Genet. Res., Camb. (1989), **54**, *pp.* 137–141 *With* 1 *text-figure* *Printed in Great Britain*

P-element transposase induces male recombination in *Drosophila melanogaster*

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(*Received 3 February 1989 and in revised form 28 March 1989*)

Summary

Male recombination in P–M dysgenic crosses has been viewed as a reflection of *P*-element transposase interacting with *P* elements. However, recent studies suggest that the transposase may catalyse double-stranded breaks in chromosomal DNA. We have, therefore, introduced *P*(*Δ2–3 ry*$^+$) (99B), a single non-mobile *P*-element transposase source, into the long-standing laboratory true *M* strains of a flanking lethal crossover selective system, thus facilitating the examination of rare male recombination events as an assay for transposase activity. We find that the rate of male recombination in the presence of this non-mobile *P* element is greater than twenty times the background rate of male recombination in the control examined prior to introduction of the transposase source.

1. Introduction

Male recombination was first reported in low but significant frequencies in *Drosophila melanogaster* by Hiraizumi in 1971. Such significant rates of male recombination are one of the manifestations of the P–M hybrid dysgenic syndrome (Kidwell, Kidwell & Sved, 1977) associated with the presence of the *P* family of transposable elements in a population (Bingham, Kidwell & Rubin, 1982). The several features of this syndrome have been considered reflections of the *P*-element transposase interacting with *P* elements, and thus male recombination has been viewed as accompanying insertion or excision of *P* elements in their interactions with transposase (see review by Engels, 1989). However, male recombination apparently does not result from a simple rejoining of non-homologous breaks resulting from *P*-element mobilizations; rather, male recombinant products more closely resemble the precise exchange products of female recombination than the products of radiation-induced breaks (Sved, 1978; Isackson, Johnson & Denell, 1981; Sinclair & Grigliatti, 1985). As Engels notes in his *P*-element review (1989), an understanding of male recombination, the first identified trait of the hybrid dysgenesis syndrome, still remains obscure.

Several lines of evidence suggest that the transposase may be able to interact with genomic DNA. *P* elements are able to occupy many sites in a newly infected true *M* strain. Although apparent randomness of insertion sites is observed, there are many reports of both 'hot' and 'cold' spots (Kidwell, 1987). This non-randomness suggests a selective awareness of prospective genomic DNA sites on the part of the insertion mechanism. The 8 bp repeat of genomic DNA at insertion sites, characteristic of *P* elements (O'Hare & Rubin, 1983), is suggestive of a staggered double-stranded DNA cut mediated by a product of the *P* element. This prompted Rio *et al.* (1988) to test a cDNA clone of transposase introduced into yeast. Their results suggest that the transposase is able to catalyse double-stranded DNA breaks.

In light of these reports we considered whether, in the absence of *P* targets or mobile *P* elements, we could assay the action of transposase on *Drosophila* genomic DNA. It seemed possible, for example, that a significant level of male recombination could be generated by such transposase/genomic site interactions, and hence subject to assay. The present report examines whether the provision of the transposase alone would induce male recombination.

2. Methods and materials

(i) *Experimental conditions*

In all experiments flies were reared at 25 °C on standard *Drosophila* cornmeal-sucrose-yeast medium

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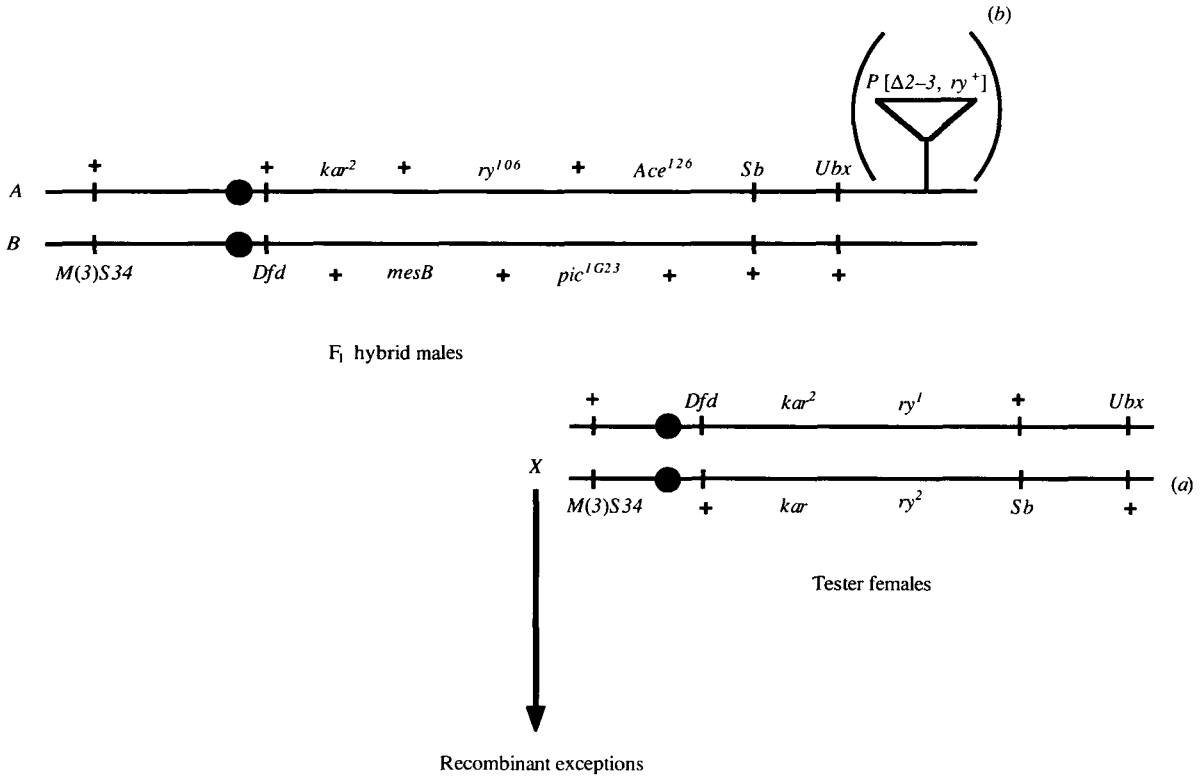

Fig. 1. Flanking lethal crossover selective system. This protocol will permit recombinant exceptions to be recovered only in one direction, with each exchange product consisting of a portion of the *A* chromosome proximal to the event and that portion of the *B* chromosome distal to the event. (*a*) Multiple break rearrangement *Tp(3)MKRS* which is used to suppress crossing over in tester females. (*b*) *P(Δ2–3, ry+)* (99B) is present on chromosome *A* only in Expt 3.

in glass vials. Single F_1 hybrid test males were mated to six tester females and rebrooded for 20 days.

(ii) *M strains*

Long-standing laboratory *M* strains used in these experiments are described in Fig. 1. Detailed descriptions of mutants and rearrangements are given in Lindsley & Grell (1968) and Hilliker *et al.* (1980).

Parental chromosome *A* is maintained over *In(3LR)Dcxf*, and parental chromosome *B* over *In(3LR) Ubx130, ry2301 Ubx130e8*. The *M(3)S34* chromosome of the tester females is the multiple break rearrangement *Tp(3)MKRS*.

Chromosome *A* and chromosome *B* stocks each yielded 0% F_1 gonadal sterility in tests of *P* potential (test male × M Female) and 100% F_1 gonadal sterility in crosses to assess cytotype (test female × P male). Thus both chromosome *A* and *B* stocks test as typical *M* or *M′* strains (Daniels *et al.* 1987). These stocks were also determined to be free of *P* elements by *in situ* hybridization and are therefore classified as true *M* strains. Tests for presence of hobo elements proved negative.

We did not search for evidence of other mobile elements. Extensive and convincing studies have now eliminated the likelihood that other known *Drosophila* transposable elements are mobilized by P–M hybrid dysgenesis (Woodruff, Blount & Thompson, 1987; Eggleston, Johnson-Schlitz & Engels, 1988), and the reciprocal control crosses 1 and 2 provide a test for unidentified factors whose activation could induce male recombination.

(iii) *P(Δ2–3, ry+) (99B)*

This is a unique *P*-element derivative that shows high transposase activity and is unusually stable in its insertion position at 99B on the right arm of the third chromosome (Laski, Rio & Rubin, 1986; Robertson *et al.* 1988). It is used only in Expt 3. *P(Δ2–3, ry+)* (99B) is marked with *ry+*. It lacks the third intron of the *P* element, consequently producing transposase in both somatic and germinal tissue (Laski *et al.* 1986). A stock of *rosy506 P(Δ2–3, ry+)* (99B) was crossed to the chromosome *A*-bearing parental stock. From the progeny of chromosome *A/ry506 P(Δ2–3, ry+)* (99B) hybrid females we selected single male recombinants placing *P(Δ2–3, ry+)* (99B) distal to *Ubx* on chromosome *A*. Each recombinant, balanced over *In(3LR)TM6, Tb*, was tested for characteristic *P(Δ2–3, ry+)* somatic transposase expression with *snw* (Robertson *et al.* 1988), and the cytological localization of the *P* element at 99B was confirmed by *in situ* hybridization. 99B responded to both *P* and *rosy* probes and no other *P* elements were

https://doi.org/10.1017/S0016672300028500 Published online by Cambridge University Press

observed. The line used in the present study was derived from a single recombinant chromosome and has been completely stable in stock and during the course of Expt 3, showing no loss of the ry^+ visible marker, no loss of transposase activity in the sn^w assay, and no *in situ* evidence of mobilization in any of the ten recombinants recovered in Expt 3. We have monitored two excisions of $P(\Delta 2\text{–}3, ry^+)$ from 99B, both occurring during out-crosses to perform other tests. Chromosome $A, P(\Delta 2\text{–}3, ry^+)$ (99B) tested positively for transposase production in both somatic and germinal tissues. 100% of F_1 males showed somatic mosaicism in tests of specific transposase action at sn^w (1208 sn mosaic males and 1 sn^+ among sons of 42 males tested) indicating active production of somatic transposase. 34 of 35 F_1 males produced sons showing F_2 germline activity at sn^w, demonstrating active transposase in the germline. Southern blots of both Expt 3 parent chromosomes revealed no *P* elements in parent chromosome *B* stock, and only one *P* element in the chromosome $A, P(\Delta 2\text{–}3, ry^+)$ (99B) stock.

(iv) *Gonadal sterility*

Gonadal sterility (GD) tests were carried out at 29 °C following the protocol of Daniels *et al.* (1987) except that both sexes were scored for GD.

(v) In situ *hybridization*

Polytene chromosome spreads were made from mature third instar larvae grown at 18 °C in uncrowded cultures. Biotinylated probe was made by nick-translation (BRL nick-translation reagent kit, 8160SB) of pπ25.7wc DNA, and *hobo* DNA. Hybridization and detection followed the method of Engels *et al.* (1986) except that coverslips were removed after freezing the slides in liquid nitrogen.

(vi) *Probes*

pπ25.7wc, the *P*-element probe used for *in situ* hybridization, contains a functional *P* element that has a small segment deleted from one of its terminal repeats (Karess & Rubin, 1984). It carries flanking genomic sequences from the 17C region of the *X*-chromosome reflecting its origin, and serving as a positive control indicator of hybridization. A 2·2 kb Dde I restriction fragment internal to the *P* sequences of pπ25.7wc was used as probe for Southern blots which were prepared as described in Rushlow *et al.* (1984). A *hobo* probe was used for *in situ* tests for presence of *hobo* sequences.

(vii) *Flanking lethal crossover selective system.*

A flanking lethal crossover selective screen (Fig. 1) was developed for the recovery of exceptional male recombinants within the region of chromosome 3 from *M(3)S34* (3.44.3) to *Ubx* (3:58.8). The experimental system is essentially the reciprocal of a selective screen that was developed earlier for genetic fine structure recombination mapping in females (Chovnick *et al.* 1962, 1964). The scheme involves a male parent heterozygous for the recessive lethal marked third chromosomes, *M(3)S34 Dfd + +/+ + Sb Ubx* mated to females carrying the recessive lethal marked third chromosomes *Tp(3), MKRS, M(3)S34 + Sb +/+ Dfd + Ubx.* In the absence of crossing over in the region from *M(3)S34* to *Ubx*, all developing zygotes will die due to homozygosity for one or another of the lethals, *M(3)S34, Dfd, Sb* and *Ubx*. Since the tester females carry the multiple break rearrangement *Tp(3), MKRS*, there is no possible crossing over within this region in females. Hence, the only zygotes that can survive and complete development are those that receive one or another of the several classes of single crossovers that might occur in the male parent. We recover 1/2 of the exchanges between *Dfd* and *Sb*: one exchange product is free of lethals and survives over either tester chromosome whereas the reciprocal product carries all four selective lethals and dies over either tester. We recover 1/4 of the exchanges in the regions *M(3)S34* to *Dfd* and *Sb* to *Ubx*: the retrievable exchange products live over only one tester chromosome and the reciprocal products die over either tester. The male parent is also heterozygous for a series of five recessive markers of the region between *Dfd* and *Sb* (*kar*, *mesB*, *ry*, pic^{1G23} and Ace^{126}) that are not selected against in the progeny. These facilitate further identification of exchange events and their assignment to specific intervals within the region bounded by the selective lethal markers. Moreover, they serve to verify the legitimacy of the surviving exceptions.

(viii) *Progeny estimates*

Estimates of total progeny are determined by crossing appropriate F_1 males individually to ry^{506} non-selective females. All progeny survive, are counted and provide a multiplier for the number of fertile experimental males in a given experiment.

3. *Results and Discussion*

Expts 1 and 2 (Table 1) provide a background male recombination rate for the monitored region in a laboratory *M* strain and in the absence of $P(\Delta 2\text{–}3, ry^+)$ (99B) transposase source. Chromosome *A* is introduced into the F_1 hybrid test males through the male parent in Expt 1 (Table 1, line 1). Matings of 99 fertile F_1 hybrid males gave no surviving exceptions in an estimated zygotic sample of 46431. In Expt 2, the reciprocal of the first experiment, chromosome *A* is introduced into the F_1 hybrid males through the female parent. Matings of 135 fertile F_1 hybrid males

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Table 1. *Summary of male recombination data*

Expt	Male[a] parent of F_1	Observed exceptions	Independent events	Extrapolated independent events	Progeny estimate	Recombination rate (cM × 10^3)
1	*A*	0	0	0	46431	0·0
2	*B*	1	1	2	69525	2·88
1,2		1	1	2	115956	1·72
3	*A*, *P*(Δ)	10	8	24	60422	39·7

[a] Indicates chromosome from Fig. 1 used as male parent in producing hybrid F_1 test males for each experiment.

produced one $kar^2 ry^+$ exception in a zygotic sample estimated to be 69525 (Table 1, line 2). Since the reciprocal crossover is lost to the lethal selective system, this survivor represents a recovery of two exceptions in a combined zygotic sample of 115956 (Table 1, line 3) giving a background male recombination rate of $1{\cdot}72 \times 10^{-3}$ cM. Expts 1 and 2 were performed in reciprocal to allow potential expression of unknown factors that might influence male recombination in these stocks. We found no evidence for the presence of such factors (Table 1).

We next assayed the effect of transposase provided by a non-mobile *P* element in this same selective system by introducing the *P*(Δ*2–3*, ry^+) (99B) construct onto chromosome *A* (Fig. 1) distal to *Ubx* and outside the monitored recombination region. This chromosome then was introduced through the male parent to produce the F_1 hybrid males for Expt 3 (Fig. 1). From 197 fertile F_1 hybrid males tested we recovered 10 exceptional offspring from 8 matings (Table 1, line 4). Of these, eight were independent events, and one of these was a cluster of three from a single male. Each exception was examined by *in situ* hybridization to check for mobilization of the *P* element from 99B. All exceptional lines were free of *P* elements, the element at 99B itself being excluded by the recombination event.

Seven exceptions including the cluster of three, occurred between *M(3)S34* and *Dfd*. Each of the four single exceptions in the *M(3)S34* to *Dfd* region represents, due to survival of exceptions over only one of the tester chromosomes and loss of the reciprocal events, four independent events. The cluster of three represents two independent events since the reciprocal cluster is lost. Adjusting for the cluster and the loss of other crossovers in this region due to lethals, we infer 18 independent events. The three events in regions *Dfd* to kar^2, kar^2 to *mesB* and *Ace* to *Sb* each represent two independent events due to loss of the reciprocal events. Thus we estimate 24 independent events in a zygotic sample of 60422, giving a transposase-induced male recombination rate of $39{\cdot}7 \times 10^{-3}$ cM, more than 20 times greater than the control rate (Expts 1 and 2).

We conclude that *P*-element transposase, in the absence of mobile *P*-elements, induces a significant rate of crossing over in *Drosophila* males. We do not conclude from these experiments that *P* targets and mobilizing *P* elements, when present, have no influence on male recombination rates. Nor have we demonstrated that the transposase has a direct and unmediated action on genomic sequences although both this report and the report of Rio *et al.* (1988) raise this possibility. We note with interest that the transposase-induced crossovers of Expt 3 may reflect upon the existence of genomic sites with sequence similarities to *P* element DNA in long-standing laboratory true *M* strains.

We wish to thank W. Gelbart for providing *hobo* DNA, S. B. Daniels for the ry^{506} *P*(Δ*2–3*, ry^+) (99B) stock, and K. Sitaraman for untiring assistance. This work was funded by National Institutes of Health research grants GM-09886 and GM-37246 to A.C.

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