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Homologue destabilization by a putative transposable element in *Drosophila melanogaster*

(transposon/mutation rate/chromosome breakage)

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ABSTRACT **We postulate the presence of a transposable element, designated the *L* factor, to explain the properties of an unstable *X* chromosome and its derivatives. These chromosomes generate recessive lethal mutations at high rates, as does a stable *X* chromosome that has been associated with them for only one generation. The stable *X* chromosome does not become highly mutable in the absence of the unstable *X* chromosome, even when autosomes from the unstable stock are present. These facts suggest that the *L* factor is confined to the *X* chromosome and that it transposes to other *X* chromosomes paired with it. We propose the term "homologue destabilization" to denote the change in the stable chromosome brought about by this transposition. The lethal mutations caused by the *L* factor occur preferentially in the region around the cut wing locus (*ct*) and are sometimes associated with recognizable chromosome aberrations. The breakpoints of these aberrations are most often in the vicinity of *ct*, implying that the *L* factor is located near *ct* on the unstable chromosome, but it may reside at other sites as well. Alternately, the *ct* region may simply be a preferred target for the insertion of this transposable element.**

Transposable elements have been identified in a number of eukaryotes, including yeast (1, 2), Drosophila (3), and maize (4). These elements are involved in the processes of gene mutation and chromosome breakage (5, 6) and may compose a significant fraction of the genome (7). It is possible that transposable elements play a role in evolution, for they can relocate genes, duplicate chromosome segments, and alter gene expression. The manner in which all of these processes take place is not fully understood.

In this paper we present further information about the properties of an unstable *X* chromosome in *Drosophila melanogaster* that seem to be due to a transposable element (8–11). These properties are the frequent occurrence of *X* chromosome-linked lethal mutations and structural rearrangements. Both properties are acquired by a stable *X* chromosome after only one generation of association with the unstable *X* chromosome, but they are acquired independently of ordinary meiotic recombination. We refer to the change in the stable *X* chromosome brought about by its association with the unstable homologue as "homologue destabilization," and propose that this phenomenon is due to the transposition of an element from the unstable chromosome to its partner. We postulate that this transposable element is responsible for the gene mutations and chromosome rearrangements that occur on the unstable chromosome and on *X* chromosomes destabilized by it. This element is designated the *L* factor, where *L* symbolizes lethal-inducing.

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MATERIALS AND METHODS

Basic X Chromosomes. The unstable *X* chromosome used in these experiments carried a reverted lethal mutation and the recessive markers y^{59b} (yellow body), *z* (zeste eyes), w^i (white-ivory eyes), ct^6 (cut wings), and *f* (forked bristles); it was derived from the unstable chromosome of Lim (8) and will be symbolized simply as *Uc-l*^{r} (unstable chromosome lethal-reverted) (11). The stable *X* chromosome that we used carried the recessive marker *m* (miniature wings) and was unrelated to *Uc-l*^{r}. The stocks that carried each of these chromosomes were tested for their properties in the *P–M* and *I–R* systems of hybrid dysgenesis (12, 13) and were found to belong to the *IM* category.

Experimental Procedures. Initially the strategy was to map the agent of *Uc-l*^{r} instability by recombination methods. The procedure was to obtain recombinants between the *m* and *Uc-l*^{r} chromosomes and then to measure their mutabilities in standard *X*-linked lethal tests. From the variation in mutation rates, we hoped to determine which segment of the *Uc-l*^{r} chromosome was responsible for high mutability.

The recombinants were generated in 10 replicate experiments. Each of these began by mating a single *Uc-l*^{r} male with *m/m* females who had the stable *X* chromosome. The *m/Uc-l*^{r} daughters and *m* sons from each mating were then crossed to one another to produce recombinants. From each cross we collected 10 males from each of seven genotypic classes, including the parental types and five types of recombinants. These were: I, y^{59b} *z* w^i ct^6 + *f* (*Uc-l*^{r}); II, + + + + *m* +; III, y^{59b} *z* w^i ct^6 *m* +; IV, + + + + + *f*; V, y^{59b} *z* w^i ct^6 + +; VI, + + + + *m f*; and VII, + + + ct^6 + *f*. We also collected *m* males from matings of *Uc-l*^{r} males to *m/m* females; these males have a set of autosomes from the *Uc-l*^{r} stock, but not *Uc-l*^{r} itself. Finally, we collected *m* males directly from the *m* stock. These last two types of males were used as controls in the *X*-linked lethal tests.

Each of the males that we collected was mated separately to *Df(1)Basc/Df(1)*w^{rJ1} females. The *Df(1)Basc* and *Df(1)*w^{rJ1} chromosomes carry nonoverlapping deficiencies that behave as recessive lethals. More complete descriptions of these and other chromosomes used can be found in ref. 14. From each mating, *Df(1)Basc/C* daughters were collected and crossed to *FM6/Y* males. Here "*C*" refers to one of the seven possible *X* chromosomes or to the control *X* chromosomes, while *Df(1)Basc* and *FM6* are balancer *X* chromosomes with the dominant marker *B* (Bar eyes). The mated *Df(1)Basc/C* females were then placed individually in culture tubes and incubated according to the procedures of ref. 15 to test if the "*C*" chromosome had acquired a recessive lethal mutation. The scoring and verification

Abbreviation: *Uc-l*^{r}, unstable chromosome lethal-reverted.

procedures of ref. 15 were followed.

Each of the lethal *X* chromosomes identified in these tests was kept in stock by mating *FM6/l* females with *FM6/Y* males. Representative lethal chromosomes were tested genetically for the location of the lethal mutation and examined cytologically for structural aberrations. A set of five deficiencies in the *X* chromosome and compensating duplications on the *Y* chromosome was used for mapping. The chromosomes used can be found in refs. 8, 11, 14, and 16; they included $Df(1)y^{75e}/y^{2}Y^{67g}$, $Df(1)TEM\text{-}1/B^{s}w^{+}y^{+}Y$, $Df(1)ct^{78}/y^{+}ct^{+}Y$, $Df(1)m^{259-4}/y^{+}Y\ v^{+}B^{s-}$, and $Df(1)mal^{3}/y^{+}Y\ mal^{106}$.

For the cytological examination, balanced lethal heterozygotes were mated individually to $y^{59b}\ z\ w^{i}\ ct^{6}\ f$ or to $y\ sn^{3}\ v$ males from stable stocks to obtain female larvae carrying the lethal chromosome. Salivary glands from these were dissected and squashed in the usual way (8).

Media. Standard Drosophila cornmeal/molasses medium was used throughout these experiments except in the tube cultures of the *X*-linked lethal tests, where a sugar/yeast medium (15) was used.

RESULTS

X-Linked Lethal Mutation Rates. We show the results of the experiments to detect *X*-linked lethal mutations in Table 1. The mutation rates were calculated by the unweighted procedure of Engels (17), which gives the minimum variance when clusters of mutants occur. There was also a small adjustment for possible lethals among the unclassifiable chromosomes (15). Five of the seven values for the parental and recombinant classes were between 2.1% and 3.3%, and the extremes of the range were 1.3% and 5.5%. A χ^2 test for heterogeneity using the number of independent mutational events showed no significant difference among the seven classes. Clearly, every class of chromosome is highly mutable when compared to the control classes given at the bottom of the table. Actually, the mutation rates of the control chromosomes are less than the conventionally cited value of 0.25% (18), but even this rate is an order of magnitude less than the experimental results.

The control classes refer to chromosomes that came from the *m* stock without having had any association with *Uc-l*^{r}; 3,619 control *X* chromosomes taken directly from the *m* stock were tested for lethal mutations, and none was found; 4,368 *m* chromosomes that had been associated in males with the autsomes of the *Uc-l*^{r} stock but not with *Uc-l*^{r} itself were tested for lethal mutations, and only 2 were found. Thus, in the absence of any association with the *Uc-l*^{r} chromosome in females, the *m* chromosome has a low mutation rate. Moreover, the autosomes of the *Uc-l*^{r} stock have no appreciable effect on the mutability of the *m* chromosome.

In contrast, the *Uc-l*^{r} chromosome and all the recombinants derived from it were highly mutable. Moreover, there was no obvious relationship between recombinant genotype and degree of mutability. All of the mutation rates for classes III–VII were increased significantly above the control rates. Thus, the agent responsible for the high mutability of the *Uc-l*^{r} chromosome cannot be localized by conventional recombination methods.

The most striking result in Table 1 is the high mutability of the *m* chromosomes of class II. These chromosomes were recovered as nonrecombinants in males from the crosses that generated recombinant classes III–VII. The data show that of all the chromosomes tested, these nonrecombinants were the most mutable. The estimated mutation rate was 5.5%. Altogether 29 of the 100 independently derived nonrecombinant *m* males that were tested produced at least one *X*-linked lethal mutation in the sperm sampled. The average number of sperm sampled per male was 31.84. These results imply that an *m* chromosome associated with *Uc-l*^{r} for only one generation can acquire the tendency to mutate to the lethal condition at a high rate. This property is apparently acquired without ordinary meiotic recombination and could be explained by the transposition of a mutator factor from *Uc-l*^{r} to its homologue.

In order to check the consistency of results among the chromosomes obtained from the 10 replicate experiments, we calculated the lethal mutation rates for each experiment. These rates pool the data from the seven genotypic classes that were obtained from each of the recombination experiments. All experiments except two gave mutation rates between 1.6% and 5.9%. The two exceptional rates were 0.57 ± 0.24% and 0.08 ± 0.06%, suggesting that in two of the initial crosses, the agent responsible for the high mutability of the *Uc-l*^{r} chromosome had been lost.

Mapping the Lethal Mutations. Altogether, over 500 lethal *X* chromosomes were identified in these experiments; some occurred in clusters in the progeny of a single male and, therefore, probably were derived from a single mutational event. We selected a sample of lethals in such a way that each cluster was represented only once and then proceeded to map the lethals using five duplication/deficiency stocks. The procedures permitted us to screen ≈20% of the euchromatic *X* chromosome for the location of each lethal. This 20% included segments around the loci *y*, *w*, *ct*, *m*, and *mal* (maroon-like eyes). Any

Table 1. *X*-linked lethal mutation rates by genotypic class

Genotype	Class	Males tested, no.	Chromosomes tested, no.	Lethal chromosomes, no.	Independent events, no.	Mutation rate ± SE
y z w ct + f	I	100	3,260	103	21	0.0329 ± 0.0096
+ + + + m +	II	100	3,184	183	29	0.0549 ± 0.0147
y z w ct m +	III	93	3,188	53	17	0.0260 ± 0.0093
+ + + + + f	IV	100	3,147	62	15	0.0212 ± 0.0069
y z w ct + +	V	100	2,769	35	16	0.0126 ± 0.0037
+ + + + m f	VI	100	3,072	79	22	0.0253 ± 0.0063
+ + + ct + f	VII	100	3,099	70	22	0.0253 ± 0.0065
	Total	693	20,719	575	142	
+ + + + m +	Control*	138	3,619	0	0	—
+ + + + m +	Control†	197	4,368	2	2	0.0004 ± 0.0003

The portion of the genotype from the stable *m* chromosome is underlined.

*These males were taken directly from the *m* stock.

†These males came from the mating *m/m* females × *Uc-l*^{r} males and, therefore, carried autosomes from the *Uc-l*^{r} stock.

Table 2. Lethal mapping data

Genotype	Class	Map region						
		y	*w*	*ct*	*m*	*mal*	Not located	Total
y z w *ct + f*	I	1	1	9			10	21
+ + + *+ m +*	II	1		3	2		22	28
y z w *ct m +*	III			3	1			12
+ + + *+ + f*	IV			3	1		11	15
y z w *ct + +*	V			2			11	13
+ + + *+ m f*	VI			2			19	21
+ + + *ct + f*	VII		2	4			11	17
	Total	2	3	26	4	0	92	127

The portion of the genotype from the stable *m* chromosome is underlined. The regions of the *X* chromosome corresponding to the duplications in the tester stocks are given at the top. The entries in the table are the number of chromosomes in each class whose lethal mutation mapped in the region.

lethal mutation lying in the remaining 80% of the euchromatin obviously could not be localized. Counting singletons, there were 142 independent mutational events causing lethals; we obtained mapping data for 127 of these.

Thirty-five of the 127 lethals could be localized to one of the five regions by using these mapping methods. None of the 35 was located in the *mal* region, but there were 2 in the *y* region, 3 in the *w* region, 4 in the *m* region, and 26 in the *ct* region. The complete data are given in Table 2, which enumerates the lethals by location and genotypic class.

Twenty-six of the lethals affected the *ct* region, which constitutes ≈4% of the euchromatic *X* chromosome, as judged by the number of polytene bands in the duplication of the *ct* tester stock. Therefore, we might expect that ≈4% of all lethals tested would map in this region; however, 20.4% actually did map there, a result that deviates significantly from the naive expectation as judged by a standard likelihood ratio test. Thus, among the chromosomes tested, the *ct* region appears to be preferentially mutable, corroborating previous data (8, 11).

None of the 127 lethals that we tested mapped in the *mal* region. This suggests that the *mal* region might be a mutational cold-spot in this mutator system. There is no strong evidence that any of the other regions we screened—*y*, *w*, and *m*—are either hot-spots or cold-spots.

The conclusion that the *ct* region is a mutational hot-spot is based on all of the mapping data. However, chromosomes of four of the seven classes used in these experiments contained a *ct* region that was derived from *Uc-l*^{r} and might, on the basis of previous work (8, 11), be expected to acquire lethals in the vicinity of *ct*. Thus, it is important to ascertain if the chromosomes that did not contain the *ct* region derived from *Uc-l*^{r} were also preferentially mutable there. This can be done by pooling the results of classes II, IV, and VI, from which 64 lethals were mapped; of these, 8 were localized to the *ct* region (and in 7 cases, also were associated coincidentally with new mutations of the *ct* locus itself). A likelihood ratio test establishes that this result deviates significantly from that expected under the hypothesis that the mutability of the *ct* region is proportional to its size. Thus, even chromosomes lacking the *Uc-l*^{r}-derived *ct* region show a propensity to acquire lethals around *ct*.

The two lethal mutations detected on the control chromosomes were also subjected to mapping tests. One mapped in the *m* region, and the other could not be localized to any of the five regions tested.

Cytological Analysis. Altogether, 140 chromosome lines were examined cytologically, but only 122 of these were mapped. The discrepancy is due to the inclusion of some incompletely lethal lines in the set examined cytologically and to sterility in some of the matings.

Throughout the cytological analysis, at least two larvae from a single carrier female of each line were examined. When an aberration was detected in one of these larvae, additional larvae were sometimes examined, including those produced by sibs of the carrier female. Altogether, *X* chromosome aberrations were detected in 13 of the 140 lines studied. These included deficiencies, inversions, duplications, a transposition within the *X* chromosome, and a translocation between the *X* and the second chromosome.

There was some variation in the structure of the *X* chromosome in the larvae of single carrier females. Four lines each segregated an aberrant *X* chromosome in one larva and normal *X* chromosomes in the other larvae examined. One line segregated two different aberrations, one of which was an *X*;2 translocation, seen only in a single larva. Three of the aberrations detected seemed to be associated with a lethal mutation in the *ct* region. Two of these were deficiencies and the third was an inversion, all with breaks in the *6F–7A* section of the chromosome. In all three cases, the *ct* region was derived from the *Uc-l*^{r} chromosome.

The distribution of the breakpoints associated with the aberrations is given in Table 3. There were 30 independent breakpoints altogether, with the *6F* and *9F* regions preferentially involved. Within each of these regions it was not possible to determine if there was more than one breakage site. Eight of the 21 breakpoints observed on chromosomes with a *Uc-l*^{r}-derived *ct* region were in the *6F* section of the chromosome; however, only one of the nine breaks that were detected on the chromosomes without a *Uc-l*^{r}-derived *ct* region was in the *6F* section. Four of the breaks on these were in the *9F* section, whereas only 2 of the 21 breaks on the chromosomes with a *ct* region from the *Uc-l*^{r} chromosome involved the *9F* section. It seems, therefore, that *6F* is a breakage hot-spot on chromo-

Table 3. Distribution of chromosome breakpoints.

	Breakpoints per chromosome region													Total breakpoints
	1A	*5A*	*5D*	*6F*	*7A*	*7E*	*8A*	*8F*	*9A*	*9F*	*11B*	*12A*	*17A*	
Frequency	1	1	1	9	3	1	1	2	2	6	1	1	1	30

somes with a *ct* region derived from *Uc-l*^{r} and that *9F* might be a warm-spot on the other chromosomes.

DISCUSSION

Previous publications (8, 9) described the properties of an unstable *X* chromosome, called *Uc*, which arose in a laboratory stock with a history of ethyl methanesulfonate treatment. This chromosome accumulated lethal mutations and structural rearrangements at a high rate, with the preponderance of these events involving sites near the *ct* locus in the *6F–7C* region of the polytene chromosome map. The mutations and rearrangements sometimes arose in clusters, indicating a premeiotic origin, and were evidently unstable, as judged by their ability to revert to the wild-type condition. We hypothesize that the properties of the *Uc* chromosome are attributable to a transposable element, designated the *L* factor.

This paper concerns a derivative of the *Uc* chromosome that had a reverted lethal mutation that had mapped in the *ct* region. This derivative, known as *Uc-l*^{r}, exhibits the same instabilities as the *Uc* chromosome (11). Our data show that stable *X* chromosomes not related to *Uc* become unstable after being associated with *Uc-l*^{r} for only one generation. We call this change in the stable chromosome brought about by its unstable partner homologue destabilization. This phenomenon could reflect the transposition of *L* factors from *Uc-l*^{r} to its stable homologue during their temporary association in females and the subsequent activation of these factors a generation later in males, leading to lethal mutations in the males' germ cells. A strong prediction of this explanation is that destabilized *X* chromosomes should be able to destabilize other *X* chromosomes in turn.

If autosomal *L* factors exist, they do not seem to act directly on the stable *X* chromosomes of males, so long as these have not been associated with *Uc-l*^{r}. The evidence is that males with the stable *X* chromosome and autosomes from the *Uc-l*^{r} stock seldom produce *X*-linked lethal mutations. This implies either that autosomal *L* factors do not exist, that they do not transpose to the *X* chromosome in males, or that, if they do transpose to it, they do not produce lethal mutations immediately. Our data do not rule out transposition of *L* factors from the autosomes to the *X* chromosome in females, followed by local mutational action in males a generation later. This situation differs from the "chromosome contamination" that occurs in the *P–M* system of hybrid dysgenesis, in which a transposable element moves readily from the autosomes to the *X* chromosome in males (19, 20); however, in the *I–R* system (19, 21), this sort of movement seems to occur only in females. Moreover, the element that is involved is mutationally inactive in males.

The preferential involvement of the *ct* region of the *Uc* and *Uc-l*^{r} chromosomes in mutational and breakage events suggests that some element or set of elements is located there and that this is the main agent of chromosome instability. Prokaryotic transposable elements generate mutations and structural rearrangements at or near their sites of insertion, as do some of the other transposable elements known in *Drosophila* (6, 10, 22, 23). In this respect, it is interesting to note that a preferentially mutated site on the destabilized chromosomes is also near *ct*. This could be explained if the *L* factor postulated to reside in the *ct* region of the *Uc-l*^{r} chromosome transposes preferentially to a corresponding location on its homologue. So far no other transposable element has been shown to have this behavior. It is also possible that the high frequency of lethal mutations in the *ct* region of the destabilized chromosome may simply reflect a general susceptibility of that region to *L* factor insertion.

Of course, it is quite possible that there may be other sites on the unstable chromosome where the *L* factor resides, such as at the breakpoints of *L*-induced rearrangements that lie outside the *ct* region. It is also possible that the *L* factor does not naturally reside near *ct* but that it preferentially transposes there.

As discussed here, the *L* factor is an entity postulated to explain the properties of the *Uc* and *Uc-l*^{r} chromosomes. Its physical nature is currently unknown. It may be similar or even identical to one of the other transposons known in *D. melanogaster*. These include the "nomadic sequences" (7) such as *copia*, *412*, and *297* (24), *FB* (25), and the elements responsible for some of the mutations of the white locus, such as w^{DZL} (23, 26) and w^c (27, 28). Only a molecular analysis can address this and other questions, such as how the *L* factor functions. This analysis is currently underway. However, on the basis of genetic experiments alone it is possible to exclude some transposons from consideration. These are the *P* and *I* factors, which are mobilized only in dysgenic hybrids (19). Our experiments were performed so as to avoid *P–M* and *I–R* dysgenic interactions, so the results are not likely to be due to the action of *P* or *I* factors.

The principle of Mendelian segregation rests on the fact that ordinarily a chromosome does not change the structure or properties of its partner. The phenomenon of homologue destabilization is an exception to this rule, but how widespread it is has yet to be determined.

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