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

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Manuscript received January 6, 1993
Accepted for publication March 31, 1993

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-1* 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-1* males, but not in the daughters of *Uc-1* 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-1* and this strain.

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 SVED 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; KARESS 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). 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

We dedicate this paper to the memory of the late Professor LEON A. SNYDER.

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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* (STRECK, MACGAFFEY and BECKENDORF 1986). The nucleotide sequence of one of the 3.0-kb elements, designated $hobo_{108}$, indicated that the entire element is 3,016 bp. It had 12-bp inverted terminal repeats, and a truncated copy of the terminal 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 short (S) repeats near the center of the element, and two tandem copies of 20 bp (GAGTATTTTTGGAAACACCC) known as long (L) repeats about 250 bp from the 5′ end.

Restriction endonuclease and Southern analyses of nine EC *hobo* clones isolated from the *Df(1)cm-In* stock indicated that the *hobo* elements in this genome are shorter than $hobo_{108}$. The sequence analysis of 186 nucleotides in the region bounded by the *Eco*RI and *Hin*dIII 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 $hobo_{108}$ (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 $hobo_{108}$ (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)cm-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 F_1 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° 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 LINDSLEY 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 polytene 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^a (white-apricot eyes); it was obtained from the Bowling Green Stock Center in the 1960s. (c) *y z spl* sn^3 (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^{59b} *z* w^i (abbreviated *Uc-1*). The *X* chromosome in this stock, called *Uc-1*, carries three recessive markers: y^{59b}, *z*, and w^i, and 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)cm-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*, l^{69a}/*Df(1)Basc*/sc^8.*Y* (abbreviated *69a/DB*), an *X* chromosome balancer stock. *FM6*, l^{69a} is an *FM6* balancer *X* chromosome that carries an unlocalized EMS-induced recessive lethal mutation, $l(1)^{69a}$. *Df(1)Basc* is a *Basc* balancer *X* chromosome with a lethal terminal deletion that is complemented by the

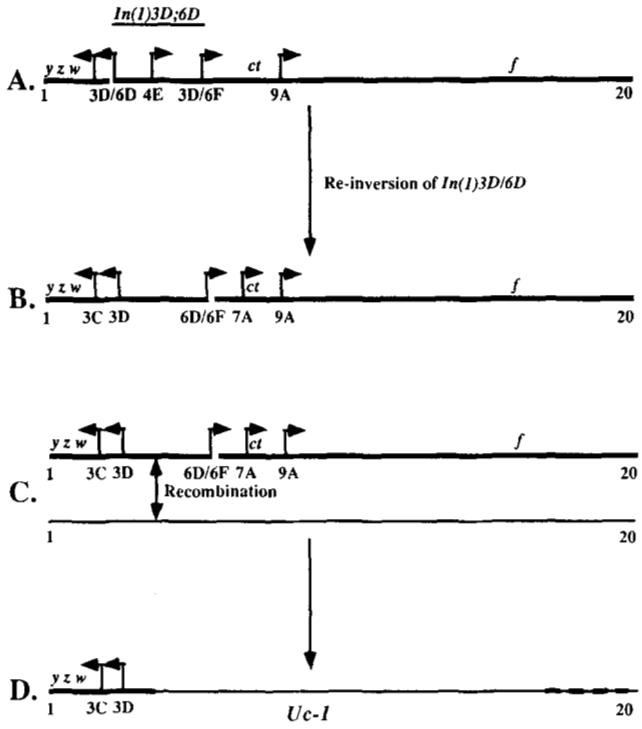

FIGURE 1.—A diagram showing the origin of the *Uc-1 X* chromosome. The thick and thin horizontal lines indicate the *Df(1)cm-In* and CS *X* chromosomes, respectively. Each of the five short arrows above the chromosomes represents a *hobo* element, with its orientation as indicated. The cytological positions of these elements are shown below the chromosomes, and the locations of several genetic markers are indicated above them. *Df(1)cm* in the *Df(1)cm-In* chromosome, which can be either *Df(1)3D/6D* or *Df(1)6D/6F* depending on the orientation of the 3D-6D region in *In(1)3D/6D*, is indicated by a gap. **A**, The *Df(1)cm-In* chromosome. **B**, A chromosome in which the inversion *In(1)3D/6D* in the *Df(1)cm-In* chromosome has reinverted; this chromosome is denoted *Df(1)cm-In*^{ri} (LIM 1988). **C**, Recombination between the *Df(1)cm-In*^{ri} chromosome and a *hobo*-free *X* chromosome from CS. **D**, The *Uc-1* recombinant *X* chromosome. The region with alternating thick and thin lines at the right indicates uncertainty about its origin.

sc^{8} duplication on the *sc*^{8}*.Y* chromosome. *In situ* hybridization with a biotinylated *hobo* probe indicates that *hobo* elements are present at six genomic sites in this stock.

CDH (for complete and defective hobo element) strains: Genomic Southern analysis has shown that these strains have both complete and defective *hobo* elements. The strains are: (a) Oregon R^{Sparrow} (abbreviated OR), a wild-type laboratory stock obtained from W. M. GELBART and (b) *FM7/C(1)DX, y f/Y* (abbreviated *FM7*), a balancer *X* chromosome stock. The markers in the *FM7 X* chromosome are *y*^{31d}, *sc*^{8}, *w*^{a} and *B*.

E (for special E)* strains: These wild-type strains were derived from hybrid females produced by crossing CS and OR flies. The hybrid females were backcrossed to CS males for two consecutive generations and then 149 sublines were established from the population by single pair matings. Genomic Southern analysis and *in situ* hybridization revealed that five of these 149 sublines lacked *hobo* sequences in their genome. These five sublines are designated *E** strains to denote empty strains that have genetic material, but not *hobo* elements, from a *CDH* strain. Two of the *E** strains used in this study, *E*28* descended from CS/OR hybrids and *E*150* from OR/CS hybrids, were sib-mated 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* males will be referred to as the *cross A* F_1 females. The hybrid daughters from the reciprocal crosses using *Uc-1* females will be designated as *cross B* F_1 females. The hybrid granddaughters produced by these F_1 females will be referred to as *cross A* F_2 females and *cross B* F_2 females, respectively.

For the F_1 hybrids, the genotype or designation of the female parent will always be indicated first. Thus, CS/*Uc-1* represents the genotype of F_1 hybrids from the cross of CS females with *Uc-1* males; F_1 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 F_1 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 F_1 females); then the same *Uc-1* females were mated with *spl* males (producing yellow and zeste F_1 females), and finally with *Basc* males (producing white-apricot and semi-Bar F_1 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 F_2 females were produced by mating normal-winged F_1 virgin females, from at least 100 F_1 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 λ 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 *Xho*I 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 *Xho*I fragment or the 0.9-kb *Eco*RI 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-

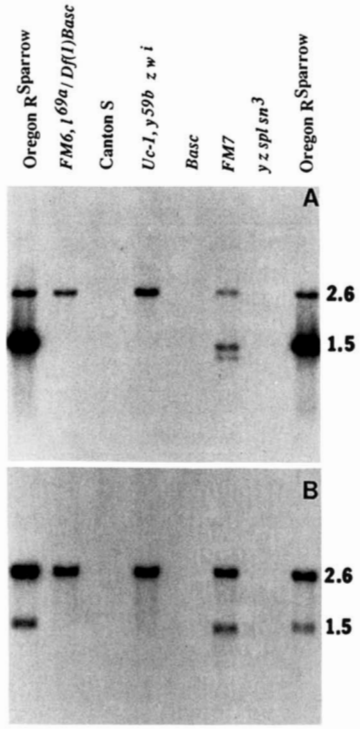

FIGURE 2.—Southern hybridization analysis of seven inbred strains with *hobo* element probes. Panel A shows the results of hybridization with a 2.6-kb *Xho*I fragment of *hobo* from a λ clone, EC296 (LIM 1988). Panel B shows the results of hybridization with a 0.9-kb *Eco*RI fragment of *hobo* from EC296. The fragment sizes in kilobases are shown at the right.

lected from each experimental cross. The Mann-Whitney-Wilcoxon rank sum test (STEEL and TORRIE 1960) was used to compare data from two samples of either the same or different crosses. If there were ties in the ranks, a correction was made in the test statistic (HAJEK 1969). The Kruskal-Wallis H test (STEEL and TORRIE 1960) was used to evaluate the homogeneity of data from a particular cross.

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 *Xho*I-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 *Xho*I 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 *Xho*I-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 *Eco*RI fragment. This was indicated by the failure of the 1.3-kb band to hybridize with the central 0.9-kb *Eco*RI 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 F_1 and the F_2 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 the 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 G_{88} to 11.89 ± 1.78% in G_{75}, 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 G_1–G_6 and in G_{91}–G_{96}, 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-1 X* chromosome**

Generation	No. of pair matings			No. of daughters		*Notch* mutation rate (%)
	Tested	≥1[a]	>1[b]	*N* and *N*$^+$	*N*	Mean ± SE
G_1	140	77	47	6,133	276	4.54 ± 0.76
G_2	115	60	39	3,210	195	7.03 ± 1.28
G_3	119	51	30	2,460	160	7.42 ± 1.60
G_4	118	54	30	2,197	153	6.08 ± 1.06
G_5	133	56	20	2,723	169	6.35 ± 1.35
G_6	120	32	15	1,618	60	4.65 ± 0.94
G_{91}	113	57	35	2,456	141	6.24 ± 1.07
G_{92}	115	63	39	2,661	165	7.00 ± 1.14
G_{93}	93	47	30	1,929	158	8.18 ± 1.69
G_{94}	94	28	16	1,660	104	5.11 ± 1.29
G_{95}	106	49	30	2,492	172	6.38 ± 1.18
G_{96}	84	28	17	1,355	67	5.40 ± 1.36
Total[c]	6,330	3,137	2,028	193,181	10,755	6.06 ± 0.15

[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 47–96).

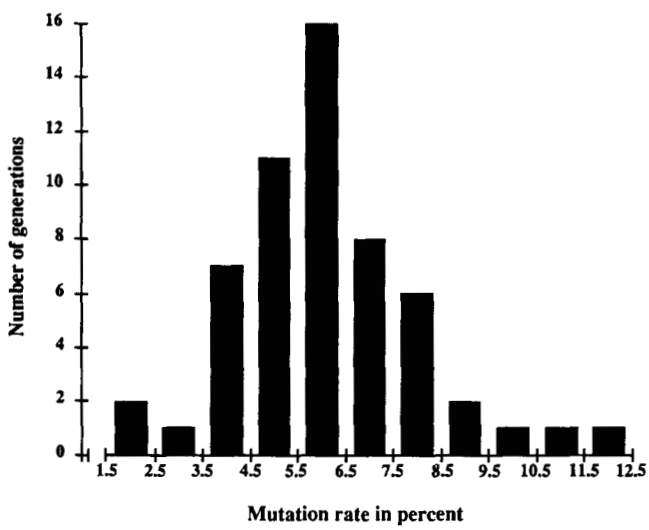

FIGURE 3.—Distribution of mutation rates estimated for the *Uc-1* strain during 56 generations of study. The rates were tallied in 11 groups shown on the abscissa. The lowest rate was 2.30%, the highest rate was 11.89%, and the mean was 6.06 ± 0.15%.

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-1* 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-1* males, and the odd-numbered crosses represent the *cross B* F_1 hybrids, the daughters of *Uc-1* females. *Notch* mutations were detected at a high rate among the F_1 daughters of all three major classes of crosses (*Uc-1* × E, *Uc-1* × CH, and *Uc-1* × 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-1* 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-1* 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-1* 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-1* 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-1* 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-1 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-1 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.

TABLE 2

***Notch* mutations detected in the F_1 hybrids carrying the *Uc-1 X* chromosome**

Crosses	No. of pair matings			No. of daughters		*Notch* mutation rate (%)
	Tested	≥1[a]	>1[b]	*N* and N^+	*N*	Mean ± SE
Uc-1 × *E* strain						
1. *Uc-1* ♀ × *CS* ♂	281	66	41	11,025	225	1.96 ± 0.37
2. CS ♀ × *Uc-1* ♂	729	108	52	29,078	267	1.23 ± 0.22
3. *Uc-1* ♀ × *Basc* ♂	571	245	154	22,393	729	3.90 ± 0.36
4. *Basc* ♀ × *Uc-1* ♂	443	155	95	9,996	535	6.14 ± 0.68
5. *Uc-1* ♀ × *spl* ♂	372	128	60	8,660	266	3.42 ± 0.41
6. *spl* ♀ × *Uc-1* ♂	662	329	313	107,191	9,892	10.25 ± 0.82
Uc-1 × *CH* strain						
7. *Uc-1* ♀ × *DB* ♂	102	45	35	3,114	145	4.56 ± 0.82
8. *69a/DB* ♀ × *Uc-1* ♂	151	68	55	9,241	740	8.09 ± 1.45
Uc-1 × *CDH* strain						
9. *Uc-1* ♀ × OR ♂	429	163	85	11,708	451	4.60 ± 0.51
10. OR ♀ × *Uc-1* ♂	427	192	162	22,204	1,287	6.52 ± 0.75

Except for crosses 7 and 8, the data for each cross represent more than one replicate experiment. These replicates have been pooled because statistical comparisons failed to reveal any significant differences among them.

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

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

First, it is clear that the *Uc-1 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 CS crosses 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-1* mutability in F_1 hybrids was assessed by examining their F_2 daughters for Notch mutants (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-1/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-1* mutability in both *cross A* and *B* F_1 hybrids was comparable to that estimated from the *Uc-1* inbred strain and from the F_1 flies. In the second, represented by crosses 5–8′, mutability was significantly reduced (less than 1%) in the germ cells of *cross A* F_1 hybrids, but not in the *cross B* F_1 hybrids. In the third pattern, represented by the crosses involving the OR strain, mutability was completely repressed in both *cross A* and *B* F_1 hybrids.

These F_2 data suggest that the production of *Notch* mutations in the *Uc-1 X* chromosome can be repressed in two different ways. In one, manifested in the results with the *Basc* and *69a/DB* strains, *Notch* mutability was repressed in the mitotic germ cells of the *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 *Basc* and *69a/DB* strains. Moreover, since the *Basc* 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 *Basc* 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 *Basc* 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

TABLE 3

***Notch* mutations detected in the F_2 progeny carrying the *Uc-1 X* chromosome**

F_1 crosses	No. of pair matings			No. of daughters[a]		*Notch* mutation rate (%)
	Tested	≥1[b]	>1[c]	*N* and *N*$^+$	*N*	Mean ± SE
Uc-1 F_2 progeny with an *E* strain genome						
1. *Uc-1*/CS ♀ × *FM7* ♂	252	82	54	10,501	314	2.85 ± 0.46
2. CS/*Uc-1* ♀ × *FM7* ♂	1,061	593	392	27,537	1,957	7.04 ± 0.39
3. *Uc-1/spl* ♀ × *FM7* ♂	357	153	109	9,735	527	6.10 ± 0.72
4. *spl/Uc-1* ♀ × *FM7* ♂	353	79	53	8,857	314	4.03 ± 0.73
5. *Uc-1/Basc* ♀ × *FM7* ♂	348	146	93	8,649	450	6.37 ± 0.83
6. *Basc/Uc-1* ♀ × *FM7* ♂	347	25	12	8,427	46	0.57 ± 0.15
Uc-1 F_2 progeny with a *CH* strain genome						
7. *Uc-1/DB* ♀ × *FM7* ♂	383	118	66	8,809	362	3.71 ± 0.53
8. *DB/Uc-1* ♀ × *FM7* ♂[d]	299	6	4	9,426	10	0.11 ± 0.45
8′. *69a/Uc-1* ♀ × *FM7* ♂[d]	297	7	4	10,927	16	0.43 ± 0.34
Uc-1 F_2 progeny with a *CDH* strain genome						
9. *Uc-1*/OR ♀ × *FM7* ♂	239	0	0	5,094	0	
10. OR/*Uc-1* ♀ × *FM7* ♂	709	0	0	19,763	0	

[a] In all F_2 progeny, only the *Uc-1/FM7* females, which could be distinguished from their sisters without the *Uc-1 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-1* and *69a/Uc-1* females shown in crosses 8 and 8′ were daughters of common *69a/DB* mothers.

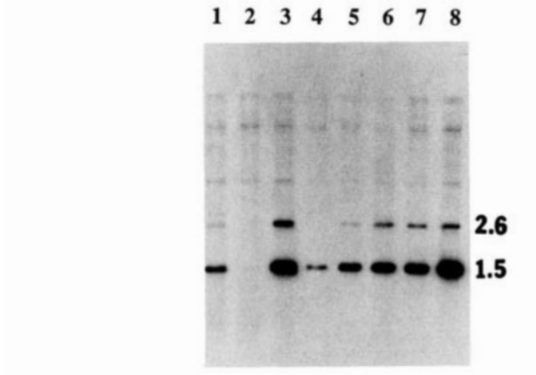

FIGURE 4.—Southern analysis of sublines derived form CS/OR hybrids segregating for *hobo* elements. The blot of genomic DNA digested with *Xho*I was hybridized with the phage clone EC296 which contains a 2.95-kb *hobo* element. The second lane from the left of the panel that shows no detectable amount 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-1* flies to produce *cross A* and *B* F_1 hybrids, and the non-Notch F_1 females that had Notch sisters were mated with *FM7* males to produce the F_2. *Uc-1/FM7* F_2 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 F_1 progeny of *Uc-1* 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-1* males producing mutations, as well as to large mutational clusters.

The mutation rates estimated from the F_2 data indicate that *hobo*-mediated mutability was repressed in the F_1 females from *cross A*, but not in the F_1 females from *cross B* (0.43% *vs.* 3.35%, respectively). This reciprocal cross effect is reminiscent of the repression observed in the F_1 hybrids derived from the *Basc* 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-1 X* chromosome.

The reduced occurrence of *Notch* mutations in the *cross A* F_1 hybrids from the *E** females could be due to a loss of *hobo* elements from the *Uc-1 X* chromosome, in particular, the *hobo* element in the *Notch* locus which is indispensable for the mutational process. To check this possibility, the F_2 males from the *E*/Uc-1* females that carried the *Uc-1* markers were mated individually with *spl* females, and their daughters were scored for *Notch* mutations. If the reduced mutability in the germ cells of *cross A* F_1 hybrids were due to a loss of *hobo* elements from the *Uc-1 X* chromosome, then the *Uc-1* sons derived from these hy-

TABLE 4

***Notch* mutations detected in the *E*/Uc-1* and *Uc-1/E** F_1 hybrids and their F_2 progeny**

Crosses	No. of pair matings			No. of daughters		*Notch* mutation rate (%)
	Tested	≥ 1[a]	>1[b]	N and N^+	N	Mean ± SE
Uc-1 × *E** line						
1. *Uc-1* ♀ × *E**[c] ♂	232	83	63	5,643	378	5.92 ± 0.91
2. *E** ♀ × *Uc-1* ♂	150	81	74	13,909	1,914	13.69 ± 2.05
Uc-1 F_2 progeny with an *E** line genome:						
3. *Uc-1/E** ♀ × *FM7* ♂	366	136	89	12,176	409	3.35 ± 0.38
4. *E*/Uc-1* ♀ × *FM7* ♂	630	26	13	13,531	65	0.43 ± 0.12
Progeny of *Uc-1* F_2 males:						
5. *spl/spl* ♀ × *Uc-1* F_2 ♂	432	76	57	19,545	437	2.30 ± 0.40

[a] Number of pair matings with one or more *Notch* daughters.
[b] Number of pair matings with more than one *Notch* daughter.
[c] The *E*28* and *E*150* 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

Class	Labeled sites in the *Uc-1 X* chromosome	No. of larvae
1	None	7
2	3C only	1
3	3C/3D breakpoint only in *Df(1)3C;3D*	1
4	3C and 3D only	52
5	3C, 3D and a third site[a]	24
6	3C, 3D and three additional sites[a]	4
7	3C, 3D and 4 additional sites[a]	1
Total		90

[a] 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.

brids should also show reduced mutability.

The resulting mutation rate of 2.30 ± 0.40% (cross 5 in Table 4) was more than fivefold higher than the rate for the *cross A* F_1 females, indicating that a significant proportion of the gametes from the *Uc-1* F_2 males had the *hobo* element required to produce *Notch* mutations. Consequently, the observed reduction in *Notch* mutability in the *cross A* F_1 hybrids appears to be due to the repression of *hobo* activity rather than to the loss of *hobo* elements.

Cytological analyses of the *Notch* mutations: *In situ* hybridization established that a majority of the *Notch* mutations occurring on the *Uc-1 X* chromosome were caused by interactions between a *hobo* element in the *Notch* locus (cytological position 3C7) and another *hobo* element at position 3D1–2, or at other sites in the *X* chromosome.

Table 5 summarizes data from *in situ* hybridization of a tritiated *hobo* probe to the polytene *X* chromosomes of 90 female larvae from the *Uc-1* stock after it had been inbred for 30 generations. *Hobo* elements were present at 3C7 (the *Notch* locus) and at 3D1–2 in 81 of the 90 *X* chromosomes (90%) and also at the breakpoints of a single deletion involving 3C and 3D.

TABLE 6

Chromosome structures observed in 80 *Uc-1 Notch* mutations of independent origin

Chromosome structure	No. of mutants
Df(1)3C7;3D	64
Other deficiencies involving 3C7[a]	4
A combination of *Df(1)3C;3D* and an inversion[b]	2
Inversions involving 3C7[c]	3
Cytologically normal *X* chromosome[d]	7
Total	80

[a] One each of *Df(1)3C7;3C10*, *Df(1)3C7;3E*, *Df(1)3C7;3F* and *Df(1)3C7;4A*.
[b] *Df(1)3C7;3D* with *In(1)3D;10B*, and *Df(1)3C7;3D* with *In(1)3D;4A*.
[c] *In(1)3C7;19E*, *In(1)3C7;6A* and *In(1)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(1)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

TABLE 7

Distribution of *hobo* elements in 25 *Uc-1 X* chromosomes with *Notch* mutations

No. of Notch mutants	Rearrangements	Labeled sites in the *X* chromosome
8	*Df(1)3C7;3D*	3C7/3D only
11	*Df(1)3C7;3D*	3C7/3D and one to three additional sites[a]
1	*Df(1)3C7;3D* + *In(1)3D;10B*	3C7/10B, 3D/10B, 5D, and 15D
1	*Df(1)3C7;3D* + *In(1)3D;4A*	3C/4A, 3D/4A, 7C, and 13C
1	*Df(1)3C7;3E*	3C/3E only
1	*In(1)3C7;19E*	3C/19E, 3C/19E, 3D, and 7C
1	*In(1)3C7;6A*	3C/6A, 3C/6A, and 3D
1	*In(1)3C7;4E*	3C/4E, 3C/4E, and 3D

[a] In addition to the 3C7/3D breakpoint, these 11 deficiency chromosomes were labeled in the following positions: 4D; 11C; 16F; 4A and 7C; 4B and 11A; 6A and 7B; 10B and 19A; 12A and 16F; 12F and 13B; 12F and 16F; and 7F, 11A and 14D.

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 strongly 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,

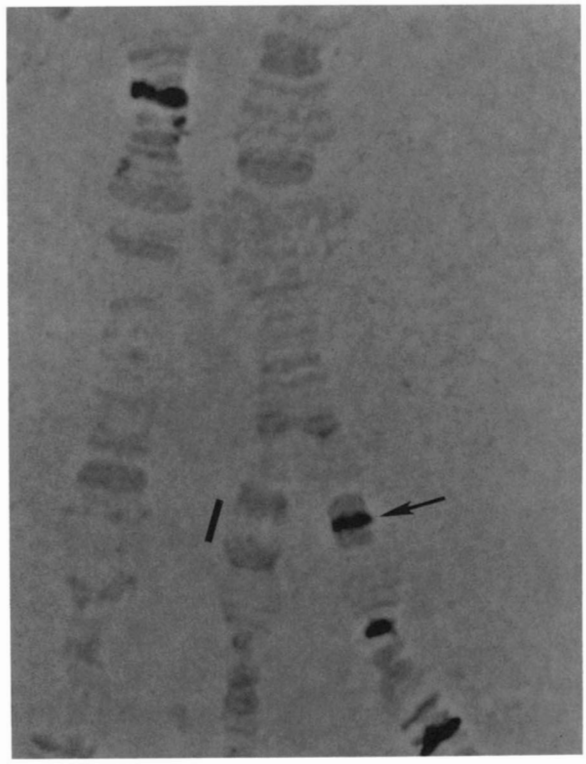

FIGURE 5.—An enlarged view of partially asynapsed *X* chromosomes that have been hybridized with a biotinylated *hobo* probe made from the phage clone EC296. The chromosomes came from a female heterozygous for a *Notch* mutation associated with a deficiency *Df(1)3C7;3D* in the *Uc-1* chromosome. The approximate region corresponding to the deficiency (3C7 to 3D1–2) in the *Df(1)3C7;3D* is shown with a short bar at the left side of the normal *X* chromosome, and the deficiency breakpoint with *hobo* label is indicated by an arrow to the right of the breakpoint. Two additional labeled sites in the *Uc-1 X* chromosome are 4A, which contains a *hobo* sequence, and 4E-F, which contains unique sequences in the probe.

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.

Polytene 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 polytene 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

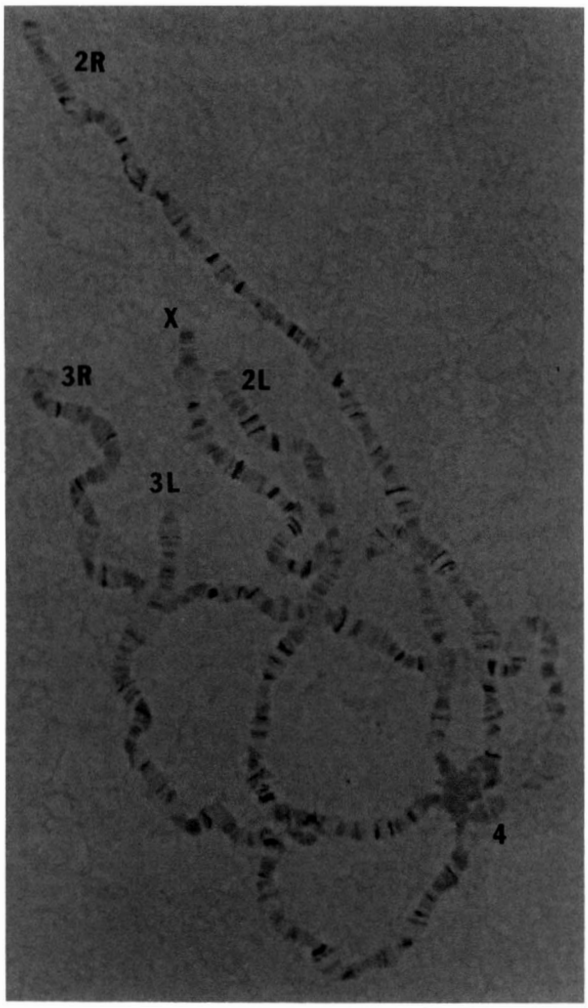

FIGURE 6.—Moderately stretched polytene chromosomes of an Oregon-R^{Sparrow} female hybridized *in situ* with a biotinylated *hobo* probe. Only the darkly labeled sites are obvious in this photograph.

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 F_1 progeny from crosses involving the *Uc-1* strain with several non-*Uc-1* inbred strains, and for the F_2 progeny from these F_1 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 F_1 females involving the *Uc-1* strain. The appearance of mutant clusters among the F_1 daughters of *Uc-1* males (*cross A* F_1) as well as those of *Uc-1* females (*cross B* F_1) 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 F_1 data that deserve further study were noted. First, the rates estimated among the F_1 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 F_1 hybrids. Second, the mutation rate estimated for the *Uc-1* inbred strain seemed lower than the expected rate based on the F_1 data. Third, a greater variability in the mutation rates was observed among the daughters of *Uc-1* males (*cross A* F_1 females) compared to the rates observed among the daughters of *Uc-1* females (*cross B* F_1 females).

Excluding the F_1 data involving the CS strain, the estimated mutation rate for the *cross A* F_1 females (daughters of *Uc-1* males) was $8.03 \pm 0.43\%$, and the rate for the *cross B* F_1 females (daughters of *Uc-1* females) was $4.03 \pm 0.24\%$. Compared to these rates, the *Notch* mutation rates estimated for the *cross A* and *B* F_1 females involving the CS strain were significantly lower, $1.23 \pm 0.22\%$ and $1.96 \pm 0.37\%$ for *CS/Uc-1* and *Uc-1/CS* F_1 females, respectively. *Uc-1* males and females were multiply mated with two or three non-*Uc-1* inbred strains for the production of the F_1 hybrids (see MATERIALS AND METHODS). Therefore, the F_1 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 F_1 females, excluding the daughters of the CS strain, are $8.03 \pm 0.43\%$ and $4.03 \pm 0.24\%$ 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 unrepairable 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* F_1 hybrids (daughters of *Uc-1* males) compared to the rate variation in the *cross B* F_1 hybrids (daughters of *Uc-1* females)? The maternal parents of *cross A* F_1 females are represented by different inbred strains whereas all of the *cross B* F_1 females had the same maternal parent, *Uc-1* females. Does the observed greater variability in the *cross A* F_1 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 F_1 females. Our F_2 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 *Basc* 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 F_1 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 F_1 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 F_1 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* F_1 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? F_1 females from reciprocal crosses between *Uc-1* and other inbred strains should have identical zygotic genotypes. However, large differences were found between reciprocal F_1 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* F_1 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 NUSSLEIN-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-1 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-1 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 *Basc* 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 *Basc* 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 *Basc/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 $R^{Sparrow}$ 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 *Basc*, *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."

We would like to thank BURKE JUDD for his many years of support and encouragement. We would also like to thank BILL ENGELS, BILL EGGLESTON, BURKE JUDD, MIKE SIMMONS and anonymous reviewers for their valuable insights and constructive suggestions. We are especially indebted to MIKE SIMMONS for generously helping us with the careful reading and editing of this manuscript. However, any errors or misinterpretations of the data in the paper are ours alone. Competent technical assistance of SUE HILLMAN, and useful suggestions for statistical analysis by DAVID LUND were also greatly appreciated. We are also grateful to the many undergraduate students who assisted us in the laboratory. They are FRED ASARE, MICHAEL BJERKE, JAMI JOHNSON, CRYSTAL MARTIN, LORI NELSON, ROBERT PETERSON, CHRISTINE PORWIT, ALLISON PRITCHETT and KIMBERLY SABELKO. Finally, we acknowledge financial support from the National Institutes of Health (GM31106), the National Science Foundation (DMB 90 18545), and a Research Augmentation Award from the Board of Regents of the University of Wisconsin System.

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Communicating editor: C. C. LAURIE