Evidence of *P*-Element-Induced Sister-Chromatid Exchange in a Ring-X Chromosome in *Drosophila*, With Implication for a High Rate of Formation of Hybrid Elements

John A. Sved¹ and Xiumei Liang

School of Biological Sciences, University of Sydney, Sydney, New South Wales 2006, Australia

Manuscript received September 6, 2005

Accepted for publication September 17, 2005

ABSTRACT

Activation of a single incomplete *P* element induces recombination at a rate of ~0.5–1% in the male germline of *Drosophila*. Male recombination rises by an order of magnitude to ~20% if homologous *P* elements are involved. The high rate of recombination suggests the possibility that sister-chromatid exchange (SCE) might be elevated to a similar extent, since homologous *P* elements must always be present in sister chromatids. This possibility was tested by recombining a single *P* element onto a ring-X chromosome and using sex-ratio distortion to measure the loss of the ring-X due to SCE in the male germline. The results confirmed a rate of loss comparable to that expected with homologous elements, although the rate of loss was variable. Both SCE and recombination results are consistent with the “hybrid element insertion” model, in which the left and right ends from different elements associate, providing that insertion occurs preferentially in the vicinity of a *P*-element end. For autosomes, hybrid element formation may thus occur at a much higher rate than the 0.5–1% implied by single element recombination, with only a small minority of hybrid element excision events being resolved by recombination.

P elements induce male recombination at a low rate in crosses between wild and laboratory strains of *Drosophila melanogaster* (HIRAIZUMI 1971). Comparable levels of recombination can be produced by a single incomplete *P* element when activated by a transposase source (SVED *et al.* 1990). Furthermore, the level of recombination rises from <1% to ~20% if *P* elements are present at homologous sites on the two chromosomes (SVED *et al.* 1991).

A likely mechanism for this recombination was elucidated by GRAY *et al.* (1996), who showed that recombination can be produced when an element containing only an intact left end is brought together with an element on the homologous chromosome containing only an intact right end. The only possibility for association in this case involves ends from different chromosomes, associating to form a “hybrid element.” PRESTON *et al.* (1996) showed that this model can account for recombination produced by a single *P* element if the left and right ends that associate in a hybrid element come from sister chromatids. The model of hybrid element insertion, together with the complementary process of “hybrid element repair,” can account for most aspects of the recombination process. The clustering of recombinant products suggests that in males these processes occur primarily in the mitotic divisions preceding meiosis. *P*-element-induced recombination

has not been studied in females owing to the high background of meiotic recombination.

The hybrid insertion model predicts that sister-chromatid exchange (SCE) should occur at comparable levels to nonsister recombination. Such SCE is, however, difficult to detect, since it has no genetical consequences. Furthermore, the high elevation of recombination caused by the presence of homologous *P* elements suggests the possibility that SCE might be similarly elevated, since both sister chromatids must contain homologous elements.

One test available for the detection of SCE is the effect on ring chromosomes. Recombination in premeiotic mitosis is expected to cause a double ring with two centromeres. This structure can be resolved only by breaking of the ring, leading to inviable products (MORGAN 1933). Such events are most easily detected in the X chromosome of males, where germline recombination results in distortion of the sex ratio.

Owing to the lack of male recombination, ring-X chromosomes are normally stable in the male germline. The ring chromosome technique has been used in the past to assess the effect of chemically and radiation-induced SCE (LEIGH 1976; see also the SCE study by GATTI *et al.* 1980). The consequences of radiation-induced breaks can be quite complex because of the potential variety of breakages (see, *e.g.*, LEIGH 1976, Figure 2). The predictions in our study, where SCE is induced by a transposon rather than chemically, are simpler. Insertion-like events are expected to cause recombination in a precise

¹Corresponding author: School of Biological Sciences, Biology A12, University of Sydney, Sydney, NSW 2006, Australia.
E-mail: jsved@usyd.edu.au

FIGURE 1.—The production of recombinant ring chromosomes in male progeny from the ring/rod heterozygous female genotype shown at left.

manner (GRAY *et al.* 1996), leading to double-ring formation, followed by chromosome breakage.

Here we report the effect of activating a single incomplete *P* element contained on a ring-X chromosome.

MATERIALS AND METHODS

Stocks used: The following stocks were used: *ClB/R(1)2. cv v f. Cal20.10 = w*, *P[CaSpeR] (17C)*, and *C(1)DX; P[Δ2-3] (99B)*. All crosses were carried out, and the progeny raised, at 25°.

Production of the ring-X chromosome containing the *P* element: Female recombination in the absence of transposase was used to recombine the *P* element from the rod-X chromosome containing a *P[CaSpeR]* insertion at 17C (*Cal20.10*; see SVED *et al.* 1990) to the ring-X chromosome. Male progeny of female flies heterozygous for the ring-X chromosome and for the *Cal20.10* chromosome were screened (Figure 1). Recombinants occurred at a low level, consistent with the notion that double recombination is needed to produce viable recombinants.

Four flies containing the *cv* marker but not *f* were chosen to found lines by crossing to attached-X females. These lines were screened by PCR for presence of the *CaSpeR* element, using one primer pair from the *P*-element left end and one pair from the right end. Three of the four, labeled *RCa1*, *RCa2*, and *RCa4*, were found to possess the element. The first two of these, *RCa1* and *RCa2*, were used in experiments reported here.

Preliminary tests: We checked whether the chromosome had some of the properties required of the ring-X chromosome (GRAF 1981). The effect on recombination was tested by crossing to a *y w* stock and checking for recombination between *y*, *w*, *cv* and *P[CaSpeR] (17C)*. Recombination was reduced to ~1% of the expected levels with a rod-X chromosome. As expected, there was a high frequency of double recombinants compared to single recombinants. We also noted that the frequency of male progeny in crosses to attached-X stocks was substantially reduced compared to crosses with rod-X chromosomes, 0.29 for *RCa1* and 0.34 for *RCa2* vs. 0.56 for the original rod chromosome containing *P[CaSpeR] (17C)*.

A mobility assay of the *P[CaSpeR]* transposon was carried out by crossing males containing the *P[Δ2-3] (99B)* transposon to *white* females. The presence of *P[CaSpeR]*-containing male offspring, indicated by an orange eye color, shows transposition of *P[CaSpeR]* to another chromosome—2, 3, 4, or Y. The estimated frequency of transposition was $8.0 \pm 2.0\%$, as compared to a rate of $10.2 \pm 2.3\%$ for the original *P[CaSpeR] (17C)*. Therefore the activity of the *P* element appears unaffected by the movement to the ring-X chromosome.

Tests for the effect of transposase on the ring-X *P* element: Figure 2 shows the experimental (e) and control crosses (c)

FIGURE 2.—Crosses used to measure the effect of transposase on ring-X chromosomes (e) compared to controls (c). X chromosome and chromosome 3 are shown. *P[Δ2-3] (99B)* and *P[CaSpeR] (17C)* are shown as solid and open symbols, respectively.

used to monitor the effect of *P[Δ2-3] (99B)* on the ring-X chromosome. F₁ test males (asterisk) were produced by crossing males from the *RCa1* and *RCa2* stocks to attached-X females homozygous for the *P[Δ2-3] (99B)* transposon (e) and lacking the transposon (c). Single F₁ males from both crosses were then crossed to either attached-X females or normal females. The cross to attached-X females ensures that the F₂ progeny containing the ring-X appear in hemizygous condition in male offspring, while the cross to normal females results in ring-X progeny in heterozygous state in female offspring. Half the (e) progeny are expected to carry the *P[Δ2-3] (99B)* transposon. Not all ring-X progeny are expected to carry the *P[CaSpeR] (17C)*, as the excision rate in the parents is of the order of 20–30% (SVED *et al.* 1990).

Two experiments were carried out, separated by several months, on each of the *RCa1* and *RCa2* stocks. Each experiment involved a mass intercross in the F₀ followed by the crosses of individual F₁ males to three females. In the first experiment, 20 individual males were used for each of the (e) and (c) crosses and tipped over twice a week up to the age of 4 weeks. The second experiment involved 40 males for each set tipped over once a week up to 4 weeks. The numbers of progeny scored in the two experiments were 89,203 and 89,813, respectively.

As an additional control of the effect of the *P[Δ2-3] (99B)* transposon, the same procedure as outlined above was carried out on the rod-X *Cal20.10* stock. No aging was attempted in this experiment, and the total number of progeny counted was 8043.

The selective effect of activation of the *P* element on the ring-X was calculated as follows. For the case of the cross to attached-X, where the ring-X appears in male progeny, let the

FIGURE 3.—Estimated selective losses caused by *P*-element activation in ring- and rod-X chromosomes in male progeny (solid bars) and female progeny (open bars). *** $P < 0.1\%$.

number of female and male progeny in the experimental set with $P[\Delta 2-3](99B)$ be $f_e:m_e$. Similarly, let the numbers in the control lacking $P[\Delta 2-3](99B)$ be $f_c:m_c$. If the selection against ring-X-carrying males is s , so that the survival rate is $1 - s$, then the expected ratio in the experimental set, m_e/f_e , is $(1 - s)m_c/f_c$. The selective loss, s , thus is estimated from $1 - (m_e/f_e)/(m_c/f_c)$. For the cross to normal unattached-X females, where the ring-X goes to the female progeny, s is estimated from $1 - (f_e/m_e)/(f_c/m_c)$. In both cases, significance of the selective loss was judged using Fisher's exact test in a 2×2 table.

RESULTS

The results for the two experiments involving two different ring-X stocks are shown in Figure 3. There is a striking difference between the cases of crossing to attached-X females, where significant loss of the ring-X is observed, and crossing to XX females, where there is no significant loss of the ring-X. This implies that damage to the ring-X chromosome, presumably attributable to recombination, is not sufficient to cause lethality if a normal chromosome masks the effect of the ring-X chromosome in female offspring. However, the selection is somewhat variable, being considerably higher for both chromosomes in the first experiment compared to the second. The possibility exists that some of the ring-X chromosomes may have opened up before the second experiment, particularly in the case of *RCa2*.

We looked at the results for the time course of the loss of the ring-X as revealed by progeny counts at different times of male aging. No simple relationship was found. The relationship over time is expected to be a complex one. An increase in recombination events is expected owing to the accumulation of events in aging stem cells (TANAKA *et al.* 1997). This is, however, expected to be balanced by a loss of *P* elements due to simple excision. The failure to detect a simple relationship may therefore be due to the canceling out of these two forces.

We noted an increased death rate in the parent flies, asterisked in Figure 2, containing the ring-X and trans-

FIGURE 4.—Death rates at half-weekly intervals of ring-X flies containing $P[CaSpeR](17C)$ in the presence and absence of $P[\Delta 2-3](99B)$ transposase source.

posase. Figure 4 shows the death rate, calculated from the number of males dying during each period as a function of the number alive at the start of the period. Although the numbers at each class are not individually significant, at each of the nine age classes the dysgenic flies died at a higher rate than the nondysgenic flies, a highly significant result.

DISCUSSION

The loss of ring-X chromosomes is predicted from *P*-element-induced recombination (see details below). Before considering implications of the recombination hypothesis, however, it is necessary to examine an alternative explanation for the loss of ring-X chromosomes. The transposase used in this study, $P[\Delta 2-3](99B)$, is known to have somatic as well as germline effects (ENGELS *et al.* 1987), leading to pupal lethality in combination with some truncated *P* elements. The possibility therefore exists that the loss of ring-X chromosomes could be a function of such pupal lethality.

Arguing against this possibility is the fact that rod-X chromosomes containing the same $P[CaSpeR](17C)$ transposon appear to be unaffected by the transposase. This explanation would also require that the F_1 males in which the germline events occur (asterisk in Figure 2) would themselves suffer somatic lethality. The results of Figure 4 show that these individuals do, in fact, die earlier than their control counterparts, but the full effect takes several weeks and could not account for sex-ratio biases appearing shortly after eclosion of the progeny when counts were made. Finally, it should be noted that the F_2 progeny containing the ring-X are expected to contain the $P[\Delta 2-3](99B)$ transposon in only 50% of cases, and the $P[CaSpeR](17C)$ transposon is lost through excision in 20–30% of such progeny (SVED *et al.* 1990). Therefore, although potential somatic effects of the $P[\Delta 2-3](99B)$ transposon are an unnecessary complication in this experiment, it seems unlikely that they play a critical role in determining the outcome.

FIGURE 5.—The model of hybrid element excision. The two sister strands are distinguished for convenience, although they are expected to be genetically identical.

The fact that almost no significant sex-ratio distortion was found in female progeny containing the ring-X also needs comment. The effects of structural changes in ring-X chromosomes are masked by the normal X chromosome, implying that any structural changes are comparatively minor ones. This seems difficult to reconcile with the model of chromosome breakage associated with the resolution of di-centromeric ring chromosomes at anaphase, unless the chromosomal bridges tend to break close to their midpoints. We have not attempted to investigate the nature of the changes. There is no easy method for separating out females containing structural changes, and it seems likely that there would be a considerable bias due to sterility of surviving females containing the more severe structural changes.

The hybrid element model: It is possible to give expectations for SCE frequencies on the basis of the hybrid element model of PRESTON *et al.* (1996). Although this model was put forward to explain nonsister recombination in autosomes, it also makes predictions for SCEs for both the sex-linked and autosomal cases. The model described below (Figure 5) is presented for the case of a sex-linked element, but the conclusions that follow are largely of interest for the case of an autosomal element.

Figure 5a shows two sister elements at the stage following DNA replication. *Pe*lement excision would normally occur at this stage, initiated by the association of left and right ends from one or another element. In this case, association occurs between the left end of one element and the right end of a sister element, indicated by dashed circles. The association of these two ends, as shown in Figure 5b, forms a hybrid element. This is followed by an "excision" event, as shown in Figure 5c, although in the case of a hybrid element, no true excision takes place.

Two sets of ends need to be considered, although, strictly speaking, the two sets cannot be considered separately. First, the excision process leaves broken ends that do not contain elements, circled in Figure 5c. GRAY *et al.* (1996) have shown, for the case of end-deleted elements, that such broken ends join at high frequency, probably using repair against the sister chromatid. Such joining would lead to SCE in this case.

The second aspect concerns the hybrid element. In the case of elements inserted on autosomes, hybrid elements may resolve by insertion into the homologous chromosome to produce recombination (PRESTON *et al.* 1996). However, this is not an option for X-linked elements in males. Even for autosomes, the majority of hybrid elements may not resolve in this way (see below).

There are three possibilities for resolving the hybrid element in Figure 5. In the first, (Figure 5d1), the element inserts back into one of the two strands from which it has excised, simply reversing the excision event, albeit with insertion or deletion. The second possibility (Figure 5d2) is for insertion into one of the strands containing the original elements. This leads to SCE, with either deletion or duplication, depending on the orientation of insertion and which strand goes into the gamete. The third possibility (Figure 5d3) is that the *Pe*lement ends disassociate. Ligation of such ends could then occur in the same way as for joining of broken ends not containing *Pe*lements (LIANG 2003). From the point of view of exchange, both of these two latter possibilities involving the hybrid element would lead to SCEs.

How frequently does hybrid element formation occur? Hybrid element formation has been inferred to explain male recombination (PRESTON *et al.* 1996). However, it has been postulated that hybrid element formation occurs at a reasonably low rate, since the rate of recombination produced by a single *Pe*lement is only of the order of 0.5–1.0% (SVED *et al.* 1990).

By contrast, the recombination frequency is ~20% when homologous elements are present at a single site (SVED *et al.* 1991). Two different explanations can be given for this large increase:

1. Hybrid element formation occurs at the same, relatively high, rate in the single and homologous chromosome cases. However, resolution of hybrid

elements results only rarely in recombination in the former case and frequently in the latter case.

2. Hybrid element formation occurs at a much higher rate (per element) in the homologous case. This could be, for example, if formation of a hybrid element on either chromatid is stimulated by the presence of an element on the homologous chromosome, although there is no obvious reason for such an effect.

The first of these explanations is supported by the results of the ring-X experiments. They imply a rate of hybrid element formation >20% even if only a single element is present. Note that this is the rate cumulated over all mitotic germline divisions rather than for a single division. The different modes by which hybrid elements are resolved (see Figure 5, d1–d3) suggest that the majority of such elements will lead to SCE.

Turning to the case of a single autosomal element, genetic recombination can be produced only by insertion of the hybrid element into the homologous strand. Under the model, although hybrid elements occur at a rate of >20%, only 0.5–1% of recombination results. Thus resolution of hybrid elements, in the majority of cases, leads to no recombination.

By contrast, the model requires that for homologous elements, resolution of the hybrid elements results in recombination in a much higher fraction of cases. The situation with homologous elements is more complicated than that for a single element, since hybrid elements may involve nonsister chromatids, in addition to sisters as shown in Figure 5.

Perhaps the biggest difference between single and homologous elements, however, is the fact that the recombination from a single element requires insertion into a non-*P* region, which is not the case with homologous elements. It has been shown that *P* elements frequently insert into sites close to existing elements (EGGLESTON 1990; TOWER *et al.* 1993). This preference for sites close to *P* elements in some cases could be due simply to constraints for the availability of sites for insertion of hybrid elements. However, such a preference for insertion close to an element could also explain the high disparity between single and homologous recombination. In addition, the possibility of simultaneous excision events on both homologs could further increase the frequency of recombination.

In conclusion, the intriguing possibility suggested by these arguments is that formation of hybrid elements as inferred from single-element recombination reveals only the “tip of the iceberg.” These considerations suggest that it is the 20% level of recombination given by homologous *P* elements that reveals the true level of

hybrid element formation. The difference in recombination rates between single elements (0.5–1%) and homologous elements (20%) would reflect not a difference in the rate of formation of hybrid elements but rather the manner of their resolution.

The formation of hybrid elements through the association of opposite sister-chromatid *P*-element ends would appear to be an aberrant event. It seems difficult to see how this type of association could be advantageous either for the *P* element or for the organism containing it. Earlier estimates that such events occur at a frequency of ≤1% reduced their potential importance. The possibility that these events occur at a much higher frequency requires that they be studied more seriously.

Our knowledge of ring-X chromosomes comes from Larry Sandler, for whose original influence we are grateful. Helpful comments from reviewers are also acknowledged. The work was supported by a grant from the Australian Research Council.

LITERATURE CITED

- EGGLESTON, W. B., 1990 *P* element transposition and excision in *Drosophila*: interactions between elements. Ph.D. Thesis, University of Wisconsin, Madison, WI.
- ENGELS, W. R., W. K. BENZ, C. R. PRESTON, P. L. GRAHAM, R. W. PHYLLIS *et al.*, 1987 Somatic effects of *P* element activity in *Drosophila melanogaster*: pupal lethality. *Genetics* **117**: 745–757.
- GATTI, M., S. PIMPINELLI and B. S. BAKER, 1980 Relationships among chromatid interchanges, sister chromatid exchanges, and meiotic recombination in *Drosophila melanogaster*. *Proc. Natl. Acad. Sci. USA* **77**: 1575–1579.
- GRAF, U., 1981 An easy way to test for ring configuration of ring-X chromosomes in *D. melanogaster*. *Dros. Inf. Serv.* **56**: 176–177.
- GRAY, Y. H. M., M. M. TANAKA and J. A. SVED, 1996 *P* element-induced recombination in *Drosophila melanogaster*: hybrid element insertion. *Genetics* **144**: 1601–1610.
- HIRAZUMI, Y., 1971 Spontaneous recombination in *Drosophila melanogaster* males. *Proc. Natl. Acad. Sci. USA* **68**: 268–270.
- LEIGH, B., 1976 Ring chromosomes and radiation induced chromosome loss, pp. 505–528 in *The Genetics and Biology of Drosophila*, edited by M. ASHBURNER and E. NOVITSKI. Academic Press, New York.
- LIANG, X., 2003 *P* element-induced recombination in *Drosophila melanogaster*. M.Sc. Thesis, University of Sydney, Sydney.
- MORGAN, L. V., 1933 A closed X chromosome in *Drosophila melanogaster*. *Genetics* **18**: 250–283.
- PRESTON, C. R., J. A. SVED and W. R. ENGELS, 1996 Flanking duplications and deletions associated with *P*-induced male recombination in *Drosophila*. *Genetics* **144**: 1623–1638.
- SVED, J. A., W. B. EGGLESTON and W. R. ENGELS, 1990 Germline and somatic recombination induced by *in vitro* modified *P* elements in *Drosophila melanogaster*. *Genetics* **124**: 331–337.
- SVED, J. A., L. M. BLACKMAN, A. S. GILCHRIST and W. R. ENGELS, 1991 High levels of recombination induced by homologous *P* elements in *Drosophila melanogaster*. *Mol. Gen. Genet.* **225**: 443–447.
- TANAKA, M. M., X. LIANG, Y. H. M. GRAY and J. A. SVED, 1997 The accumulation of *P*-element-induced recombinants in the germline of male *Drosophila melanogaster*. *Genetics* **147**: 1769–1782.
- TOWER, J., G. H. KARPEN, N. CRAIG and A. C. SPRADLING, 1993 Preferential transposition of *Drosophila P* elements to nearby chromosomal sites. *Genetics* **133**: 347–359.

Communicating editor: M. J. SIMMONS