Interacting hobo Transposons in an Inbred Strain and Interaction Regulation in Hybrids of Drosophila melanogaster

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ABSTRACT

A transposable hobo element in the Notch locus of the Uc-1 X chromosome, which does not interfere with the normal expression of the locus, interacts with other hobo elements in the same X chromosome to produce Notch mutations. Almost all of these mutations are associated with deficiencies, inversions or other rearrangements, and hobo elements are present at each of the breakpoints. The Uc-1 X chromosome produces the Notch mutations at a rate of 4–8% in both sexes of flies in a strain that has been inbred for 96 generations. At least two-thirds of the mutations are produced in clusters suggesting that they have originated in mitotic (premeiotic) germ cells of the Uc-1 inbred strain. The interaction of hobo elements in the Uc-1 X chromosome can be repressed by at least two different mechanisms. One found in three inbred strains not related to the Uc-I strain involves a maternal effect that is not attributable to the actions or products of hobo elements. Repression by this mechanism is manifested by a clear reciprocal cross effect so that the production of Notch mutations is repressed in the daughters of Uc-I males, but not in the daughters of Uc-I females. The other mechanism apparently requires genetic factors and/or hobo elements in a particular strain of Oregon-R; complete repression is present in both types of hybrids between Uc-I and this strain.

The instability of the Uc X chromosome is manifested by its ability to produce X-linked recessive lethal mutations at a high rate, frequent reversion of the lethal mutations, accumulation of structural rearrangements confined to the X chromosome, and reinversions of existing inversions (LIM 1979, 1981a). In addition, simple rearrangements in this X chromosome, such as inversions and deletions, have a tendency to become more complex and existing rearrangement breakpoints are usually involved in the process (LIM 1981a). The Uc X chromosome produces attached-X chromosomes in male germ cells (LIM 1981b; MORRISON et al. 1988), and apparent transposition of an unstable site within the Uc X chromosome has also been documented (LAVERTY and LIM 1982). All of these studies suggested transposable elements as the causative agents for the genetic instability in the Uc X chromosome. However, for technical reasons, our recent studies have focused on a derivative of the original Uc called Df(1)cm-In (JOHNSON-SCHLITZ and LIM 1987; LIM 1988).

Cytogenetic analysis of the Df(1)cm-In chromosome showed recurring site-specific deletions involving the Notch locus in this chromosome. This observation suggested the involvement of insertion sequences in the process of mutation and rearrangement formation (JOHNSON-SCHLITZ and LIM 1987). Additional studies with in situ hybridization and molecular analysis showed that rearrangements in the Df(1)cm-In chromosome can be accounted for by intrachromosomal

The remarkable advances that had taken place during the last decade in all aspects of Drosophila genetics depended heavily on the P element-mediated transformation technique pioneered by RUBIN and SPRADLING (1982) and SPRADLING and RUBIN (1982). The establishment of this technique in the early 1980s was a timely one, since another new tool, recombinant DNA technology, had become widely appreciated and practiced. However, the studies that led to the development of P element-mediated transformation had a modest objective: to define the conditions necessary for mobilization and repression of P element activity (KIDWELL, KIDWELL and SYED 1977; ENGELS 1979a, 1989). Subsequent studies, some using the transformation technique, have provided many insights into the nature of P element regulation (O’HARE and RUBIN 1983; KAESS and RUBIN 1984; LASKI, RIO and RUBIN 1986), but a full understanding of the phenomenon has not yet been achieved. Information on the regulation of other transposable elements in Drosophila is even more incomplete.

Our studies of the hobo transposable elements began with the discovery of a mutable X chromosome designated Uc. for unstable chromosome. The instability of this chromosome has persisted in an inbred genetic background from its discovery in 1978 (LIM 1979). We dedicate this paper to the memory of the late Professor LEON A. SYNDER.

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recombinations mediated by hobo transposable elements. Furthermore, the studies showed that the type of rearrangements produced were dependent on the orientation of the elements involved in the recombination events (LIM 1988). The results from molecular analysis of recessive lethal mutations localized in the cm-Sxl region (6E-7A) of the X chromosomes, derived from the Uc stock, support the idea that rearrangements in Uc and its derivatives are generated through orientation-dependent intrachromosomal recombination mediated by hobo transposons (SHEEN 1990; SHEEN, LIM and SIMMONS 1993).

The transposable element hobo of Drosophila melanogaster was first isolated as a 1.3-kb insert in the Sgs-4 glue protein gene (MCGINNIS, SHERMOEN and BECKENDORF 1983). The results of a systematic search and molecular analysis indicated that a family of 3.0-kb elements and their deletion derivatives homologous to the 1.3-kb element are present in many strains of D. melanogaster, Drosophila simulans and Drosophila mauritiana (STREECK, MACGAFFEY and BECKENDORF 1986). The nucleotide sequence of one of the 3.0-kb elements, designated hobo108, indicated that the entire element is 3,016 bp. It had 12-bp inverted terminal repeats, and a truncated copy of the terminal repeats referred to as short (S) repeats near the center of the element, and two tandem copies of 20 bp (GAG-TATTTTTGGAAACCC) known as long (L) repeats about 240 bp upstream from the 3' end. In addition to these repeats are 10 perfect and 5 degenerate copies of 9-bp (ACTCCAGAA) tandem repeats referred to as perfect (P) repeats that are not present in hobo108. The sequence analysis of 186 nucleotides in the region bounded by the EcoRI and HindIII sites of the hobo element in the EC245 clone indicated that the element has only 3 perfect S repeats rather than 10 such repeats found in hobo108 (LIM 1988). The nucleotide sequence analysis of another hobo element, HFL1, has shown that this element also has only 3 perfect S repeats, but it also has 6 nucleotides that are not present in hobo108 (CALVI et al. 1991).

The recurrence of hobo-mediated rearrangements in the Uc X chromosome and its derivatives in an inbred genetic background suggested that a regulatory mechanism for hobo activity may be quite different from that known for P elements. To learn about the mechanisms that regulate interaction of hobo transposons, we focused our attention on the production of hobo-mediated rearrangements in a derivative of Df(1)cem-In called Uc-1 in inbred and in hybrid backgrounds. We first estimated the Notch mutation rates in the Uc-1 X chromosome in a strain that has been inbred for 96 generations. The association of Notch mutations with chromosome rearrangements involving a hobo element in the Notch locus was established through cytological analysis of the mutations and in situ hybridization of rearranged chromosomes. Mutations produced by individual females and males carrying the Uc-1 X chromosome suggested that the rearrangement-associated mutations were produced in premeiotic (mitotic) germ cells. The effect of genomes not related to the Uc-1 in the F1 females suggested the presence of two distinct repression mechanisms. One of these mechanisms is attributable to putative maternally expressed genes in three inbred strains not related to the Uc-1 stock; the hobo activity is repressed in the hybrid daughters of Uc-1 males, but not in the hybrids of Uc-1 females. The second mechanism is apparently associated with genetic factors and/or hobo elements in a particular Oregon-R strain; the Uc-1 X hobo activity in the hybrid daughters of both Oregon-R males and females is completely repressed.

MATERIALS AND METHODS

Genetic stocks: All stocks and experimental cultures were raised at 22–25°C in eight dram shell vials or half-pint milk bottles on a standard cornmeal-molasses-agar medium. Additional information about the chromosomes and genetic markers in the stocks can be found in LINDSEY and ZIMM (1992).

E (empty) strains: These strains do not have detectable hobo elements as determined by genomic Southern analysis and by in situ hybridization of polypytene chromosomes with hobo sequence probes. Strains are as follows. (a) Canton S (abbreviated CS), a wild-type laboratory stock, obtained from the Bowling Green Stock Center in the 1960s. (b) Basc, an X chromosome balancer stock with the X-linked markers B (Bar eyes) and w+ (white-apricot eyes); it was obtained from the Bowling Green Stock Center in the 1960s. (c) y z spi snl (abbreviated spl), an inbred strain with an X chromosome homozygous for four recessive markers: yellow (y at 0.0), zeste (z at 1.0), split (spl at 3.0), and singed (sn at 21.0). This stock was obtained from B. H. JUDD who synthesized it in the 1970s.

CH (for complete hobo) strains: These contain only complete 2.95-kb hobo elements as determined by genomic Southern analysis. Strains are as follows. (a) Uc-1, y w+. (b) Uc-1, y w+ (abbreviated UC-1). The X chromosome in this stock, called Uc-1, carries three recessive markers: y+, z, and w+. (c) Complete hobo elements at cytological positions 3C7 (within the Notch locus, but without an effect on Notch function) and 3D. A previous study (LIM 1988) indicated that these two hobo elements are oriented in the same direction and that interaction between them caused deletion mutations of the Notch locus. The Uc-1 X chromosome was derived from a rearranged unstable X chromosome called Df(1)cem-In (see Figure 1; JOHNSON-SCHLITZ and LIM 1987; LIM 1988). Sublines of a homozygous Uc-1 stock were maintained by sib or single pair matings and were regularly checked for the production of hobo-mediated Notch mutations. Only non-Notch females were used to propagate the sublines. (b) FM6, 69Y/Df(1)Basc/sexY (abbreviated 69Y/DB), an X chromosome balancer stock. FM6, 69Y is an FM6 balancer X chromosome that carries an unlocalized EMS-induced recessive lethal mutation, l(1)69Y. Df(1)Basc is a balancer X chromosome with a lethal terminal deletion that is complemented by the
for 15 to 18 generations after establishing the sublines.

**Nomenclature and terminology:** Notch mutability was determined in the hybrid daughters and granddaughters of different crosses. The hybrid daughters from crosses using Uc-1 flies will be referred to as the cross A F₁ females. The hybrid daughters from the reciprocal crosses using Uc-1 females will be designated as cross B F₁ females. The hybrid granddaughters produced by these F₁ females will be referred to as cross A F₂ females and cross B F₂ females, respectively.

For the F₁ hybrids, the genotype or designation of the female parent will always be indicated first. Thus, CS/Uc-1 represents the genotype of F₁ hybrids from the cross of CS females with Uc-1 males; F₁ hybrids from the reciprocal cross will be designated Uc-1/CS. All individuals in a group of progeny from a single pair mating will be referred to as a progeny group.

**Mating schemes:** The mating schemes for experimental crosses were designed to estimate the rate of Notch mutations produced by the hobo elements in the Uc-1 X chromosome. Because the Notch mutations are generated in mitotic (premeiotic) Uc-1 germ cells, all experimental matings were performed with individual pairs of flies. The female progeny from each cross were scored for Notch mutations on days 14 and 19 after the crosses were made.

The F₁ hybrid females were produced by successively mating individual Uc-1 flies with flies from three or four different strains, as long as the progeny from these matings were phenotypically distinguishable. For example, Uc-1 females were mated individually with CS males (producing wild-type F₁ females); then the same Uc-1 females were mated with sbl males (producing yellow and zeste F₁ females), and finally with Basic males (producing white-apricot and semi-Bar F₁ females). Uc-1 males were also mated with three to four different types of females. This procedure was practiced to minimize differences attributable to parentage among the crosses. The serial matings were done in different orders to avoid any effect that may be attributable to the age of the Uc-1 flies.

The F₁ females were produced by mating normal-winged F₁ virgin females, from at least 100 F₁ progeny groups, individually with FM7 males.

**Cytological analysis and in situ hybridization:** The detailed protocols for cytological analysis and in situ hybridization with tritiated probes can be found in LIM and SNYDER (1968) and in JOHNSON-SCHLITZ and LIM (1987), respectively. A detailed protocol for in situ hybridization with biotinylated probes is available upon request. Probes were made from the A phage clone EC296 (LIM 1988). This clone contains a 2.95-kb hobo element and about 10 kb of DNA from cytological position 4E-F of the X chromosome.

**Genomic DNA/Southern analysis:** With minor modifications, the method of BENDER, SPIERER and HOGNESS (1983) was used for hybridization analysis of genomic DNA. Genomic DNA isolated from four females from each strain (eight males from FM7) was digested to completion with XhoI which cuts near the ends of hobo elements. The restriction fragments were separated electrophoretically in 0.7% agarose gels, transferred onto Genescreen plus filters (NEN Research Products) and hybridized with nick-translated hobo element probes. The probes were prepared from either the 2.6-kb XhoI fragment or the 0.9-kb EcoRI fragment from the complete hobo element in the clone EC296 (LIM 1988).

The genomic DNA of all strains used in the studies was analyzed at least three times, and the results of repeated analyses were consistent.

**Data and statistical analysis:** The unweighted mutation rate of ENGELS (1979b) was estimated from the data col-
Figure 2.—Southern hybridization analysis of seven inbred strains with hobo element probes. Panel A shows the results of hybridization with a 2.6-kb XhoI fragment of hobo from a λ clone, EC296 (LIM 1988). Panel B shows the results of hybridization with a 0.9-kb EcoRI fragment of hobo from EC296. The fragment sizes in kilobases are shown at the right.

RESULTS

Southern analysis of the experimental strains: The results of genomic DNA Southern blot analyses are shown in Figure 2. We failed to detect hobo element sequences in three long-established laboratory strains, CS, Basc and spl. However, hobo elements were seen in the other strains. The XhoI-digested genomic DNA blot of Uc-1 and 69a/DB had a 2.6-kb band only. This 2.6-kb band was interpreted to be the canonical 2.95-kb complete hobo element with its XhoI sites at 286 bp and 110 bp from the 5' and 3' termini, respectively. Since the 2.95-kb hobo element is widely accepted as a complete element (STRECK, MACGAFFEY and BECKENDORF 1986; LIM 1988; BLACKMAN and GELBART 1989; CALVI et al. 1991), these two strains were designated as CH (complete hobo) strains.

Both OR and FM7 had complete and defective hobo elements in their genome. In the OR strain, the defective hobo elements were 1.9 kb long, and judging from the intensity of the bands, they were several times more numerous than the 2.95-kb elements. These 1.9-kb defective elements may correspond to a particular defective hobo element called Th (PERIQUET et al. 1989). Three distinct classes of hobo elements (2.95-, 1.9- and 1.7-kb elements corresponding to 2.6-, 1.5- and 1.3-kb bands in the XhoI-digested DNA blot, respectively) were present in the FM7 genome. The intensity of the bands indicated that the most abundant was the 1.9-kb class, and the least abundant was the 1.7-kb class. The deleted region in the 1.7-kb elements included the central 0.9-kb EcoRI fragment. This was indicated by the failure of the 1.3-kb band to hybridize with the central 0.9-kb EcoRI fragment of the hobo element. The OR and FM7 strains will be referred to as CDH (complete and defective hobo) strains.

Notch mutations in the Uc-1 X chromosomes: The following three sections summarize the occurrence of Notch mutations in females of the inbred Uc-1 stock, and in the F1 and the F2 females that carried the Uc-1 X chromosome.

Mutations in the inbred Uc-1 stock: Notch mutations in the Uc-1 stock were tallied for 56 generations of pair matings, including generations 1–6 and generations 47–96. Altogether 6,330 progeny groups were tallied with an average of 30.5 daughters in each. Among these were 3,137 progeny groups that had at least one Notch daughter, including 2,028 progeny groups that had more than one. Seventy-five of these progeny groups had more than half of the daughters with Notch mutations, and eight of these progeny groups had all daughters with Notch mutations. Occurrence of these large clusters of mutations suggested that most of the mutations in the Uc-1 stock were produced in the mitotic (premeiotic) germ cells. A total of 193,181 females were tallied in the 6,330 progeny groups and 10,755 of these had Notch mutations. The mutation rates ranged from 2.30 ± 0.60% in G88 to 11.89 ± 1.78% in G75, and the mean was 6.06 ± 0.15%. This fivefold difference in the range may represent the instability of the locus due to apparent mobility of hobo transposons in the stock. Table 1 summarizes the Notch mutations that were detected in G91–G96 and in G91–G96, and Figure 3 shows the distribution of mutation rates compiled from all 56 generations. The mutation rates fluctuated around 6% but there was no obvious trend over time. The data conclusively demonstrate that Notch mutability is a recurrent property of the Uc-1 stock.

Production of Notch mutations in the Uc-1 X chromosome is apparently a complex process that reflects hobo activity in the Notch locus as well as many uncontrollable variables and conditions yet to be defined and identified. The wide range of mutation rates within the Uc-1 inbred stock itself need to be taken
TABLE 1

Notch mutations detected in a strain homozygous for the Uc-I X chromosome

<table>
<thead>
<tr>
<th>Generation</th>
<th>Tested</th>
<th>( \geq 1^a )</th>
<th>( &gt; 1^b )</th>
<th>No. of daughters</th>
<th>No. notches</th>
<th>Mutation rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>G_1</td>
<td>140</td>
<td>77</td>
<td>47</td>
<td>6,133</td>
<td>276</td>
<td>4.54 ± 0.76</td>
</tr>
<tr>
<td>G_2</td>
<td>115</td>
<td>60</td>
<td>59</td>
<td>3,210</td>
<td>195</td>
<td>7.03 ± 1.28</td>
</tr>
<tr>
<td>G_3</td>
<td>119</td>
<td>51</td>
<td>50</td>
<td>2,460</td>
<td>160</td>
<td>7.42 ± 1.60</td>
</tr>
<tr>
<td>G_4</td>
<td>118</td>
<td>54</td>
<td>50</td>
<td>2,197</td>
<td>153</td>
<td>6.08 ± 1.06</td>
</tr>
<tr>
<td>G_5</td>
<td>133</td>
<td>56</td>
<td>20</td>
<td>9,723</td>
<td>169</td>
<td>6.35 ± 1.35</td>
</tr>
<tr>
<td>G_6</td>
<td>120</td>
<td>32</td>
<td>15</td>
<td>1,618</td>
<td>60</td>
<td>4.65 ± 0.94</td>
</tr>
<tr>
<td>G_{20}</td>
<td>115</td>
<td>57</td>
<td>35</td>
<td>2,456</td>
<td>141</td>
<td>6.24 ± 1.07</td>
</tr>
<tr>
<td>G_{42}</td>
<td>115</td>
<td>63</td>
<td>39</td>
<td>2,661</td>
<td>165</td>
<td>7.00 ± 1.15</td>
</tr>
<tr>
<td>G_{95}</td>
<td>93</td>
<td>47</td>
<td>30</td>
<td>1,929</td>
<td>158</td>
<td>8.18 ± 1.69</td>
</tr>
<tr>
<td>G_{94}</td>
<td>94</td>
<td>28</td>
<td>16</td>
<td>1,660</td>
<td>104</td>
<td>5.11 ± 1.29</td>
</tr>
<tr>
<td>G_{16}</td>
<td>106</td>
<td>49</td>
<td>30</td>
<td>2,492</td>
<td>172</td>
<td>6.38 ± 1.18</td>
</tr>
<tr>
<td>G_{29}</td>
<td>84</td>
<td>28</td>
<td>17</td>
<td>1,555</td>
<td>67</td>
<td>5.40 ± 1.36</td>
</tr>
<tr>
<td>Total (^c)</td>
<td>6,330</td>
<td>3,137</td>
<td>2,028</td>
<td>193,181</td>
<td>10,755</td>
<td>6.06 ± 0.15</td>
</tr>
</tbody>
</table>

\(^{a}\) Number of pair matings with one or more Notch daughters.

\(^{b}\) Number of pair matings with more than one Notch daughter.

\(^{c}\) Total based on the data from 56 generations (generations 1–6 and generations 47–56).

FIGURE 3.—Distribution of mutation rates estimated for the Uc-I strain during 56 generations of study. The rates were tallied in 11 groups shown on the abscissa. The lowest rate was 2.30%, the highest rate was 11.89%, and the mean was 6.06 ± 0.15%.

into account in interpreting the data involving the hybrids and their progeny.

Mutations in the F_1 hybrids: The data on the Notch mutations detected in the Uc-I F_1 hybrids are summarized in Table 2. In this table, the even-numbered crosses represent the cross A F_1 hybrids, the daughters of Uc-I males, and the odd-numbered crosses represent the cross B F_1 hybrids, the daughters of Uc-I females. Notch mutations were detected at a high rate among the F_1 daughters of all three major classes of crosses (Uc-I × E, Uc-I × CH, and Uc-I × CDH) and in both cross A and cross B. The presence of Notch daughters in both cross A and cross B F_1 daughters indicates that mutations occurred in the germ cells of both sexes of Uc-I parents. As in the inbred strain itself, a majority of the mutations appeared in clusters, indicating a premeiotic origin.

There was considerable variability among the mutation rates estimated from the cross A F_1 data, and the rates ranged from 1.23 ± 0.22% for the daughters of CS females to 10.25 ± 0.82% for the daughters of spl females. Pair-wise statistical comparisons indicated that 1.23 ± 0.22% was significantly lower (\( P < 0.001 \)) than the rates for the crosses involving the other strains (Basc, spl, 69a/DB and OR). Although the rate of 10.25 ± 0.82% observed among the daughters of spl females was significantly higher than that observed among the daughters of Basc (\( P < 0.001 \)) and OR females (\( P < 0.01 \)), we note that all rates, except that for the daughters of CS females, were within the range of rates seen in the Uc-I inbred strain itself. No statistical differences were seen in the other pairwise comparisons (spl vs. 69a/DB, 69a/DB vs. Basc, 69a/DB vs. OR, and Basc vs. OR). The average value of 5.97 ± 0.31% based on all cross A F_1 hybrids, including those from the CS daughters, was comparable to the mutation rate estimated from the inbred Uc-I stock itself (6.06 ± 0.15%). The average mutation rate for the cross A F_1 hybrids was 8.03 ± 0.43% when the rate was calculated without the daughters of the CS females; this rate was also well within the range of the rates for the inbred Uc-I stock itself.

Among the cross B F_1 hybrids, the mutation rates were less variable than those observed among the cross A F_1 hybrids, ranging from 1.96 ± 0.37% for the daughters of CS males to 4.60 ± 0.51% for the daughters of OR males. The rate of 1.96 ± 0.37% for the daughters of CS males was again significantly lower (\( P < 0.001 \)) than the rates observed among the daughters of the other strains (OR, DB, Basc and spl). The remaining six pairwise comparisons (OR vs. spl, OR vs. Basc, OR vs. DB, DB vs. spl, DB vs. Basc, and Basc vs. spl) did not reveal any significant statistical differences (\( P > 0.05 \)). The average mutation rate for the cross B F_1 hybrids was 3.40 ± 0.22% and 4.03 ± 0.24% for the data with and without the daughters of CS males, respectively, and both rates were within the range for the Uc-I inbred strain.

The results of testcrosses with a sample of the mutations detected among the F_1 and F_2 females indicated that they are produced exclusively in the Uc-I X chromosome; 99% of the Notch daughters from 491 F_1 Notch females that had been mated with FM7 males were yellow-2, white-apricot, and semi-Bar, indicating tight linkage between the Notch mutations and the markers on the Uc-I X chromosome. In addition, 99% of the F_2 Notch females from normal-winged F_1 females mated with FM7 males were phenotypically yellow-2, white-apricot and semi-Bar. Three conclusions can be drawn from these F_1 data.
First, it is clear that the *Uc-I* X chromosome recurrently generates *Notch* mutations in the mitotic germ cells of both sexes. Second, in both the *cross A* and *cross B* *F*1 hybrids, the mutation rates from the *cross* *A* and *cross B* *F*1 hybrids were significantly less than the rates from all other crosses. Third, a greater range of mutation rates was observed among the *cross A* hybrids than among the *cross B* hybrids.

**Mutations in the *F*2 females:** The impact of different genetic backgrounds on *Uc-I* mutability in *F*1 hybrids was assessed by examining their *F*2 daughters for *Notch* mutations (Table 3). These daughters were derived from *F*1 females from both *cross A* (even-numbered rows in Table 2) and *cross B* (odd-numbered rows in Table 2). In all cases, the *F*1 females had been mated to *FM7* males and their *Uc-I/* *FM7* daughters, recognized by their phenotypes, were scored for *Notch* mutants.

The data in Table 3 reveal three distinguishable patterns of mutability. In one, represented by the data from crosses 1–4, *Uc-I* mutability in both *cross A* and *cross B* *F*1 hybrids was comparable to that estimated from the *Uc-I* inbred strain and from the *F*1 flies. In the second, represented by crosses 5–8, mutation was significantly reduced (less than 1%) in the germ cells of both *cross A* *F*1 hybrids, but not in the *cross B* *F*1 hybrids. This type of repression, designated by the term maternal repression, indicates that *Notch* mutability can be influenced by a maternal effect of the *Base* and *69a/DB* strains. Moreover, since the *Base* strain is devoid of *hobo* elements, this effect cannot be attributed to a *hobo* encoded product. Rather, it must be caused by factors in the *Base* genome itself.

The second type of repression, exhibited by the *F*1 hybrids from the *OR* strain, was not due to a maternal effect since *Notch* mutability was repressed in the germ cells of both types of hybrids. Also, this type of repression seemed to be stronger than the repression seen with the *Base* and *69a/DB* strains. This suggests a different repressing agent, possibly by genetic factors and/or *hobo* elements or by their products in the *OR* strain. We propose the term zygotic repression to designate this type of repression.

**Reexamination of genetic factors that repress *hobo* activity:** The zygotic repression manifested by the *OR* strain was reexamined with experiments using inbred strains that were derived from *OR* and *CS* hybrid females; however these strains, designated *E*, lacked *hobo* elements. The derivation of these *E* strains is given in MATERIALS AND METHODS. Genomic Southern analysis showed that among the 149 lines surveyed, 122 had both complete and defective *hobo* elements, 22 had only defective elements, and 5 had no *hobo* elements at all. The absence of *hobo* elements in the last 5 lines was also checked by in situ hybridization. Figure 4 is a sample blot involving eight of the lines. Two of the *hobo-free* *E* lines, *E*28 and *E*150, were selected for further study. *E*28 was derived
digested with XhoI was hybridized with the phage clone EC296 shown at the right of the panel. The blot of genomic DNA is which contains hybrids segregating for hobo elements. The blot of genomic DNA tallied for Notch mutations.

The mutation rates estimated from the F1 progeny of matings with more than one Notch daughter were unusually high. This high rate seems to be due to a large proportion of F2 males producing mutations, as well as to large mutational clusters.

The mutation rates estimated from the F2 data indicate that hobo-mediated mutability was repressed in the F1 females from cross A, but not in the F1 females from cross B (0.43% vs. 3.35%, respectively). This reciprocal cross effect is reminiscent of the repression observed in the F1 hybrids derived from the Bascl strain genome and OR strains. Since the CS strain does not have the ability to repress hobo activity (see Table 3), it is possible that these maternally expressed genetic factors may have originated in the E* genome. Alternatively, these E* strains may represent combinations of maternally expressed genetic factors from the hybrids that can repress hobo activity in the Uc-I X chromosome.

The reduced occurrence of Notch mutations in the cross A F1 hybrids from the E* females could be due to a loss of hobo elements from the Uc-I X chromosome, in particular, the hobo element in the Notch locus which is indispensable for the mutational process. To check this possibility, the F2 males from the E*/Uc-I females that carried the Uc-I markers were mated individually with females, and their daughters were scored for Notch mutations. If the reduced mutability in the germ cells of cross A F1 hybrids were due to a loss of hobo elements from the Uc-I X chromosome, then the Uc-I sons derived from these hy-

<table>
<thead>
<tr>
<th>F1 crosses</th>
<th>No. of pair matings</th>
<th>No. of daughters</th>
<th>Notch mutation rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TexEl ±1^b &gt;1^c</td>
<td>N and N*</td>
<td>Mean ± se</td>
</tr>
<tr>
<td>Uc-I F2 progeny with an E strain genome</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. Uc-I/CS B × FM7 6</td>
<td>252 82 54</td>
<td>10,501 314</td>
<td>2.85 ± 0.46</td>
</tr>
<tr>
<td>2. CS/Uc-I B × FM7 6</td>
<td>1,061 593 392</td>
<td>27,537 1,957</td>
<td>7.04 ± 0.39</td>
</tr>
<tr>
<td>3. Uc-I/F1 B × FM7 6</td>
<td>357 135 109</td>
<td>9,735 527</td>
<td>6.10 ± 0.72</td>
</tr>
<tr>
<td>4. spl/Uc-I B × FM7 6</td>
<td>353 79 53</td>
<td>8,857 314</td>
<td>4.03 ± 0.73</td>
</tr>
<tr>
<td>5. Uc-I/Bascl B × FM7 6</td>
<td>348 146 93</td>
<td>8,649 450</td>
<td>6.37 ± 0.83</td>
</tr>
<tr>
<td>6. Bascl/Uc-I B × FM7 6</td>
<td>347 25 12</td>
<td>8,427 46</td>
<td>0.57 ± 0.15</td>
</tr>
<tr>
<td>Uc-I F2 progeny with a CH strain genome</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7. Uc-I/Ds B × FM7 6</td>
<td>388 118 66</td>
<td>8,809 362</td>
<td>3.71 ± 0.53</td>
</tr>
<tr>
<td>8. DB/Uc-I B × FM7 6</td>
<td>299 6 4</td>
<td>9,426 10</td>
<td>0.11 ± 0.45</td>
</tr>
<tr>
<td>8'. 69a/Uc-I B × FM7 6</td>
<td>297 7 4</td>
<td>10,927 16</td>
<td>0.43 ± 0.34</td>
</tr>
<tr>
<td>Uc-I F2 progeny with a CDH strain genome</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9. Uc-I/OR B × FM7 6</td>
<td>239 0 0</td>
<td>5,094 0</td>
<td></td>
</tr>
<tr>
<td>10. OR/Uc-I B × FM7 6</td>
<td>709 0 0</td>
<td>19,763 0</td>
<td></td>
</tr>
</tbody>
</table>

^a In all F2 progeny, only the Uc-I/FM7 females, which could be distinguished from their sisters without the Uc-I X chromosome, were tallied for Notch mutations.

^b Number of pair matings with one or more Notch daughters.

^c Number of pair matings with more than one Notch daughter.

^d The DB/Uc-I and 69a/Uc-I females shown in crosses 8 and 8' were daughters of common 69a/DB mothers.

Figure 4.—Southern analysis of sublines derived form CS/OR hybrids segregating for hobo elements. The blot of genomic DNA digested with XhoI was hybridized with the phage clone EC296 which contains a 2.89-kb hobo element. The second lane from the left of the panel that shows no detectable amount of hobo sequence is E*28. The two faint bands common to all the lanes are unique sequences from the 4E-F region. The hobo fragments in kb are shown at the right of the panel.

from CS/OR hybrids with a CS mother, and E*150 was isolated from the OR/CS hybrids produced by OR mother.

Each E* line was mated with Uc-I flies to produce cross A and B F1 hybrids, and the non-Notch F1 females that had Notch sisters were mated with FM7 males to produce the F2, Uc-I/FM7 F2 females, identified by their phenotypes (yellow-2, white-apricot and semi-Bar), were then scored for Notch mutations. Table 4 summarizes the results of these mutation screens.

The mutation rates estimated from the F1 progeny of Uc-I females and males mated to the E* flies were 5.92 ± 0.91% and 13.69 ± 2.05%, respectively. The former was within the usual range, but the later was unusually high. This high rate seems to be due to a large proportion of Uc-I males producing mutations, as well as to large mutational clusters.

The mutation rates estimated from the F2 data indicate that hobo-mediated mutability was repressed in the F1 females from cross A, but not in the F1 females from cross B (0.43% vs. 3.35%, respectively). This reciprocal cross effect is reminiscent of the repression observed in the F1 hybrids derived from the Bascl and 69a/DB strains and suggests that maternally expressed genetic factors, that can influence hobo activity, are also present in the E* genomes. The E* strains were derived from hybrids between the CS and OR strains. Since the CS strain does not have the ability to repress hobo activity (see Table 3), it is possible that these maternally expressed genetic factors may have originated in the OR genome. Alternatively, these E* strains may represent combinations of maternally expressed genetic factors from the hybrids that can repress hobo activity in the Uc-I X chromosome.
Notch appears to be due to the repression of the following sites:

- Breakpoints of a single deletion involving 3C and 3D. Notch hybrids should also show reduced mutability.

81 of the 90 stock after it had been inbred for 30 generations. Some of the seven mutants with structurally larger than the common deletion, with breakpoints in 3C7 and 3D. Such inversions would be difficult to recognize in unstained polytene chromosomes because the only prominent bands in

### TABLE 4
Notch mutations detected in the E*/Uc-1 and Uc-1/E* F1 hybrids and their F2 progeny

<table>
<thead>
<tr>
<th>Crosses</th>
<th>No. of pair matings</th>
<th>No. of daughters</th>
<th>Notch mutation rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Tested</td>
<td>≥f²</td>
<td>&gt;f³</td>
</tr>
<tr>
<td>Uc-1 X E⁺ line</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. Uc-1 X E⁺</td>
<td>232</td>
<td>83</td>
<td>63</td>
</tr>
<tr>
<td>2. E⁺ X Uc-1 δ</td>
<td>150</td>
<td>81</td>
<td>74</td>
</tr>
<tr>
<td>Uc-1 F2 progeny with an E⁺ line genome:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3. Uc-1/E⁺ X FM7 δ</td>
<td>366</td>
<td>136</td>
<td>89</td>
</tr>
<tr>
<td>4. E⁺/Uc-1 X FM7 δ</td>
<td>680</td>
<td>26</td>
<td>13</td>
</tr>
<tr>
<td>Progeny of Uc-1 F2 males:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5. spl/spi X Uc-1 F2 δ</td>
<td>482</td>
<td>76</td>
<td>57</td>
</tr>
</tbody>
</table>

* Number of pair matings with one or more Notch daughters.

** Number of pair matings with more than one Notch daughter.

† The E*28 and E*130 data were pooled because statistical comparisons indicated no significant differences between them.

### TABLE 5
Distribution of hobo elements in the Uc-1 X chromosome in a strain inbred for 30 generations

<table>
<thead>
<tr>
<th>Class</th>
<th>Labeled sites in the Uc-1 X chromosome</th>
<th>No. of larvae</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>None</td>
<td>7</td>
</tr>
<tr>
<td>2</td>
<td>3C only</td>
<td>1</td>
</tr>
<tr>
<td>3</td>
<td>3C/3D breakpoint only in Df(I)3C;3D</td>
<td>1</td>
</tr>
<tr>
<td>4</td>
<td>3C and 3D only</td>
<td>52</td>
</tr>
<tr>
<td>5</td>
<td>3C, 3D and a third sitea</td>
<td>24</td>
</tr>
<tr>
<td>6</td>
<td>3C, 3D and three additional sitesa</td>
<td>4</td>
</tr>
<tr>
<td>7</td>
<td>3C, 3D and 4 additional sitesa</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>90</td>
</tr>
</tbody>
</table>

* Additional labeled sites were at either one or a combination of the following sites: 3E, 4A, 4E, 5D, 6A, 6E, 7B, 7C, 10B, 10E, 12A, 12F, 13A, 15D, 16A, 16F and 17A.

### TABLE 6
Chromosome structures observed in 80 Uc-1 Notch mutations of independent origin

<table>
<thead>
<tr>
<th>Chromosome structure</th>
<th>No. of mutants</th>
</tr>
</thead>
<tbody>
<tr>
<td>Df(I)3C7;3D</td>
<td>64</td>
</tr>
<tr>
<td>Other deficiencies involving 3C7a</td>
<td>4</td>
</tr>
<tr>
<td>A combination of Df(I)3C;3D and an inversionb</td>
<td>2</td>
</tr>
<tr>
<td>Inversions involving 3C7c</td>
<td>3</td>
</tr>
<tr>
<td>Cytologically normal X chromosomed</td>
<td>7</td>
</tr>
<tr>
<td>Total</td>
<td>80</td>
</tr>
</tbody>
</table>

* One each of Df(I)3C7;3C10, Df(I)3C7;3E, Df(I)3C7;3F and Df(I)3C7;4A.

b | Df(I)3C7;3D with In(1)3D;10B, and Df(I)3C7;3D with In(1)3D;4A.

c | In(I)3C7:19E, In(I)3C7:6A and In(I)3C7:4E.

d | Two of these mutants have had episodes of reversion to N⁺.

Although the Uc-1 X chromosome was derived from a recombinant X chromosome that had only the 3C and 3D hobo elements, after 30 generations of inbreeding, about 8% of the chromosomes in the Uc-1 stock had lost both of these elements and about one-third of them had acquired additional elements.

Table 6 summarizes the cytological analysis of unstained polytene chromosomes from 80 Notch mutations that arose independently in the Uc-1 stock. Seven had a cytologically normal X chromosome, but the remaining 73 had a deletion or an inversion involving the Notch locus (3C7); 66 of the chromosomes had a deletion of the 3C7–3D interval, and in two of these, there was an inversion in addition to the deletion. Four deletions that were either slightly smaller or larger than the common deletion, Df(I)3C7;3D, and three inversions involving the Notch locus were also observed. Some of the seven mutants with structurally normal X chromosomes may have had an inversion with breakpoints in 3C7 and 3D. Such inversions would be difficult to recognize in unstained polytene chromosomes because the only prominent bands in
the 3C7 to 3D1–2 interval are the centrally located 3C9–10 doublets.

From the 80 unstained slides used for cytological analysis, 25 were selected for in situ hybridization with a hobo probe. These were 19 Df(1)3C7;3D, two Df(1)3C7;3D with an inversion, Df(1)3C7;3E representing one of the larger-sized deletions, and all three of the inversions involving 3C7. The results are shown in Table 7.

Labels were clearly present in the breakpoints of all the deficiencies, whether they were Df(1)3C7;3D or other deficiencies involving 3C7, and in both breakpoints of all three of the inversions. These results, together with the distribution of hobo elements in N'Uc-1 X chromosomes strongly support the idea that most of the Notch mutations detected in this X chromosome are caused by hobo-mediated arrangements. Figure 5 shows an example of the Df(1)3C7;3D breakpoints labeled with a biotinylated probe. This was the most common type of rearrangement seen in the chromosomes examined, suggesting that a majority of the Notch mutations produced in the Uc-1 X chromosome are deletions resulting from the interaction between the hobo elements in 3C7 and 3D. The presence of a hobo element in all of the breakpoints in these rearrangements supports the view that interaction between hobo elements in the Uc-1 X chromosome, possibly through homologous recombination between the elements, may give rise to the rearrangements.

Df(1)3C7;3E is slightly larger than Df(1)3C7;3D and may have been produced by interaction between the 3C7 element and another hobo in 3E. The 3C7 element clearly has the ability to interact with hobo elements at sites other than 3D. This is indicated by three inversions detected in this study. In these inversions, the 3D element was present within the inverted region of the chromosome and a hobo element was present at each of the inversion breakpoints. These three inversions support the idea that 3C7 hobo element can occasionally interact with a hobo element other than the one located in 3D.

Polyprene chromosomes from another 25 independent Notch mutants were hybridized with the clone NR311 which contains about 15 kb from the 3' end of the Notch locus (KIDD, LOCKETT and YOUNG 1983). None of the 25 Df(1)3C7;3D chromosomes probed with NR311 showed label at the deficiency breakpoint. This demonstrates that the 3' end of the Notch locus was indeed deleted in each of the deficiencies examined. Furthermore, it indicates that the hobo element in the Notch locus, 3C7, of the Uc-1 X chromosome is situated to the left of the NR311 sequence.

Because of its importance in this study, the polyprene chromosomes of the OR strain were also hybridized in situ with a biotinylated hobo probe. The results show at least 85–92 labeled sites in the genome (Figure 6). Many of the darkest positions consist of clusters...
of 2–3 labeled sites indicating a nonrandom distribution of hobo insertions.

DISCUSSION

Notch mutability in the Uc-1 X chromosome: The Uc-1 X chromosome has a 2.95-kb hobo element in its Notch locus that does not interfere with the normal expression of the locus. However, it does interact with other hobo elements in the same X chromosome to produce Notch mutations associated with chromosomal rearrangements. Therefore, the production of Notch mutations can be related to the interactions of hobo elements in the Uc-1 X chromosome. The Notch mutation rates were estimated for the Uc-1 inbred strain, for the F1 progeny from crosses involving the Uc-1 strain with several non-Uc-1 inbred strains, and for the F2 progeny from these F1 hybrid females. The results suggest that the hobo activity in the Uc-1 X chromosome is affected by the genetic background of the flies.

The hobo elements in the Uc-1 inbred strain were very active. They produce Notch mutations at a rate of 4 to 8%, suggesting that the Uc-1 genetic background supports the activity of the hobo elements. The frequent appearance of very large clusters of mutations produced by individual flies suggests that most of the mutations are produced in the mitotic (premeiotic) germ cells. The Notch mutants also appear in large clusters among the F1 females involving the Uc-1 strain. The appearance of mutant clusters among the F1 daughters of Uc-1 males (cross A F1) as well as those of Uc-1 females (cross B F1) suggests that Notch mutations are indeed produced in the mitotic germ cells of both sexes of Uc-1 flies.

Three features of the inbred and F1 data that deserve further study were noted. First, the rates estimated among the F1 hybrids from crosses involving the Uc-1 and CS strains, regardless of the sex of the Uc-1 strain in the cross, were significantly lower than those estimated for other F1 hybrids. Second, the mutation rate estimated for the Uc-1 inbred strain seemed lower than the expected rate based on the F1 data. Third, a greater variability in the mutation rates was observed among the daughters of Uc-1 males (cross A F1 females) compared to the rates observed among the daughters of Uc-1 females (cross B F1 females).

Excluding the F1 data involving the CS strain, the estimated mutation rate for the cross A F1 females (daughters of Uc-1 males) was 8.03 ± 0.45%, and the rate for the cross B F1 females (daughters of Uc-1 females) was 4.03 ± 0.24%. Compared to these rates, the Notch mutation rates estimated for the cross A and B F1 females involving the CS strain were significantly lower, 1.23 ± 0.22% and 1.96 ± 0.37% for CS/Uc-1 and Uc-1/CS F1 females, respectively. Uc-1 males and females were multiply mated with two or three non-Uc-1 inbred strains for the production of the F1 hybrids (see MATERIALS AND METHODS). Therefore, the F1 hybrid females have had common Uc-1 parents. What is the cause of the lower rates? Selective elimination of zygotes or embryos heterozygous for Notch deletions seems to take place in the presence of the CS genome. However, our data do not provide a reasonable basis for explanation of this selective killing.

Our data clearly indicate that the Notch mutations in the Uc-1 X chromosome are produced in the mitotic germ cells of both sexes of the Uc-1 inbred strain. Therefore, the expected Notch mutation rate for the Uc-1 strain itself should approximate the sum of the Notch mutation rates estimated for the daughters of Uc-1 males and females, or $r + t - 2rt$, where $r$ and $t$ are the mutation rates for the daughters of Uc-1 males and of Uc-1 females, respectively. The mutation rates for the F1 females, excluding the daughters of the CS strain, are 8.03 ± 0.43% and 4.03 ± 0.24% for the daughters of Uc-1 males and Uc-1 females, respec-
tively. Therefore, the mutation rate for the Uc-1 strain itself should be about 12% rather than the observed 4–8%. We suggest that double-strand breaks are often generated in or around hobo elements in the Uc-1 X chromosome. Furthermore, we suggest that the unreparable gaps in the 3C to 3D region of the Uc-1 X chromosome are responsible for the death of some of the Uc-1 females heterozygous for the deficiency during the early stages of development.

What about the greater variability of the rates in the cross A F1 hybrids (daughters of Uc-1 males) compared to the rate variation in the cross B F1 hybrids (daughters of Uc-1 females)? The maternal parents of cross A F1 females are represented by different inbred strains whereas all of the cross B F1 females had the same maternal parent, Uc-1 females. Does the observed greater variability in the cross A F1 hybrids reflect the differences in the maternal genetic background? A well-planned experiment focused on this problem may provide the answer. Experiments designed to test whether or not the Notch mutations can be produced after fertilization are desirable to clearly define the roles the maternal parents play in the production of the mutations.

The effects of non-Uc-1 genomes on the hobo activity in the Uc-1 X chromosome can be assessed by studying the production of Notch daughters by the F1 females. Our F1 data show that hobo activity was not affected by the CS or spl genome. However, hobo activity in the Uc-1 X chromosome can be repressed by at least two different mechanisms. The first one, found in the Bas and 69a/DB strains, show a clear reciprocal cross effect. Hobo activity in the daughters of Basc or 69a/DB females was repressed, but the activity in the F1 hybrids of Basc or DB males was not. This situation was indicated by the production of only 0.1–0.6% Notch daughters by the F1 hybrid females produced by Basc or 69a/DB mothers (see rows 6, 8, and 8' in Table 3), as contrasted to 6.3% and 3.7% by their reciprocal F1 hybrid females (rows 5 and 7 in Table 3). This phenomenon can be explained by the presence of maternally influencing factors in these strains that can repress hobo activity. We propose the term maternal repression to denote the repressive effect the maternal parent has on the hobo activity in their progeny, as demonstrated by Basc and 69a/DB mothers.

The second repression mechanism, found in the OR strain, was effective in both cross A and B F1 hybrids, suggesting the influence of zygotic gene expression in the germ cells of the hybrids. The term zygotic repression will be used to signify the hobo activity repression in the progeny by the parental genome, as shown by the OR strain. This strain of OR has almost 100 hobo elements, most of which are defective. Since the Uc-1 genome does not repress hobo activity in its X chromosome, the putative factors that are responsible for the zygotic repression must be associated with the OR genome and possibly with its hobo elements.

To check the possibility that the repressive effect the OR genome has on the hobo activity in the Uc-1 X chromosome, hybrids between OR and CS (an E strain) were backcrossed to CS for two consecutive generations, and lines without hobo elements (E*28 and E*150 lines) were isolated. The E* lines were unable to manifest the zygotic repression, suggesting that the genetic factors and/or hobo elements in the OR strain are likely responsible for this repression. However, the E* females were found to exhibit maternal repression (see rows 3 and 4, Table 4).

Although maternal repression was not obvious in the hybrids between the OR and Uc-1 strains, isolation of E* strains with the maternal repression mode suggests that this mode of repression may be latent in the genetic material of the OR strain. One of the E* lines, E*28, was derived from CS/OR hybrids in which the CS females used in the cross were known not to repress hobo activity in the germ cells of their daughters. Therefore, the ability of E*28 females to repress the hobo activity must have originated from the OR genome or a genotype resulting from recombination of the CS and OR genomes in the hybrids. In addition, since CS mothers do not repress hobo activity, the maternal repression expressed by the E*28 line cannot be attributed to a cytoplasmic factor in the CS strain or to the maternally expressed genes in the CS strain. These observations and situations are the basis for our suggestion that the factor(s) responsible for maternal repression is maternally expressed genetic material rather than a cytoplasmic factor(s), and that such maternally expressed genes must have been latent in the OR genome. Further studies are desired to identify and characterize the factor(s) that is responsible for the maternal repression of hobo activity.

These results from the crosses involving E* lines suggest that the OR strain has both maternal and zygotic repression mechanisms, and that genetic factors and/or hobo elements in the OR strain are the most likely agents responsible for the zygotic repression. These results also suggest that in a strain with both maternal and zygotic repression, such as the OR strain, the maternal repression may not be seen because the zygotic repression is so strong.

The nature of hobo activity in the Uc-1 X chromosome: The results from cytological analysis indicated that most of the Notch mutations were associated with deletions or inversions with one of their breakpoints in the Notch locus. In situ hybridization with a hobo probe showed that most of the Uc-1 X chromosomes, before the production of Notch mutations, had hobo elements at the sites corresponding to the dele-
tion and inversion breakpoints in the Notch rearrangements. This finding suggested that the hobo element in the Notch locus interacted with other hobo elements in the Uc-1 X chromosome to produce the rearrangements. The detailed nature of the interactions is not known. However, previous studies (LIM 1979, 1981a, 1988; SHEEN 1990; SHEEN, LIM and SIMMONS 1993) have suggested that intramolecular recombination through homology provided by hobo elements is the most likely mechanism for the production of the rearrangements. The results from the present study are consistent with this view.

According to this view, rearrangement formation minimally requires the ability of a hobo element to find another hobo sequence, breakage of DNA molecules at the sites of these hobo elements, exchange of the broken pieces followed by ligation and resolution that permit the rearranged molecules to replicate. The recombined molecules must then be transmitted mitotically and meiotically, and the cells carrying such molecules must survive during the development of the flies. The Notch mutation rates must, therefore, reflect the influence of different genetic backgrounds on these basic cellular functions. Lack of any of these functions would prevent Notch mutations from occurring in the germ cells and survival of the flies during the development.

Double-strand gaps generated by P transposase in Drosophila can be repaired by use of a homologous sequence at an ectopic site, as well as by a site in a homolog or in a sister chromatid (ENGELS et al. 1990; GLOOR et al. 1991). Available information, though minimal, suggests that rearrangement formation in the Uc-1 X chromosome is dependent on the orientation of hobo elements involved in the rearrangement events (LIM 1988; SHEEN 1990; SHEEN, LIM and SIMMONS 1993). Orientation-dependent rearrangement formation, as well as the presence of hobo elements at rearrangement sites, strongly favors homologous recombination as a basic underlying mechanism for the production of rearrangements in the Uc-1 X chromosome.

The repression modes: We observed two basically different ways in which hobo activity in the Uc-1 X chromosome can be repressed: maternal repression and zygotic repression. What is the genetic basis for the maternal repression of hobo activity? F1 females from reciprocal crosses between Uc-I and other inbred strains should have identical zygotic genotypes. However, large differences were found between reciprocal F1 females with regards to their ability to produce Notch mutations. Notch mutations produced by the daughters of Basc, 69a/DB or E* females (cross A F1 females) were significantly less frequent than Notch mutations produced by the daughters of the corresponding males. One of the strains, 69a/DB, was a CH strain, but both the Basc and E* strains did not have hobo elements. Therefore, hobo elements are not essential for this mode of repression. Uc-1 is also a CH strain whose hobo elements are very active, and this situation is also consistent with the idea that the presence or absence of hobo elements is not related to maternal repression.

These findings suggest a maternally expressed genetic factor(s) that is common to the Basc, 69a/DB and E* strains. Several Drosophila genes that are necessary for germ plasm and oocyte formation, collectively known as maternal-effect genes or maternally active genes, have been identified (BOSWELL and MAHOWALD 1985; LEHMANN and NUSSLIN-VOLHARD 1986; SCHÜPBACH and WIESCHAUS 1989; EPHRUSSI and LEHMANN 1992; JONGENS et al. 1992). It is possible that relatively stable and long-lasting products of some yet to be discovered maternally active genes are responsible for the repression of hobo activity in the mitotic cells. Products from these genes may be deposited in the germ plasm and function as repressors in the germ cells of the next generation. Since the presence or absence of hobo elements in the genome is not essential for maternal repression, further testing of a large number of inbred strains for maternal effect on hobo activity would help to elucidate this matter.

The second mode of repression, zygotic repression, was observed in the hybrids involving the OR strain. The hobo activity in the Uc-I X chromosome is repressed in the hybrid daughters of both OR fathers and mothers. Such repression is absent in the daughters of the E* strains, the inbred strains without hobo elements, which were derived from backcrossing OR/CS and CS/OR hybrids to CS strain (E strain) for two consecutive generations. As shown in Figure 6, almost 100 hobo elements are distributed throughout the OR genome. Therefore, the elimination of hobo elements in the hybrids through backcrossings would have also effectively eliminated most of the OR genome, except the hobo-free telomeric regions. In such an E* genetic background we failed to detect the zygotic repression of the Uc-I hobo activity characteristic of the OR strain. This observation suggests the involvement of genetic factors and/or hobo elements of the OR strain in the zygotic repression.

Whither hobo hybrid dysgenesis? The P-M system of hybrid dysgenesis is the paradigm for the regulation of transposable elements in D. melanogaster, and other systems of hybrid dysgenesis have been compared to this paradigm [see FINNEGAN (1989) for a review on the I-R system]. The P-M system of hybrid dysgenesis can induce several genetic conditions, such as enhanced mutability, sterility, chromosome breakage, segregation distortion, pupal lethality and male recombination [see ENGELS (1989) for a review]. These conditions are induced by making the right type of
cross: females from a strain without P elements (M strain) to males from a strain with transposase-producing P elements (P strain). The hybrids from such a cross exhibit the dysgenic conditions listed above, but the reciprocal cross hybrids, with P strain mothers and M strain fathers, are not dysgenic. For this reason, the P-M system of hybrid dysgenesis is said to exhibit a reciprocal cross effect.

The hybrids involving E strain females (analogous to M strain) and CH strain males (comparable to P strain) also show a reciprocal cross effect. For example, hobo activity in the hybrids from Base or E* females (E strains) mated with Uc-1 males (CH strain) is repressed, but no repression is apparent in the hybrids from Uc-1 females mated with Base or E* males. However, this situation is opposite that of the P-M systems of hybrid dysgenesis, because according to the rules of the P-M system of hybrid dysgenesis (Engels 1979a), the E/CH hybrids (analogous to M/P hybrids) should show enhanced hobo activity rather than repression, as was observed in Base/Uc-1 and E*/Uc-1 females. In the Uc-1 system, the maternal repression of hobo activity is not associated with the excision of hobo elements from the Uc-1 X chromosome or degradation of the elements, so that when the repression is eliminated or lessened, hobo activity can be restored (see rows 4 and 5, Table 4).

In addition to the maternal repression, our data clearly indicate the zygotic repression of hobo activity in the Uc-1 X chromosome. Although the mechanism involved is not clear, the Uc-1 hobo activity is repressed by the OR gene, and complete regression occurs regardless of the sex of the OR flies in the cross. This is the basis for our suggestion of the term zygotic repression, and is a feature quite unique for the regulation of hobo activity in the Uc-1 X chromosome.

Suggestions have been made that the high mutability observed in the hybrids between a strain with hobo elements (H strain) and a strain without hobo elements (E strain) constitutes another system of hybrid dysgenesis (Blackman et al. 1987; Yannopoulos et al. 1987). The very notion that hybrids have dysgenic qualities implies that the dysgenic qualities are either absent or less frequent in inbreds. Since neither of these reports provides information concerning hobo activity in inbred strains, their claims cannot be assessed. However, their data clearly show that active hobo elements are present in the hybrids and that deletions and other rearrangement breakpoints are associated with hobo inserts.

Based on the results of our studies, we can interpret hobo activity in the dpp locus in the Oregon Rstraw strain (Blackman et al. 1987) as de-repression of a repressed hobo element in the dpp locus. We note that the Canton S strain in their study is one of the inbred strains in our study that lacked the ability for maternal repression. The results with the 23.5 MRF strain (Yannopoulos et al. 1987) are also consistent with our finding because, as we have shown, many inbred strains are not capable of repressing hobo activity. Furthermore, some inbred strains, like Uc-1, permit hobo activity in an inbred background as well as in some hybrid backgrounds.

The Uc-1 hobo elements associated with chromosome rearrangements, enhanced mutability, and recombination in males can now be compared with the P-M system of hybrid dysgenesis. First, unlike the P-M system of hybrid dysgenesis, the hobo elements in an inbred strain are very active. Second, some hybrids, like those involving Base, 69a/DB and E* strains mated with the Uc-1 strain, clearly exhibit a maternal effect or reciprocal cross effect of repression, but the effect is opposite that documented for both the P-M and I-R system of hybrid dysgenesis. Third, maternal repression does not involve hobo elements, rather maternally expressed genes are the best candidate for the repressing agents. Finally, the hobo activity in the Uc-1 X chromosome can be repressed in the hybrid daughters of both OR fathers and mothers suggesting the involvement of the expression of zygotic genetic factors and/or hobo elements contributed by the OR strain. What is the nature or identity of the zygotic repressor from the OR genome? The answer to this question may settle many puzzling situations associated with the genetic instability caused by hobo elements in the Uc-1 X chromosome.

Our study clearly shows that, in at least one inbred strain, the dysgenic cross is not necessary to mobilize hobo elements. Until more data are available, it is wise to be careful with the term "hobo hybrid dysgenesis."

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