Intrachromosomal rearrangements mediated by \textit{hobo} transposons in \textit{Drosophila melanogaster} (transposable elements/mutations/chromosome rearrangements/recombination)

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ABSTRACT The recurring intrachromosomal rearrangements observed in an unstable X chromosome, designated Uc, of \textit{Drosophila melanogaster} are shown to be mediated by \textit{hobo} transposable elements. Each of 29 chromosome rearrangement breakpoints in 16 gross aberrations detected in the Uc-derived X chromosomes had a \textit{hobo} element. In one particular unstable X chromosome line selected for detailed studies, a \textit{hobo} element was found in each of the five hot spots for rearrangements. Furthermore, \textit{hobo} elements at deletion hot spots were found to lie in the same orientation, whereas those \textit{hobo} elements at inversion hot spots were in the opposite orientation. The restriction maps of two phase \textit{hobo} clones containing rearrangement breakpoints indicated that a \textit{hobo} element was inserted exactly at the breakpoints. Pairing of \textit{hobo} elements in the same chromosome followed by recombination between the paired \textit{hobo} elements is suggested as the explanation for the intrachromosomal aberrations observed in the Uc X chromosomes. A clear qualitative difference among the \textit{hobo} elements in their ability to participate in rearrangement formation was noted. It was also found that each of the 11 recessive lethal mutations mapped in the 6F1-2 doublet had a \textit{hobo} element in the doublet, whereas none of the 16 independent revertants of the mutation had a \textit{hobo} element in the site. This observation indicates that \textit{hobo} movement is responsible for production and subsequent instability of recessive lethal mutations in the 6F region of the Uc X chromosomes.

In \textit{Drosophila melanogaster}, three general classes of transposable elements have been described—elements with long inverted repeats (\textit{FB} elements), \textit{copia}-like elements with long terminal repeats (LTR), and elements with neither LTR nor long inverted repeats flanking the element (refs. 1 and 2). The best known of all transposable elements in \textit{Drosophila}, \textit{P}, is without LTR or long inverted repeats (3). The \textit{hobo} transposable element isolated from the \textit{Sgs} gene of \textit{Drosophila} (4), is similar to \textit{P} element in its size and structure (5). It has been shown to cause genetic instability in \textit{Drosophila} (6, 7).

The unstable X chromosome, designated Uc, exhibits a set of peculiar site-specific chromosome instabilities (8), and involvement of transposable elements has been suggested (8–12). In this report, I show that every one of the chromosome breakpoints in the Uc X chromosome that I have sampled has a \textit{hobo} transposable element. I suggest that intrachromosomal rearrangements in the Uc X chromosome are caused by homologous association of \textit{hobo} elements located at different sites in the chromosome. I also show that unstable recessive lethal mutations in the cytological region 6F of the Uc X chromosome can be explained by insertion of \textit{hobo} transposable elements, and their reversions are correlated with \textit{hobo} excisions.

MATERIALS AND METHODS

Genetic Stocks. The unstable X chromosome used in these experiments was derived from strain described in previous studies (8, 12). It carries an inversion, In(1)3D:6F, along with a small deficiency, Df(1)6D:6F, at one end of the inversion. This deficiency removes the \textit{cm} (carmine eyes) locus, so the entire chromosome is symbolized as Df(1)cm-In. Other unstable X chromosomes with gross chromosome aberrations or lethal mutations in cytological bands 6F1-2 were also derived from previous work (8, 9). Because all of these chromosomes have recessive lethal effects, they were maintained with either the FM6 w, FM6, FM7, or the Df(1)Basc chromosome. X chromosomes that carried reversions of the 6F1-2 lethal mutations were maintained with C(1)DX, y f compound-X females. Complete descriptions of gene symbols and chromosomes can be found in refs. 8 and 12 or Lindsey and Grell (13).

For cytological analysis or \textit{in situ} hybridization, females carrying aberrations or lethal mutations were mated to males carrying the \textit{hobo}-free X chromosome marked with \textit{y}^\text{9th} z. Female larvae with light-brown mouth parts (heterozygous for the gross aberration or the lethal mutations and for \textit{y}^\text{9th} z) were then selected for slide preparation. Males carrying reversions of the 6F1-2 lethals were mated to \textit{hobo}-free Canton S females, and the progeny female larvae were sampled for cytological and \textit{in situ} hybridization studies.

Genomic Library, Subclones, and Molecular Techniques. Genomic DNA was isolated from females of the constitution Df(1)cm-In/FM6 by the method of Bingham et al. (14). The DNA was partially digested with \textit{Sau}3A to yield fragments about 15 kilobase pairs (kbp) long and cloned into EMBL 3 arms (15) by the method of Shrimpton et al. (16). The resulting recombinant phage library was screened for \textit{hobo} sequences by using a central 0.8-kbp EcoRI fragment from EC245 (Fig. 1) as the probe. pUC119 (17) was used for subcloning smaller DNA fragments within the phage clones.

The procedures described by Maniatis et al. (18) were used for molecular cloning and characterization. Packaged single-stranded plasmid DNA and template DNA for base sequencing were prepared as described by McMullen et al. (19). The Sequenase kit (United States Biochemical) was used to prepare the sequencing reactions. Genomic DNA blots (DNA from two or three flies) were prepared by the method of Bender et al. (20) with a minor modification. The protocols for cytological analysis and \textit{in situ} hybridization have been described (12, 21).

RESULTS

Identification of a \textit{hobo} Insertion in the Notch (N) Locus. Previous work (12) established that a derivative of the Uc X chromosome, Df(1)cm-In, produced deficiencies in the Notch locus (cytological location in 3C7) at a high rate.

Abbreviation: LTR, long terminal repeat.
Moreover, one of the breaks in each deficiency was mapped to a 2.2-kbp EcoRI fragment in the Notch locus. This finding prompted the examination of DNA corresponding to the 2.2-kbp EcoRI segment in the Df(1)cm-In chromosome. EcoRI-digested genomic DNA blots of Df(1)cm-In/FM6 females, probed with the 2.2-kbp EcoRI fragment, indicated that an insertion =3 kbp long was present in the EcoRI fragment of the Df(1)cm-In chromosome.

A genomic library from Df(1)cm-In/FM6 was then screened with the 2.2-kbp EcoRI fragment to isolate a clone with the insertion sequence. A map of the resulting clone, designated EC245, is shown in Fig. 1. The restriction sites in this clone were similar to those of the N2 clone of Kidd et al. (22) except for the insertion and two additional, apparently polymorphic, SalI sites. Examination of the restriction sites within the insert in EC245 suggested that it is a hobo element described by Streck et al. (5); more detailed mapping with additional restriction enzymes verified this.

**Association of hobo Elements with Chromosome Aberrations and Recessive Lethal Mutations.** The results of hybridizing Df(1)cm-In/y59b z chromosomes with EC245 indicated that five hobo elements were present in the Df(1)cm-In chromosome. The number of hobo elements in the autosomes was not constant, ranging from one to four for the second chromosome and one to eight for the third chromosome. The average number of hobo elements in the genome was 16, with a range of 9–19. Moreover, each of the two rearrangement breakpoints in the Df(1)cm-In chromosome, 3D/6D and 3D/6F, had a sequence homologous to the hobo element. These results led to the examination of 15 additional aberrations that are related in origin to IIA and IJD lines of the Uc X chromosome (8). Table 1 shows a summary of the results from the in situ hybridization of 63 slides. The most remarkable feature of the results shown in Table 1 is that a hobo element was present in every one of the rearrangement breakpoints.

Examples of the in situ labeling of some of these are shown in Fig. 2. A few asynapsed X chromosomes, such as the ones shown in Fig. 2, were observed in most of the slides. This permitted the rearranged (Uc) and nonrearranged (y59b) chromosomes to be distinguished in each of the asynapsed complexes containing the hobo element. Each insert is represented by a horizontal line with the position of the hobo sequence indicated by a thick line. The direction of hobo transcription is shown by a long arrow. All insertions, except EC296, are oriented so that the centromere is at their left and the telomere is at their right. The orientation of EC296 has not been determined. The site marked with an asterisk is a tentative placement.

### Table 1. Distribution of hobo elements in the Uc-derived X chromosomes with gross rearrangements

<table>
<thead>
<tr>
<th>Aberration(s)</th>
<th>No. of larvae sample</th>
<th>Cytological loci with hobo</th>
</tr>
</thead>
<tbody>
<tr>
<td>In(1)6F;6E*</td>
<td>1</td>
<td>4E, 6A, 6F/8E, 7E, 7C, 6F/8E, 9A, 9C, 11A, 13B, 13D, 15D, 16A, 16F</td>
</tr>
<tr>
<td>Df(1)6F1-2</td>
<td>2</td>
<td>6F/6F, 10B</td>
</tr>
<tr>
<td>Df(1)6D;6F</td>
<td>4</td>
<td>6D/6F, 17A</td>
</tr>
<tr>
<td>In(1)3D;6F</td>
<td>3</td>
<td>3D/6F, 3D/6F, 7C, 16F, 19A</td>
</tr>
<tr>
<td>In(1)6F;7C</td>
<td>3</td>
<td>4E, 6F/7C, 6F/7C, 7E, 9E, 10B</td>
</tr>
<tr>
<td>In(1)6A;7C</td>
<td>3</td>
<td>6A/7C, 6A/7C</td>
</tr>
<tr>
<td>In(1)4D;6F</td>
<td>2</td>
<td>4D/6F, 4D/6F, 8A, 9A</td>
</tr>
<tr>
<td>In(1)9F;11E</td>
<td>2</td>
<td>6F, 7D, 8E, 9A, 9F/11E, 9F/11E</td>
</tr>
<tr>
<td>In(1)3D/6F plus Df(1)6F;7C</td>
<td>1</td>
<td>3D/6F, 3D/7C, 16F, 19A</td>
</tr>
<tr>
<td>Tp(1)6F;7C to 3E</td>
<td>11</td>
<td>3E/6F, 7C/3E, 6F/7C, 13B, 16F, 17A</td>
</tr>
<tr>
<td>Df(1)cm-In+</td>
<td>8</td>
<td>3C, 3D/6D, 4E, 3D/6F, 9A</td>
</tr>
<tr>
<td>Df(1)cm-In+si</td>
<td>5</td>
<td>3C, 3D, 6D/6F, 7A, 9A</td>
</tr>
<tr>
<td>In(1)3D;9A plus Df(1)6D;6F+</td>
<td>3</td>
<td>3D/9A, 6D/6D, 5C, 3D/9A, 18C, 19A</td>
</tr>
<tr>
<td>Complex 1§</td>
<td>10</td>
<td>3D/6D, 3D/19A, 7C/19A</td>
</tr>
<tr>
<td>Complex 2§</td>
<td>1</td>
<td>6A, 6F/19E, 19A, 11A/6F, 8E, 9C, 11A/19E</td>
</tr>
<tr>
<td>Complex 3§</td>
<td>4</td>
<td>4C, 6D/6F, 4E/18A, 17A, 9A, 7C/18A</td>
</tr>
</tbody>
</table>

*In(1)6F;8E is nonlethal, and Df(1)cm-In+§ represents a rearrangement of In(1)3D/6F to the normal sequence.

†See Fig. 3 for the relationship of these rearrangements.

§Complex 1 = tip—3D/6D—3D/19A—7C/19A—base.

‡Complex 2 = tip—6F/19E—11A/6F—11A/19E—base.

§Complex 3 = tip—6D/6F—4E/18A—7C/18A—base.
and mapped. The resulting restriction maps are shown in Fig. 1. All of the clones, except EC296 from 4E, can be oriented as they exist in the Df(1)cm-In chromosome. With the centromere of the chromosome placed at the right, the hobo elements at 3C (Notch) and at the 3D/6D breakpoint are in orientation 1 (HindIII site of the element is to the left of the element’s central point). The hobo elements at 9A and at the 3D/6F breakpoint are in orientation 2 (opposite in relation to orientation 1).

The following statements summarize the manner by which the orientation of hobo elements was determined in the Df(1)cm-In chromosome. (i) EC245 is the clone of hobo in the Notch locus. The orientation of the element as it exists in the locus can be determined from the restriction map of the Notch region. (ii) Hybridization of EC312 onto the hobo-free Canton S chromosomes resulted in a strong label at 6D. This result indicated that the longer of the two flanking sequences corresponds to the 6D region of the 3D/6D breakpoint. (iii) EC296 labeled the 4E region of the Canton S chromosome only. However, the element in this clone cannot be oriented because neither a suitable aberration involving the hobo element at this site nor restriction map data for the normal 4E region are available. (iv) Hybridization of EC291 onto Canton S chromosomes showed a strong label at 6F and a weak label at 3D, indicating correspondence of the 6F region with the longer flanking sequence and the 3D region with the shorter flanking sequence in this clone. (v) Hybridizing the Canton S chromosomes in situ with EC294 indicated that this clone is from 9A. Its orientation was determined by hybridizing it with the In(1)3D:9A + Df(1)6D:6F chromosome derived from Df(1)cm-In (see Fig. 3). The proximal 3D:9A breakpoint showed at least 15 silver grains, whereas the distal breakpoint had only 4 or 5 silver grains. These results suggest that the longer of the two flanking sequences in this clone is homologous to the 9A region in the proximal breakpoint of the In(1)3D:9A.

In addition to the five X-linked hobo clones, four autosomal clones (EC300, EC284, EC282, and EC278), each containing one hobo element, were isolated. The genomic locations of these elements were determined by in situ hybridization of the cloned DNA to the chromosomes of the Canton S strain, which lacks hobo elements.

The sizes of all nine cloned hobo elements were determined by electrophoresis and blotting of restriction enzyme digests alongside the digests of pH108 hobo clone of Streck et al. (5). The hobo elements in the EC clones were very similar, if not identical, to each other. The results of the experiment with EcoRI are shown in Fig. 4. The central 0.85-kbp EcoRI fragment in pH108 is about 100 bp longer than the corresponding fragments in the nine EC clones. The size difference was traced to a 170-bp segment, bounded by the EcoRI and HindIII sites, containing the S repeat region of Streck et al. (5). A total of 186 nucleotides (including the S repeat region) in EC245 was sequenced. The results indicate that the 186 nucleotides correspond to base positions 1772-2059 of the hobo sequence determined by Streck et al. (5), with 0 mismatch and with 63 unmatchted nucleotides. The unmatched nucleotides were represented by seven repeats of ACTCCAGAA (S repeats).

Rapid Changes in Chromosome Structure and hobo Distribution. Previous studies (8, 9) have shown that Uc X chromosome undergoes rapid restructuring. One case of restructuring involving the Df(1)cm-In chromosome was examined here (Fig. 3). The origin of the Df(1)cm-In chromosome is shown in the upper part of the figure. The Uc X chromosome with normal sequence has undergone two rearrangements, each involving two breaks; the first rearrangement is an inversion with the breakpoints at 3D and 6F, and the second rearrangement is a deletion from 6D to 6F at the distal 3D/6F breakpoint. These changes can be

Fig. 2. In situ hybridization of polytene chromosomes with EC245 probe. The chromosomes were analyzed and photographed with bright field optics with a neutral filter. Panels are identified in the lower left corner. (A) Salivary gland chromosomes of In(1)4D:6F, y<sup>90</sup> z female hybridized with EC245 probe. No label is apparent in the nonrearranged chromosome (y<sup>90</sup> z). The 3C label, at the lower right corner of A, represents hybridization between the 3C unique sequence in the probe (EC245) and the complementary sequence in the Notch locus in both chromosomes. (B) Salivary gland chromosome of In(1)6F;7C chromosome (from the 5D to 8C region) asynapsed from y<sup>90</sup> z chromosome. (C) Salivary gland chromosome of Tp(1)6F;7C to 3E, asynapsed from y<sup>90</sup> z chromosome. A short horizontal bar in each panel represents about 10 μm. The period of exposure was 6 days.

figures, the y<sup>90</sup> z chromosome was free of label except in 3C, indicating that it did not carry any hobo elements.

One of the characteristics of Uc X chromosomes is independent recurrence of cytologically identical rearrangements (8, 9, 11, 12). This situation made it difficult to determine the precise number of unique breakpoints that were analyzed. Assuming that the cytologically indistinguishable breakpoints had a common origin, the minimum number of unique breakpoints observed in this study would be 29, and a hobo element was present in every one of these breakpoints.

A total of 11 chromosomes with 6F lethal mutations that are not associated with aberrations and 16 with independent reversions of these lethal mutations were analyzed by in situ hybridization using EC245 as the probe. All of the 6F lethal mutations had label in the 6F1-2 doublet, but the 16 revertants had no label. These results suggest that the 6F lethal mutations were associated with the insertion of a hobo element, which excised when the lethal mutation reverted.

hobo Clones from Df(1)cm-In Genomic Library. The five hobo elements in the Df(1)cm-In chromosome were isolated
accounted for by intrachromosomal pairing and exchange between hobo elements at these sites: opposite orientation of hobo elements at 3D and 6F would form an inversion, and the hobo elements oriented in the same direction at the 3D/6F breakpoint and at 6D would produce Df(1)6D;6F.

Cytological analysis indicated that the Df(1)cm-In chromosome produces further structural aberrations, each involving two breaks. This is one of the characteristics of the Uc X chromosome that has been well documented (9, 12). Thus, the In(1)3D;6F reverts to yield an X chromosome with a deficiency extending from 6F to 6D, which is indicated in Fig. 3, line 5, as Df(1)cm-In\textsuperscript{rei}. The 3D and 9A regions in the Df(1)cm-In\textsuperscript{rei}, in turn, are involved in producing a new inversion, In(1)3D;9A, so that the resulting chromosome will have two simple aberrations: In(1)3D;9A and Df(1)6D;6F (sixth line in Fig. 3). The Df(1)cm-In also produces In(1)3D;9A, giving rise to Df(1)cm-In + In(1)3D;9A (Fig. 3, line 7). Finally, the Df(1)cm-In produces another deficiency involving the Notch locus, Df(1)N, resulting in Df(1)cm-In + Df(1)N (Fig. 3, bottom line).

Df(1)cm-In and two of its derivatives, Df(1)cm-In\textsuperscript{rei} and In(1)3D;9A + Df(1)6D;6F, were hybridized in situ with EC245 and EC294. The latter is a hobo clone with 9A flanking sequences. The results indicated that the reversion of In(1)3D;6F was accompanied by the loss of one hobo element at 4E and the appearance of a hobo element at 7A in Df(1)cm-In\textsuperscript{rei}. The restructuring of Df(1)cm-In\textsuperscript{rei} into In(1)3D;9A + Df(1)6D;6F was associated with the loss of elements at 3C and 7A and the appearance of hobo elements at four new sites (5C, 8E, 18C, and 19A). It is important to note that the Df(1)cm-In\textsuperscript{rei} chromosome had hobo elements at 3D and 9A before generating In(1)3D;9A (see Table 1). The chromosome restructuring and loss—gain of hobo elements may be independent events. However, both of these events can take place during a short period of time, since the restructuring of Df(1)cm-In\textsuperscript{rei} into In(1)3D;9A + Df(1)6D;6F took place during one generation.

**DISCUSSION**

Examination of hobo elements in the Df(1)cm-In chromosome and the additional aberrations generated in this chromosome suggests a mechanism for generating the intrachromosomal pairing and exchange between hobo elements at these sites: opposite orientation of hobo elements at 3D and 6F would form an inversion, and the hobo elements oriented in the same direction at the 3D/6F breakpoint and at 6D would produce Df(1)6D;6F.

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mosomal rearrangements observed in the Uc X chromosome. All rearrangements can be accounted for by intrachromosomal pairwise exchange between hobo elements as indicated by the following observations: (i) production of each additional aberration involves two hobo sites, and (ii) the hobo pair involved in each event is in a specific orientation, deleting a region bounded by a hobo pair in the same orientation (3C and 3D hobo) and inverting a region bounded by a hobo pair in opposite orientation (3D/6F with 3D/6F, 3D/6D with 9A and 3D with 9A).

A hobo transposable element characterized by Streck et al. (5) is a 3-kbp element. It has 12-bp inverted repeats and is flanked by an 8-bp duplication of the target DNA sequence. However, the constitution of a functional hobo element has not been defined. A unique structural feature of hobo element is its tandem repeats of ACTTCCAGAA (S repeats) which lies within its open reading frame. The functional significance of the repeats, however, is not known.

The proposed mechanism for the restructuring of the Uc X chromosome is based on the synaptic pairing between hobo elements in which the elements provide the necessary DNA sequence homology for synaptic association. One crucial requirement for synaptic pairing is the proper orientation of the participating element pair. Two elements in a chromosome in the same proximal-to-distal orientation pair to form a loop configuration, and recombination between them will result in a deletion. Pairing between two elements in the opposite orientation results in a bend back configuration of the chromosome, and recombination between them will produce an inversion. In the Df(1)cm-In chromosome, the deletion hot spots are marked by hobo elements oriented in the same direction (3C and 3D/6D breakpoint). No inversion of this interval was observed. The hobo pairs in the inversion hot spots in the same chromosome (3D with 6F and 3D with 9A) are in the opposite orientation. Deletions involving the 3D-6F and 3D-9A regions have not been observed, even though repeated inversions involving these sites have been observed. However, it is possible that these deletions have a dominant lethal effect, precluding detection in these studies.

As shown in Fig. 3, the Df(1)cm-In chromosome is related to six independent two-break restructuring events. The hobo at cytological position 3D (or at the 3D/6D breakpoint) is involved in every one of these events. The hobo at 3D in the Df(1)cm-In chromosome is the only active element (the element which serves as an initiator of the hobo interaction) in this chromosome, and the remaining four hobo elements are passive elements. Each of the passive elements can interact with the active element at 3D.

The qualitative difference among the hobo elements in the Df(1)cm-In chromosome is also suggested by absence of inversions involving 3C hobo. Since the 3C and 3D hobo elements are structurally similar elements, are close together in the same chromosome, and are oriented in the same direction, inversions involving 3C hobo should be as frequent as those inversions in which 3D hobo is involved. However, inversions with one break in 3C have not been observed in this chromosome. This observation supports the idea about possible existence of qualitative difference among the hobo elements.

The active role played by the 3D hobo in the Df(1)cm-In chromosome is one of many examples of the nonrandom participation of hobo elements (breakpoints) in restructuring the chromosome. Examination of previously published data on the restructuring of the Uc X chromosome (see the lineage I–IV in ref. 9) also suggests the presence of one active breakpoint (hobo at 6F) that is repeatedly involved in restructuring the X chromosome.

The intrachromosomal recombinant model proposed here is consistent with the topological isolation of chromosome arms (23). The presence of active element(s) in one chromosome together with the isolation of chromosome arms explains the observation that restructuring is confined to the chromosome arm carrying the active element. Perhaps this is the reason why the X chromosome is the only unstable chromosome in the Uc stocks.

Intrachromosomal interactions of hobo elements are not the only source of instability in the Uc X chromosome. Although little is known, excision and transposition (insertion) do take place in this X chromosome. The loss of hobo elements will most likely result in stabilization of the chromosome. Perhaps this is the reason for the observed stabilization of previously unstable chromosomes (24, 25).

It has been demonstrated that reciprocal duplication and deficiency products can be generated by asymmetrical pairing and recombination between a transposable element (26, 27), and intrachromosomal recombination mediated by transposable elements has been suggested as a possible mechanism for chromosome restructuring (6, 9). Much needs to be learned, however, before we can assess the impact of transposable elements on a genome.

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