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Hybrid Dysgenesis in *D. melanogaster* Is Not a General Release Mechanism for DNA Transpositions

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Many spontaneous mutations are caused by the insertion or excision of DNA elements. Since most mutations are deleterious, evolution should favor a mechanism for genetically controlling the rate of movement of transposable elements in most, if not all, organisms. In *Drosophila melanogaster* a syndrome of correlated genetic changes, including mutation, chromosome breakage, and sterility, is observed in the hybrid progeny of crosses between different strains. This syndrome, which is termed hybrid dysgenesis, results from the movement of P-DNA elements. What is not clear is whether the movement of other types of transposable elements is under the same coordinated control. In this study the ability of hybrid dysgenesis to increase the rate of excision of 12 DNA elements at 16 mutant alleles and to induce insertion-bearing mutations to change to other mutant states was tested. The data show that hybrid dysgenesis caused by P-element transpositions does not act as a general stimulus for the movement of other Drosophila transposable elements.

TRANSPOSITIONS OF DNA ELEMENTS are a major cause of spontaneous genetic change in many prokaryotic and eukaryotic organisms (*1*). In *Drosophila melanogaster*, where about one-tenth of the genome is mobile, the majority of spontaneous mutant alleles that have been analyzed at the DNA level contain inserts. These inserts have been localized to introns, exons, and regulatory sequences of genes (*2*); when mobilized, these elements are often imprecisely excised (*3*). In addition to gene changes, transposable elements also cause chromosome breakage, nondisjunction, male recombination, and sterility. With this potential for cellular and genetic damage by transpositions, it is not surprising that genetic control of the rate of movement of these nomadic DNA sequences occurs in higher organisms.

An excellent model system for studying this control is the hybrid dysgenesis syndrome in *D. melanogaster*, where a variety of genetic changes (including high frequencies of mutation, chromosome breakage, sterility, and distortion in the ratio of transmission of alleles in gametes) occur in hybrid progeny of controlled crosses between independent population lines (*4, 5*). Frequently, this syndrome results from the insertion and excision of a family of P-DNA elements in germ cells (*6*). Hybrid dysgenesis releases the suppression of P-element movement, which leads to increased production of aberrant genetic traits. Both chromosomal and cytoplasmic components are involved; lines that have complete elements are called *P* lines and lines with no P elements or defective P elements are called *M* or pseudo-*M* lines (*7*).

Although it is clear that mobile DNA elements can disrupt genetic systems, it still remains to be determined whether different transposable elements move under the direction of element-specific enzymes or whether a number of different elements may move under a coordinated control mechanism. For example, a transposase that is coded by one type of element might be used only by these elements for transposition; alternatively, a transposase coded by one element might be used by a number of different elements for transposition. The latter mechanism would have the potential for inducing a larger amount of genetic damage. There is conflicting evidence in the literature on these two possible mechanisms of transposition in *D. melanogaster*. It has been reported that hybrid dysgenesis is a stimulus for the movement of DNA elements other than P, including foldback, copia, mdg, and gypsy sequences (*8*). This suggests that the P-element transposase can be used by other DNA elements, or that P-element–induced genetic damage acts as a general mobilizer of transposons. On the other hand, negative results have been reported for interactions between hybrid dysgenesis and the movement of I, TE, B104, copia, and foldback elements (*9*).

Since transpositions induce a variety of changes that may affect the genetic structure of natural populations, it is clearly important to define any interactions between different mobile elements. This is especially true if different transposons can be activated by similar genetic or environmental events (*10*). In this study we have measured the ability of hybrid dysgenesis in three well-characterized strains of *D. melanogaster* to increase the rate of excision of 12 DNA inserts at 16 mutant alleles. The data from this study do not support the hypothesis that hybrid dysgenesis is a general stimulus for excision of different DNA elements.

We used the natural-population *P* lines *OK1*, *W8D*, and *π2*, and the *M* line Canton-S (*5*). In addition, three *P* lines contained P-element inserts at the white locus ($w^{hd\,81c2}$, $w^{hd\,81b9}$ and $w^{hd\,80k17}$) (*6*). The other lines that carried visible mutations containing DNA inserts were *M* and were obtained from the Mid-America Drosophila Stock Center, except for $r^{MR\,2}$, which was obtained from C. Osgood (Old Dominion University, Norfolk, Virginia). The DNA insert at each mutant allele is shown in Table 1. All stocks were tested for *M* or *P* activity by measuring gonadal dysgenesis in hybrid progeny raised at 29°C (*7*). In addition, the *OK1*, *W8D*, *π2*, and Canton-S genomes were probed for P elements by Southern blot analysis; the *OK1*, *W8D*, and *π2* lines contained 20 to 30 DNA sequences that hybridized to the P probe, whereas Canton-S contained eight such sequences that are defective in hybrid dysgenesis activity (Canton-S is therefore a pseudo-*M* line) (*11*).

Mutation events in the absence of hybrid dysgenesis were scored by one of two methods: (i) by mating individual males that contained visible mutations with attached-X (*M*) females (females whose X chromosomes are joined by a single centromere) and screening F1 males for reversions to wild type and for changes to other mutant phenotypes, or (ii) by mating mutation-bearing females to Canton-S (*M*) males, mating individual F1 males to attached-X females, and scoring F2 males for genetic changes. Mutation events in the presence of hybrid dysgenesis were scored by mating

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mutation-bearing *M* females to *P*-line males, mating individual hybrid males to attached-X females, and scoring F2 males for genetic changes. All presumptive reversions and mutations were retested to confirm that they bred true. The reversion results from these crosses are shown in Table 1.

Hybrid dysgenesis caused a significant tenfold increase in P-element excision events in the crosses with w^{hd} lines. In addition, we recovered visible mutations at the *singed* locus from the w^{hd} crosses; these mutations are commonly due to P insertions (*7*). Hybrid dysgenesis also produced chromosome breakage and frequent visible mutations at the *singed*, *white*, *scalloped*, and *Minute* loci in all crosses in this study, whereas only one *Minute* mutation was recovered from the crosses in the absence of hybrid dysgenesis.

However, hybrid dysgenesis did not act as a general activator of DNA excision events. The data in Table 1 show that hybrid dysgenesis did not cause a significant increase in reversion of any allele containing the 11 DNA elements other than P.

Hybrid dysgenesis also failed to cause insertion-bearing mutations to change to other mutant states. A significant increase in genetic changes to other mutant states was only observed at the w^c locus (w^c to white or white-ivory eye color; in the absence of hybrid dysgenesis, 448/53,668 = 0.83%; in the presence of hybrid dysgenesis, 847/79,890 = 1.06%; $P < 0.01$). Genetic changes were also observed at the w^a, w^e and w^h alleles, but the frequencies were not significantly greater in the presence of hybrid dysgenesis (*12*). Further experiments will be needed to determine the molecular causes of the eye color changes at the *white* locus.

The results from this study are in contrast to the previous reports which suggested that hybrid dysgenesis can cause transpositional burst of a number of different DNA elements (*8*). This is notably apparent in the case of the gypsy insertion mutation, ct^6, where 20 ct^6 to ct^+ events were observed by Gerasimova and colleagues (*13*) among 2000 progeny in the presence of hybrid dysgenesis, whereas we found no revertants of ct^6 among 17,233 progeny (Table 1). It may be that the effect of hybrid dysgenesis on the movement of transposable elements other than P in the former study is strain-specific, or some other genetic factor acting alone or with P elements caused the observed gene changes. In the group of experiments where ct^6 and other insertion mutations were unstable (*8, 13*), only the *MRh12/Cy* P-containing line was used, whereas the *P* lines *OK1*, *W8D*, and *π2* were used in this study.

The data in this study do not rule out a possible influence of hybrid dysgenesis associated with the *OK1*, *W8D*, and *π2* lines on the insertion rates of DNA elements other than P. Hybrid dysgenesis induced by the *MRh12/Cy* line does increase the frequency of insertions of different elements (*8*), and the same may be true for other *P* lines, including *OK1*, *W8D*, and *π2*. Molecular analyses of the mutations induced by a number of *P* lines may lead to a better understanding of the impact of hybrid dysgenesis on transpositions. This is especially true if excisions and transpositions occur by different mechanisms, as appears to be the case in bacteria (*14*).

Table 1. Hybrid dysgenesis and the movement of transposable DNA elements in *D. melanogaster*.

DNA insertion mutation tested	Reversion rate (%)		Fisher exact probability of an effect associated with hybrid dysgenesis†
	Absence of hybrid dysgenesis	Presence of hybrid dysgenesis*	
P element			
$w^{hd\,81c2}$	2/11,875 (0.02)	10/7,096 (0.14)	<0.01
$w^{hd\,81b9}$	1/30,140 (0.003)	12/11,065 (0.11)	<0.001
$w^{hd\,80k17}$	0/12,229	7/9,698 (0.08)	<0.01
Total	3/54,244 (0.01)	29/27,859 (0.10)	<0.001
gypsy			
y^2	0/43,774	0/93,323	N.S.
ct^6	0/7,717	0/17,233	N.S.
Total	0/51,491	0/110,556	N.S.
copia			
w^a	0/43,774	0/93,323	N.S.
B104 (roo)			
w^{bf}	0/5,979	1/12,789 (0.01)	N.S.
ct^n	0/3,927	0/11,516	N.S.
Total	0/9,906	1/24,305 (0.004)	N.S.
foldback			
w^c	299/53,668 (0.56)	377/79,890 (0.47)	N.S.
F element			
w^h	0/7,095	0/14,172	N.S.
F-like element			
w^e	0/5,443	0/19,491	N.S.
BEL element			
$w^{a\,4}$	0/6,387	2/12,764 (0.02)	N.S.
Hobo			
r^{MR2}	0/2,532	0/6,710	N.S.
Other DNA inserts			
w^1	Not tested	0/15,017	
w^{ch}	Not tested	0/12,985	
w^{bl}	Not tested	0/29,775	

*Except in the w^{hd} crosses, the totals represent the sums of runs with *OK1*, *W8D*, and *π2*. †N.S., not significant ($P > 0.05$).

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11. P elements were identified in the *OK1*, *W8D*, *π2*, and Canton-S lines by hybridizing biotin-labeled p25.1WC DNA to genomic DNA on Southern blots. The hybridized sequences were visualized by an alkaline phosphatase–strepavidin reaction. *Drosophila simulans* DNA, which has been reported to be devoid of P elements (*7*), was used as a negative control and was observed to contain no sequences that hybridized to P elements (there was one band of hybridization between *D. simulans* DNA and pπ25.1WC due to the inclusion of X-chromosomal DNA in the pπ25.1WC probe). It should be noted that some of the hybridization sequences in the *OK1*, *W8D*, and *π2* lines could be defective P elements.
12. w^a to white or ruby eye color = 9/43,774 (0.02%) in the absence of hybrid dysgenesis (HD) and 7/93,323 (0.01%) in the presence of HD

($P > 0.05$). w^e to white eye color = 0/5,443 in absence of HD and 12/19,491 (0.06%) in the presence of HD ($P > 0.05$). w^h to white eye color = 0/7,095 in absence of HD and 2/14,172 (0.01%) in presence of HD ($P > 0.05$).
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Corresponding Spatial Gradients of TOP Molecules in the Developing Retina and Optic Tectum

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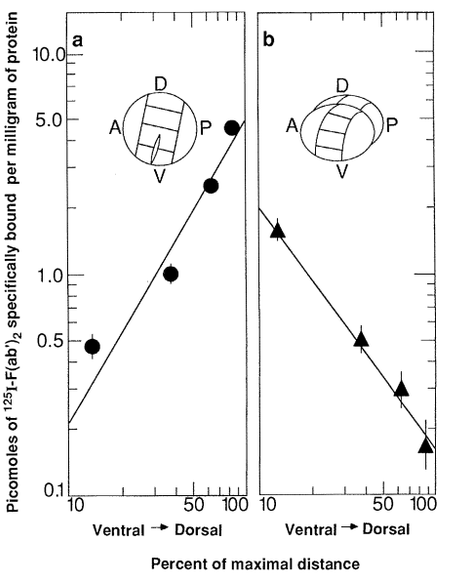
The topographic map of cell position in the avian retina is inverted in its projection to the optic tectum. Dorsal retinal ganglion cell axons project to ventral tectum, and ventral retinal ganglion cells project to dorsal tectum. Topographic gradients of toponymic (TOP) cell surface molecules along the dorsoventral axes of retina and tectum also are inverted. TOP molecules are most abundant in dorsal retina and ventral tectum and least abundant in ventral retina and dorsal tectum during the period of initial retinal-tectal interaction. Thus, TOP molecules may be involved in orienting the retinotectal map.

NEURONAL PROJECTION MAPS ARE formed when one group of neurons connects with a second group so that the spatial order of cells in the first group is represented in the second. In the retinotectal projection, the spatial order of retinal ganglion cells is preserved in the order of their terminals in the optic tectum (*1, 2*). Such projections are thought to require corresponding location-dependent signals. A location-dependent signal could result from a graded distribution of one or more molecules along corresponding axes of each neuronal group. There is a gradient of cell surface molecules, termed TOP for toponymic—a marker of cell position, along the dorsoventral axis of the developing chick retina (*3*). We demonstrate here that the developing chick optic tectum also exhibits a tenfold gradient of TOP antigen along its dorsoventral axis, although inverted with respect to the retinal gradient, as are the cellular projections.

The distribution of TOP molecules in retina and tectum of chick embryos on embryonic day 5 (E5) was determined by radioimmunoassay with a monoclonal antibody to TOP (*3*). Strips of tissue along the dorsoventral axis were removed and cut into pieces, and cells from each region were assayed for TOP. A gradient of TOP was detected along the dorsoventral axis of both retina (Fig. 1a) and optic tectum (Fig. 1b).

Fig. 1. Gradients of TOP molecules in (**a**) retina and (**b**) optic tectum of White Leghorn chicken (*Gallus gallus*) embryos from E5. (a) Strips of retina (5.0 by 2.0 mm), extending from the ventroanterior (0%) to the dorsoposterior (100%) retinal margins, were removed from 48 eyes (24 embryos) and cut into four segments (1.25 by 2.0 mm), as shown (A, anterior; D, dorsal; P, posterior; and V, ventral). The choroid fissure, shown extending from the ventroanterior margin of retina, was used as a landmark for dissection. (b) Strips of optic tectum (3.0 by 2.0 mm), extending from ventral (0%) to dorsal (100%) tectum, were removed from 72 tectal lobes (36 embryos) and cut into four segments (0.75 by 2.0 mm), as illustrated. Radioimmunoassay of the binding of TOP monoclonal antibody was performed at 4°C as described (*3*). Retinal and tectal cells were mechanically dissociated in phosphate-buffered saline (PBS). Cell suspension (100 μl containing 150 μg of cell protein) was added to each well of polyvinyl chloride 96-well V-bottom plates (Dynatech) pretreated with solution B (PBS containing 1 mg of gelatin per milliliter of solution). Plates were centrifuged at 1300*g* for 5 minutes, and the supernatant was decanted. Pellets were washed (resuspended and repelleted) three times in 150 μl of solution B (150 μl each wash) and suspended for 1 hour in 50 μl of solution B containing 1 μl of ascites fluid (6 μg of antibody to TOP or P3X63Ag8 antibody). Cells were washed three times as above, then incubated for 30 minutes in 50 μl of solution B containing 440 n*M* ^{125}I-labeled F(ab′)$_2$ (5×10^4 cpm) fragments of sheep immunoglobulin directed against mouse immunoglobulin and 500 μg of bovine serum albumin. Cells were washed three times as above, wells were separated, and radioactivity was determined. The protein was measured by a modification of the method of Lowry *et al.* (*9*). Specific binding of antibody to TOP was calculated by subtracting the number of picomoles of ^{125}I-labeled F(ab′)$_2$ bound in the presence P3X63Ag8 myeloma antibody from the number of picomoles of ^{125}I-labeled F(ab′)$_2$ bound in the presence of antibody to TOP; 0.2 pmol of ^{125}I-labeled F(ab′)$_2$ bound per milligram of tectal cell protein in the presence of P3X63Ag8 antibody. No topographical differences in P3X63Ag8 antibody binding were detected in retina and tectum. Each point is the mean of nine determinations. Percent maximal circumferential distance from ventral to dorsal retina and tectal lobe is shown on the abscissa. Bars represent the standard error of the mean.



In retina, the highest binding to TOP was present dorsally; in optic tectum, the highest binding was present ventrally. The difference between dorsal and ventral levels of binding to TOP indicates at least a tenfold gradient in both retina and tectum in E5 embryos. Extrapolation of these values to the poles of the gradients suggests that the actual gradient may be considerably larger.

Immunofluorescence of antibody binding to cells of tissue sections taken along the dorsoventral axis of retina and optic tectum of E5 embryos was consistent with the results of radioimmunoassay (Fig. 2). TOP was most abundant in dorsal retina and ventral tectum, intermediate in middle retina and tectum, and least abundant in ventral retina and dorsal tectum. Visual scanning of fluorescently stained transverse sections of whole retina and optic tectum showed that TOP was continuously graded in each. The ring pattern of fluorescence around each cell is consistent with the fact that TOP is a cell surface molecule (*3*). As previously shown in retina (*3*), most or all cells across the thickness of tectum, that is, from the pial surface to the ventricular surface, express similar levels of TOP in any region along the dorsoventral axis (Fig. 2f inset). This

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