Diverse transposable elements are mobilized in hybrid dysgenesis in *Drosophila virilis*

(regulation of transposition)

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**ABSTRACT** We describe a system of hybrid dysgenesis in *Drosophila virilis* in which at least four unrelated transposable elements are all mobilized following a dysgenic cross. The data are largely consistent with the superposition of at least three different systems of hybrid dysgenesis, each repressing a different transposable element, which break down following the hybrid cross, possibly because they share a common pathway in the host. The data are also consistent with a mechanism in which mobilization of a single element triggers that of others, perhaps through chromosome breakage. The mobilization of multiple, unrelated elements in hybrid dysgenesis is reminiscent of McClintock's evidence [McClintock, B. (1955) *Brookhaven Symp. Biol.* 8, 58-74] for simultaneous mobilization of different transposable elements in maize.

The activity of transposable elements is a major source of genetic change (1). Insertions of transposable elements account for a significant number of spontaneous hypomorphic mutations. Transposable elements can also promote other types of mutations, including the creation of novel genes, changes in gene expression in development, transpositions of large pieces of DNA, and the formation of deletions, inversions, and other DNA rearrangements. Such mutations probably play a significant role in adaptive evolution.

An important feature of transposable elements is that their activity is regulated. McClintock's early work (2) on transposable elements demonstrated not only the existence of mobile elements but also the complexity of their regulation. Her experiments showed that transposition takes place in a precise, developmentally regulated manner. She concluded that normally quiescent elements can be activated when the cell is challenged by repeated chromosomal breakage in either the chromosome or chromatic types of breakage–fusion–bridge cycle. Subsequent studies have shown that at least two different elements can be activated in this manner (3, 4). Other sources of chromosomal damage, including ultraviolet light, x-rays, and γ-radiation, can also activate normally dormant elements in maize (5).

A tight regulation of transposition was a key feature of McClintock's vision of a "dynamic and responsive" genome in which transposable elements were integral components (4). If there is a mechanism of genomic regulation, then functionally unrelated transposable elements might be mobilized when the regulatory system is disrupted, as McClintock inferred was taking place in maize cells subjected to "genomic stress" by chromosome breakage. The possibility of simultaneous mobilization is suggested by several examples in *Drosophila melanogaster* in which certain genetically unstable strains exhibit elevated levels of mobilization of more than one type of element. For example, a strain designated Uc shows high levels of *hobo* and *gypsy* mobilization (6). A different strain showing high rates of gypsy excision from an insertion in the cut locus (**cr**MR2) also seems to have an elevated level of activity of transposable elements other than *gypsy* (7).

*Drosophila* also offers several examples of genetic instability of transposable elements associated with hybridization ("hybrid dysgenesis") (8, 9). Each type of hybrid dysgenesis is thought to result in the mobilization of one and only one transposable element, for example, the *P* element, the *I* element, or *hobo*. However, the initial studies of hybrid dysgenesis mobilizing the *P* element also gave evidence for the simultaneous mobilization of other elements. For example, two of seven dysgenically induced mutations in the white locus contained insertions of *copia* rather than *P* (10). The mobilization of transposable elements such as *copia*, 412, and *F* have also been reported in dysgenic *P-M* crosses (11). However, these studies have been criticized on the grounds that they failed to control for preexisting genetic polymorphisms in the distribution of transposable elements in the genome (8).

**MATERIALS AND METHODS**

***Drosophila Strains.*** *D. virilis* strain B9 is a wild-type strain collected in 1970 in Batumi, Georgia (former U.S.S.R.), and maintained in laboratory culture since that time. Strain L160 is an old, established laboratory strain containing mutations in each of the large autosomes as follows: chromosome 2, 2b (broken crossovers); chromosome 3, 3b (tiny bristles) and *gp-L2* (gap in longitudinal wing vein 2); chromosome 4, 4cd (cardinal eye color); chromosome 5, pe (peach eye color); and chromosome 6, 6gl (glossy eye surface) (14).

***Mutant Strains.*** Three different alleles of singed bristles (*sn*7, *sn*10, *sn*25) and one allele of white eyes (*w*17) were examined in this study. These mutations were isolated independently from the progeny of the dysgenic cross between females of B9 and males of L160. Each mutant strain was established by crossing a single mutant male with B9 females.

***Cloning of Helena.*** The *Helena* element was identified in the *sn*25 allele as an insertion resulting in an altered electrophoretic

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Abbreviations: ORF, open reading frame; LTR, long terminal repeat.

The sequences reported in this paper have been deposited in the GenBank data base [accession nos. U26928 (Par) and U26847 (Helena)].
mobility of an EcoRI restriction fragment containing the last four exons of sn. The size of the restriction fragment increased from 1.7 kb to 2.4 kb. DNA isolated from flies homozygous for sn25 was completely digested with EcoRI and separated on a 0.8% agarose gel. DNA fragments ranging in size from 2.2 kb to 2.8 kb were extracted from the gel and ligated with the arms of the AZap II vector (Stratagene) predigested with EcoRI. Ligation was carried out overnight and the ligation products were packaged using Gigapack Plus packaging extract (Stratagene). The resulting library was screened with the 1.6-kb EcoRI fragment containing the wild-type sn sequence.

Cloning of Paris. A genomic library was constructed using the AGEM-11 vector (Promega). DNA isolated from flies homozygous for sn10 was partially digested with Sau3A. The resulting Sau3A overhanging ends were partially filled in with dGTP and dATP using the Klenow fragment of DNA polymerase and ligated overnight with the AGEM-11 vector arms predigested with Xho I and filled in with dCTP and dTTP. A DNA was packaged using Gigapack XL packaging extracts (Stratagene). The resulting genomic library was plated on Escherichia coli cells of strain LE392 and screened with a probe derived from the sn locus of D. virilis.

DNA Sequencing. Preparation of sequencing templates utilized the y6 system (15). Sequencing was carried out in an Applied Biosystems model 373A automated DNA sequencer using the Taq DyeDeoxy terminator cycle-sequencing kit.

Cytological Procedures. Localization of DNA probes was carried out by in situ hybridization to polytene chromosomes (16). The chromosomes were pretreated in 2× SSC at 65°C for 30 min (1× SSC is 0.15 M NaCl/0.015 M sodium citrate), dehydrated in 70% and 95% ethanol, denatured in 0.07 M NaOH for 2.5 min, washed twice in 2× SSC, dehydrated again, and dried in air. DNA probes were labeled with biotin derivatives of dNTPs (GIBCO/BRL) by primer extension of random hexamers. Hybridization of labeled DNA to polytene chromosome squashes in situ was carried out overnight at 37°C in 1.4× SSC/7% dextran sulfate/35% formamide/0.6 mg of sonicated denatured salmon sperm DNA per ml. Hybridization was detected with the Detekt I-HRP signal generation system (ENZO Diagnostics) and 3,3′-diaminobenzidine (Sigma). Chromosomes were stained with Giemsa and embedded in Permount.

RESULTS

Hybrid dysgenesis in D. virilis is observed in crosses between females of strain B9 and males of strain L160 (14). Strain B9 is a wild-type strain collected from Batumi in Georgia, former U.S.S.R.; strain L160 is an old laboratory strain containing multiple genetic markers. The F1 progeny of the dysgenic cross exhibit gonadal dysphoria and a high degree of sterility. The progeny of such crosses also show frequent new mutations, male recombination, chromosome nondisjunction, and transmission ratio distortion. These traits often indicate the mobilization of transposable elements. Neither the reciprocal cross of L160 females with B9 males nor the B9×B9 or L160×L160 intrasex crosses show any evidence of hybrid dysgenesis.

Four Different Transposable Elements in Four Dysgenic Mutations. Approximately 50 independent mutations have been isolated from the F2 or later generations of the dysgenic cross (17). Four of these dysgenic mutations have been examined at the molecular level. Each has an insertion of a different transposable element: Ulysses was isolated from the mutation w11, Penelope from y4, Paris from sn10, and Helena from sn22.

The molecular structures of the elements are shown in Fig. 1. They are structurally very diverse. Ulysses and Penelope have been described previously (18, 20, 21). Ulysses is a retroelement most closely related to the Ty3-gypsy superfamily and Penelope is structurally most similar to retroelements of the orphan class (22). Two previously undescribed elements are Paris and Helena.

Paris is in the mariner/Tcl superfamly of elements that transpose without an RNA intermediate (23). Paris was isolated from the second intron of the sn10 allele. The insertion in sn10 is longer than 10 kb and has a complex structure (Fig. 1). It is a composite transposon consisting of two virtually identical copies of Paris flanking a nonrepetitive sequence originating from the X chromosome (polytene chromosome region 4AB). The copies of Paris differ in a single base pair in one of the terminal inverted repeats. The insertion of Paris in sn10 resulted in a TA dinucleotide duplication, characteristic of mariner/Tcl transposition (Fig. 2a). Both copies of Paris possess an uninterrupted ORF, which encodes a putative protein with strong similarities to transposases of the mariner/Tcl type (Fig. 2b).

Helena is a LINE-like element (24) isolated from the third exon in the sn25 allele. Its insertion in sn25 resulted in a 10-bp-long nucleotide duplication of the target sequence (Fig. 3e). The insertion is not flanked by direct or inverted repeats. The single ORF encodes an apparently 5′ truncated reverse transcriptase (Fig. 3b).

Further Evidence for Multiple Mobilization. The distribution of the D. virilis elements in the genomes of the parental strains L160 and B9 is of interest in comparison with D. melanogaster, in which certain transposable elements are mobilized by hybridization when the male genome contains one or more functional copies of the element, whereas the female genome carries only defective copies (8, 9). Three of four elements in Fig. 1 have an asymmetric distribution in strains L160 and B9. Penelope is carried only by strain L160 (18). Helena and Paris are abundant in L160 but also present in B9. In hybridization with polytene chromosomes in situ, Helena hybridizes with 26 euchromatic sites in L160 and 4 sites in B9; Paris hybridizes with 29 euchromatic sites in L160 and 1 site in B9. In addition, colony hybridization of a P1 library from strain B9 (16) indicates at least 13 copies of Paris and 4 copies of Helena in the heterochromatin of B9 (data not shown). Only Ulysses is distributed about equally, with 15 euchromatic sites in L160 and 19 sites in B9. In addition, Ulysses, Helena, and Paris all hybridize with the chromocenter in B9 and are therefore present also in the pericentromeric heterochromatin and/or the Y chromosome.

To assess the degree to which the transposable elements are mobilized in hybrid dysgenesis, we carried out in situ hybridization of Paris, Helena, and Ulysses with polytene chromosomes from two independent lines, each of which originated from a cross of a B9 female with a single male carrying a new mutation, either w11 or sn7. w11 and sn7 were isolated in the progeny of the dysgenic cross. Few insertion sites of the transposable elements are polymorphic in the parental strains: Ulysses is found at four polymorphic sites, Helena at six, and Paris at three. All of the polymorphic sites were excluded from the analysis. With these sites excluded, it is likely that any new sites of insertion in w11 or sn7 result from transposition after the dysgenic cross. The data in Table 1 indicate an apparently high level of mobilization of Ulysses, Paris, and Helena. Mobilization of Penelope could not be examined because a probe is not available. Among 48 transposition events, approximately 64% were Helena, 23% Paris, and 13% Ulysses. In support of the interpretation that each of the new insertion sites results from a recent transposition, none of the new insertion sites was coincident between w11 and sn7. The data also show a striking tendency for Helena to become inserted at multiple nearby sites. For example, in sn7, Helena has new insertion sites in polytene regions 24A, 24D, 24E, and 24F. Although close cytologically, the sites are distant in the DNA because each lettered subdivision in the D. virilis genome contains approximately 2 megabases.
**DISCUSSION**

Although the mobilization of diverse transposable elements following a dysgenic cross appears to be a genuine phenomenon, its experimental investigation has been hindered by the lack of an easily reproduced procedure for mobilizing multiple elements at high frequency. In the example described here, the mating of strain B9 females with strain L160 males regularly yields progeny among which male sterility, male recombination, chromosome nondisjunction, transmission ratio distortion, and a high frequency of new mutations are reliably produced (14). These effects are typically associated with the mobilization of transposable elements. Following the dysgenic cross, at least four structurally diverse transposable elements are mobilized at high frequency. Because each of four dysgenesis-induced mutations obtained from B9 × L160 crosses proved to result from insertion of a different transposable element, it is reasonable to expect that still more elements will be identified as additional mutations are examined.

The molecular organization of the elements identified to date, among mutants arising from the B9 × L160 dysgenic cross, is remarkably varied. They include representatives of...
nearly all of the major types of transposable elements found in eukaryotes: an LTR-containing retrotransposon (Ulysses), an inserted repeat containing retrotransposon reminiscent of DIRS-1 element of Dictyostelium (Penelope), a LINE-like element (Helena), and a mariner/Tcl-like element (Paris). Some of them differ fundamentally in the details of their transpositional pathways—for example, the retroelements and the mariner/Tcl elements.

Simultaneous mobilization of transposable elements might be expected if a functional element of one type were able to complement some shared defect in the transposition pathways of the others. This possibility seems unlikely in view of the great diversity of transposition mechanisms employed by the elements. The dysgenically induced insertions of Ulysses, Helena, and Paris all show the sequence characteristics of their respective transposition mechanisms. For example, Helena is structurally a LINE element, and its insertion in sn25 clearly shows signs of retrotransposition of a LINE-like element: the inserted element is truncated at the 5' end and is polyadenylated at the 3' end (Fig. 1b). Paris is structurally a Tcl-like element, and its insertion in sn10 displays the characteristic features of elements in the mariner/Tcl superfamily: the target site is a TA dinucleotide and insertion generates a TA duplication (Fig. 1a). There is no indication that the mobilized copy of Paris transposed through an RNA intermediate, nor is there any evidence that Helena or Ulysses has transposed through a pathway resembling mariner/Tcl transposition.

Simultaneous mobilization would also be expected if strains L160 and B9 differ in several completely different systems of hybrid dysgenesis. This model is not free of difficulties. For example, if the hybrid dysgenesis is of the PM or IR type described in D. melanogaster (8, 9), then the elements mobilized in the dysgenic cross should have a grossly asymmetrical distribution in B9 and L160. Only Penelope is present in L160 but absent in B9 (18). On the other hand, Paris and Helena almost fit the same pattern: both have an asymmetrical distribution with many more copies in L160 than in B9. In principle, assuming that the few copies of Helena and Paris present in strain B9 are nonfunctional, one type of hybrid dysgenesis might mobilize Helena and a different type of hybrid dysgenesis might mobilize Paris. One problem with this model is that hybrid dysgenesis associated with the mariner/Tcl superfamily, of which Paris is a member, has not previously been reported. Ulysses is also a problem because its distribution in L160 and B9 is nearly symmetrical.

The supposition of several systems of hybrid dysgenesis seems less implausible if the repression is mediated through a common process in the host. In higher plants, for example, transgenes are regulated in part by a remarkable mechanism of homology-dependent gene silencing in which high levels of transcription result in posttranscriptional degradation of all species of mRNA sufficiently similar in sequence (25). Because silencing affects mRNA stability and depends only on sequence homology, it could result in simultaneous repression of transposable elements regardless of the mechanism of their transposition. A breakdown of homology-dependent silencing would then lead to simultaneous derepression of unrelated transposable elements. Although there is no evidence for homology-dependent silencing in animals, the formal analogy with repressing "cytotype" (8, 9) is clear. If the cytoplasm of L160 oocytes is considered as "repressive," then the cytoplasm of B9 oocytes may be considered as "permissive." The cytoplasm of B9 females with L160 males therefore yields a nucleus with multiple transposable elements in a background of permissive cytoplasm. However, the reciprocal cross retains the repressive cytoplasm of L160 and so represses mobilization of the transposable elements. The mobilization of Ulysses is a problem for
any model based on different systems of hybrid dysgenesis because *Ulysses* is distributed approximately symmetrically in the parental strains. A conventional hybrid dysgenesis model would have to assume that either (i) all copies of *Ulysses* in strain B9 are also nonfunctional or (ii) *Ulysses* can be mobilized even when both parental strains contain functional copies of the element.

One might also entertain a model for *D. virilis* hybrid dysgenesis in which all four elements (and perhaps others still to be discovered) are mobilized by a sort of "genomic stress" brought about by the dysgenic B9 × L160 cross. The genomic stress could be as simple as double-stranded DNA breakage. Indeed, the stress employed in McClintock’s experiments included chromosome breakage. From her experiments, she concluded that at least two different transposable elements were activated in cells of maize undergoing repeated chromosome breakage induced by the breakage–fusion–bridge cycle (4). Environmental agents associated with the creation of double-stranded breaks, including hydroxyurea and exposure to ultraviolet light, have also been reported to increase transcription and/or mobilization of some retroelements (26–28). Genetic differences may also result in elevated levels of mobilization (6, 7), and one of the important effects of the transposable element P is the production of double-stranded breaks (29). Applied to hybrid dysgenesis in *D. virilis*, these observations suggest that the production of double-stranded breaks from the mobilization of a single transposable element might induce a cellular response that releases other transposable elements from repression. In this manner, a single system of hybrid dysgenesis (possibly controlling one of the elements in Fig. 1) could mobilize multiple unrelated elements.

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