therefore the parental *Iy1-2* elements are flanked by P element sequences and, after I element transposition, should not remain associated with P sequences. In order to study whether the putative transposed copies of *Iy1-2* are still associated with the inverted repeats of the P factor, the effect of the P transposase on the [y^{+}] phenotype of the lines derived from G2 flies obtained in the experiments described in Fig. 3 was studied. P transposase synthesized by the PΔ2·3 element can cause somatic excisions of other P elements (Engels,

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Table 1. *Summary of the properties of lines showing the* [y⁺] *phenotype obtained in the experiments described in Fig. 3*

Experiment	Parental strain	[y^{c4}; *B*; *Cy* G2 flies	[y^+; *B*; *Cy*] G2 flies	Number of lines established from G2 flies	Location of the gene responsible for the [y^+] phenotype	Somatic excisions
a (SF)	*S2H*	2018	27	16	15 X chromosome 1 chromosome 2	−(15) +(1)
a (SF)	*E1H*	2102	83	34	6 X chromosome 28 chromosome 2	−(3) +(15)
b (RSF)	*E1H*	1520	12	7	2 X chromosome 5 chromosome 2	n.d.

In the last column, + indicates lines from which *yellow* mosaics were obtained in the presence of the P transposase, and − indicates lines which were not sensitive to the P transposase (see text). The number of lines studied for somatic excisions of the P element is indicated in brackets. n.d.: not determined.

1989). Excision of P elements containing the *Iy1-2* construct should result in males with a dark abdomen with *yellow* mosaic patches. Thirty-four putative transposed *Iy1-2* elements were studied in this way (Table 1). Females carrying a *Iy1-2* element were crossed with males containing the PΔ2·3 element. Their male progeny was observed for the presence of *yellow* mosaics. Sixteen were sensitive to the P transposase, indicating that they are still associated with P sequences. No *yellow* mosaics were observed for the 18 other lines exhibiting a [y^+] phenotype.

Localization of the *yellow* allele responsible of the [y^+] phenotype of all lines was also determined (crosses not shown). In 23 cases the sequences responsible for the [y^+] phenotype were located on the X chromosome and in 34 cases they were located on the second chromosome (Table 1). Lines affected by the P transposase are located on the second chromosome, whereas those which are stable in the presence of the P transposase are on the X chromosome. Within all the lines studied, there is a correlation between the location of the sequences conferring the [y^+] phenotype and the capacity of these sequences to be excised by the transposase produced by the PΔ2·3 element (Table 1). This suggests that the *Iy1-2* elements located on the second chromosome are still bounded by P element sequences whereas those located on the X chromosome are not. This was confirmed by Southern blot experiments, in which the same restriction fragments are observed in the parental strains (*S2H* or *E1H*) and in their selected descendants, when the [y^+] phenotype maps to the second chromosome. A sample of the results is given in Fig. 4. Southern blot experiments also indicate that when the [y^+] phenotype maps to the X chromosome, the restriction map is identical to that of the *yellow* wild type gene of the *Cy/Pm; H/Sb-(I)* parental strain (Fig. 4). Therefore no *bona fide* transposition event of the *Iy1-2* element was observed, indicating that the frequency of transposition is lower than 10^{-4}.

(iv) *The frequency of recombination is increased in the germ-line of SF females*

In the experiments presented in Fig. 3*a* the frequencies of events affecting the X chromosome are approximatively 1·2 and 0·7% for lines *S2H* and *E1H*, respectively (see Table 1). This frequency is 0·2% in the experiment described in Fig. 3*b* for *E1H*. Genetic and molecular analyses indicate that in these experiments (Figs. 3 and 4) [y^+; *Cy*] G2 flies have acquired the *yellow* wild-type gene from the paternal *Cy/Pm; H/Sb-(I)* strain although they were selected for the *B* mutation marking the *Binscy* balancer (see above). This suggests that recombination occurred frequently between the *yellow* and *Bar* markers in the germ-line of SF females, and, to a less extent, of RSF females. In order to estimate the frequency of recombination involving the *Binscy* balancer in non-dysgenic crosses, an experiment similar to those described in Fig. 3 was done in a complete reactive background. *Binscy* reactive females were crossed with males of the reactive strain *Cy/Pm; H/Dcxf-(R)* which has the y^+ allele (Fig. 5). G1 females heterozygous for the y^+ and y^{c4} alleles were mated with *Binscy* males. Only two G2 individuals showed a [y^+ *B*] phenotype, corresponding to flies resulting from a recombination event. The frequency of recombination between the *yellow* and *Bar* markers in crosses involving the *Binscy* balancer is therefore only 0·1% in a completely reactive background. Chi square analysis showed that this value is significantly lower than the percentage of recombination between these markers in the experiments described in Fig. 3*a* ($p < 0.01$). Therefore dysgenic crosses increase the frequencies of recombination on the X chromosome.

A similar phenomenon was also observed for the second chromosome. The frequencies of [y^+] G2 flies due to the presence of the transposon containing *Iy1-2* were 0·08 and 3·2% for lines *S2H* and *E1H* respectively in the experiments described in Fig. 3*a* and 0·6% for the experiment reported in Fig. 3*b* (see Table 1). The position of the *Iy1-2* element on the

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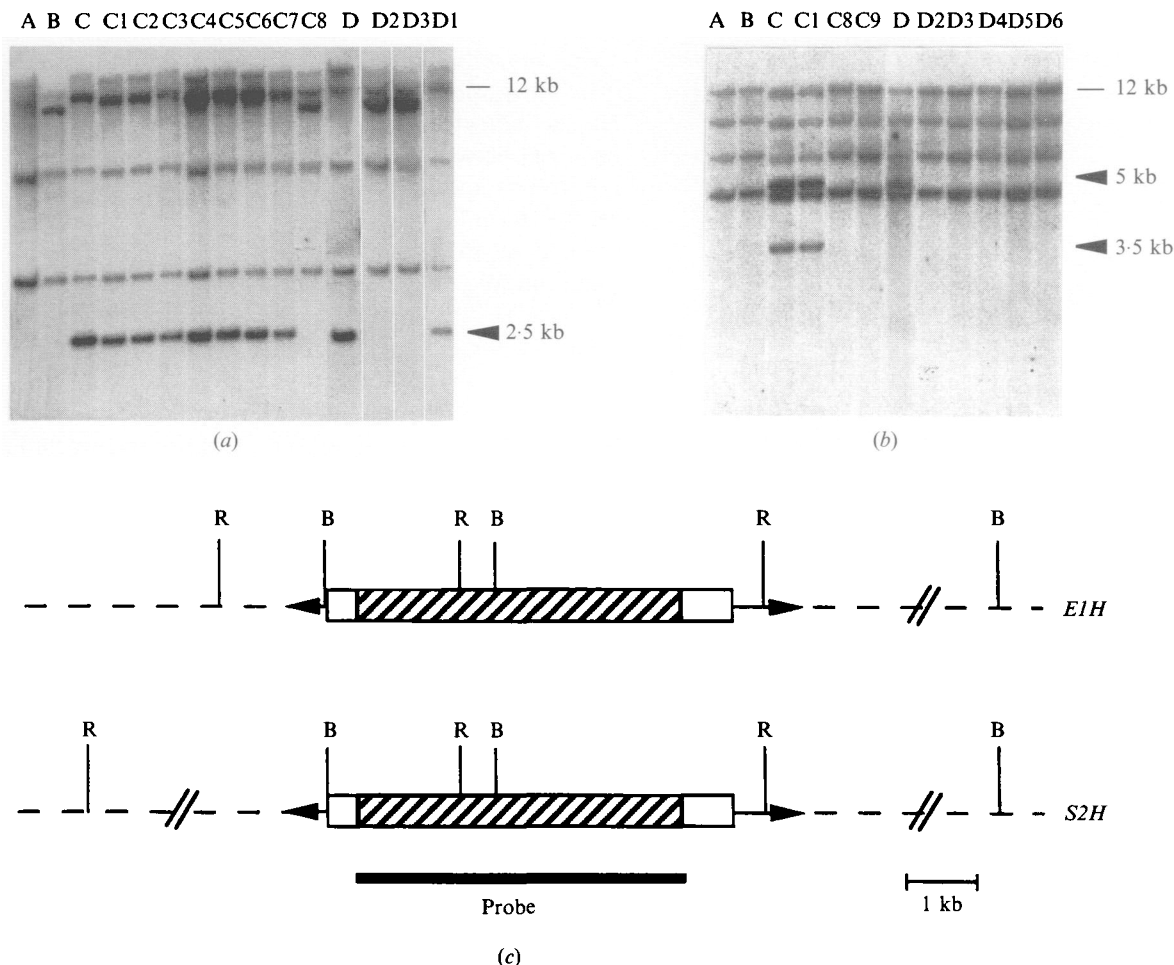

Fig. 4. Molecular analysis of lines exhibiting a [y^+] phenotype obtained in the progeny of the crosses described in Fig. 3*a*. DNAs were digested with *Bam*H I (*a*) or *Eco*R I (*b*), electrophoresed on 1% agarose gels, transferred to nylon membranes, and hybridized with the probe shown in (*c*). DNAs were from *Binscy* (A), *Cy/Pm; H/Sb-(I)* (B), *E1H* (C), *S2H* (D), lines bearing the y^+ marker on the second chromosome derived from *E1H* (C1 to C7) and *S2H* (D1), and lines bearing the y^+ marker on the X chromosome derived from *E1H* (C8 and C9) and *S2H* (D2–D6) (see Fig. 3(*a*) and text). (*a*) Digestion of DNAs from parental transgenic lines *E1H* and *S2H* with *Bam*H I gives a 2·5 kb fragment contained within the transgene and an external fragment which is more than 12 kb long in both *E1H* and *S2H* (C and D). All lines containing the y^+ marker on the second chromosome give the 2·5 kb fragment typical of the transgene (C1–C7, D1). This fragment is not observed in lines having the y^+ marker on the X chromosome, indicating that they do not possess the transgene (C8, D2 and D3). They show a pattern of hybridization identical to that of the *Cy/Pm; H/Sb-(I)* original stock (B), indicating that they have the y^+ allele of this strain. (*b*) Digestion of DNAs from *E1H* and *S2H* with *Eco*R I produces a 5 kb fragment internal to the transgene, and an external fragment which is more than 12 kb long in *S2H* and is 3·5 kb long in *E1H* (D and C). As observed with *Bam*H I digests, lines in which the y^+ marker maps on the second chromosome exhibit the 5 kb fragment typical of the transgene and the 3·5 kb external fragment when they derive from *E1H*, indicating that the transgene is at its original location (C1). Lines bearing the y^+ marker on the X chromosome do not show these fragments (C8 and C9, D2–D6) and are similar to the *Binscy* and *Cy/Pm; H/Sb-(I)* strains. This indicates that they do not have the transgene and must contain the y^+ allele from *Cy/Pm; H/Sb-(I)*. (*c*) Organization of the transgene containing the *Iy1-2* element in *E1H* and *S2H*. B: *Bam*H I, R: *Eco*R I. Arrowheads indicate the inverted repeats of the P element. Hatched and white boxes correspond to sequences of the *yellow* gene and of the I element respectively. Dotted lines show chromosomal sequences flanking the transgene. The thick line below the maps indicate the probe used in the experiments.

second chromosome was determined by *in situ* hybridization to salivary gland chromosomes of larvae. Analysis of polytene chromosomes showed no detectable chromosomal rearrangements. The transgene was located at the same place in parental strains and in their descendants. This position was 34E for *S2H* and 53F for *E1H*. Therefore the [y^+] descendants resulted probably from recombination events between the *Cy* mutation and the *Iy1-2* element that occurred in the germ-line of SF females. The differences in the frequencies of recombination in the experiments involving transgenic lines *S2H* and *E1H* can be explained by the fact that the *Cy* mutation is closer to the *Iy1-2* element in *S2H* than in *E1H*. The frequencies of recombination between *Iy1-2* located in 53F (experiments with *E1H*) and the *Cy* mutation of the *Cy* balancer were 0·6% in an RSF background and about 3·2% in dysgenic crosses (see Fig. 3 and Table 1). All these results indicate that the recombination frequencies are increased by IR hybrid dysgenesis.

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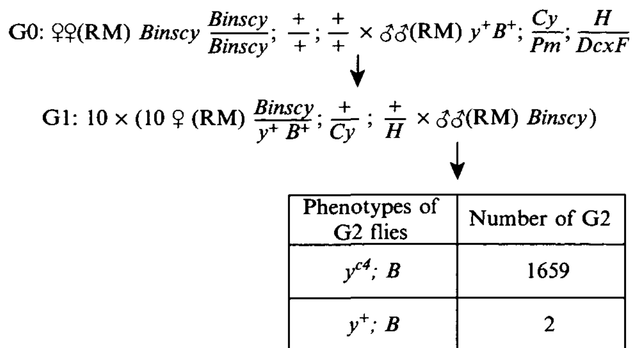

Phenotypes of G2 flies	Number of G2
y^{c4}; *B*	1659
y^{+}; *B*	2

Fig. 5. Frequency of recombination in crosses involving the *Binscy* balancer in a reactive background. The letters in parentheses correspond to the category of strains according to the IR and PM systems of hybrid dysgenesis. *Iy1-2*:*Iy1-2* element. The [y^{c4}] phenotype is due to the y^{c4} allele of the *Binscy* chromosome.

4. Discussion

We have detected no transposition of the marked *Iy1-2* element in which most coding sequences were substituted by the *yellow* reporter gene, even in the conditions of I-R hybrid dysgenesis. We can estimate that its transposition frequency is less than 10^{-4} in SF females. This low frequency of transposition could result from the fact that complementation of the products of both ORF1 and ORF2 is a very rare event, if it occurs. However the inability of *Iy1-2* to transpose might also be due to the presence of sequences of the reporter gene. Transposition of I factors requires synthesis of a full-length RNA intermediate (Chaboissier *et al.* 1990). The sequence of the *yellow* gene was introduced in a transcriptional orientation opposite to that of the I element (see Fig. 1). The antisense non coding strand of this gene contains potential polyadenylation sites. It is therefore possible that immobility of the *Iy1-2* element results from premature arrest of transcription. This would result in an I element transcript with an unusual 3′ end that could not be recognized by the polypeptides of the retrotransposition complex. However, it must be noted that the sequence of the I factor itself contains 17 potential polyadenylation sites that do not interfere with transcription. The fact that the *Iy1-2* element cannot be complemented by transposing I factors might also result from inefficient transcription due to the presence of the reporter gene in opposite orientation.

In the experiments presented in Fig. 3, G2 flies exhibiting a [y^{+}; *B*; *Cy*] phenotype were selected in the progeny of y^{c4} *B*/y^{+} B^{+}; *Cy*/*Iy1-2* SF females. Such flies are expected to carry the *Binscy* and *Cy* balancer chromosomes. Genetic and molecular analyses of lines derived from these G2 flies showed that their [y^{+}; *B*; *Cy*] phenotype resulted from the association either of the y^{+} allele and the *B* mutation on the X chromosome or of the *Iy1-2* element and *Cy* mutation on the second chromosome. In the last case, the element was still flanked by P sequences and *in situ* hybridization experiments to salivary gland chromosomes of larvae indicated that it was still located at its original position on the chromosome. All these results indicate that in SF females recombinations occurred between the *yellow* and *Bar* markers on the X chromosome, and between the *Iy1-2* and *Cy* markers on the second chromosome, although balancers were used in all experiments in order to prevent recombination. We conclude that IR hybrid dysgenesis is characterized by another abnormality not already reported, which is an increase of the frequency of recombination in dysgenic females.

Some previous results suggested that IR hybrid dysgenesis might increase recombination (Hiraizumi, 1981). However this could not be clearly attributed to the IR system. In addition increased recombination in these experiments was observed in the centric heterochromatin. In the present experiments, increased recombination occurred at least in part in euchromatin since the genetic markers that recombined were located on the same chromosomal arms in most cases. *yellow* and *Bar* are located on the X chromosome, and *Cy* and the *Iy1-2* element located at position 34E are on the same chromosomal arm. The only cases where recombinations might have occurred in centric heterochromatin as well as in euchromatin involve the *Iy1-2* element that is located at position 53F, therefore not on the same chromosomal arm as *Cy*.

The I element belongs to the class of non-LTR retrotransposons or LINEs that are widely distributed among species. Mammals contain only one class of LINEs known as L1 elements. In human, some diseases result from insertions of L1 elements into genes (Kazazian *et al.* 1988; Dombroski *et al.* 1991, 1993; Narita *et al.* 1993), and some cases of cancer have been shown to be associated with L1 insertions (Morse *et al.* 1988; Bratthauer & Fanning, 1992; Miki *et al.* 1992). However, transposition is not always deleterious. Transposition of elements related to LINEs in *Drosophila melanogaster* can serve to balance telomeric loss occurring at each replication (Biessman *et al.* 1992; Levis *et al.* 1993) indicating that they can play a crucial role in chromosome structure and organization. It has also been suggested that LINEs, which are middle repetitive DNA, could be helpful for pairing of chromosomes and exchange during the recombination mechanism. It is interesting to note that in the rat 80% of the synaptonemal complex associated DNA corresponds to middle repetitive sequences such as LINEs and SINEs, suggesting that these elements might play a role in recombination (Pearlman *et al.* 1992). It has also been shown that recombination can occur between integrating I elements in SF females, leading to chromosomal rearrangements (Busseau *et al.* 1989*b*; Proust *et al.* 1992). Transposition of I elements involving breakages can lead to chromatid exchange. Increased recombination observed in the germ-line of dysgenic females might be another consequence of such events.

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We thank Sylvie Aulard and Marie-Hélène Muchielli for their help in various experiments. This work has been financed by grants from the Commission of the European Communities (Contract ERBSC1* CT920811) and the Association pour la Recherche sur le Cancer. M.C.C. was supported by an A.R.C. fellowship.

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