Cytogenetics of *Notch* Mutations Arising in the Unstable *X* Chromosome *Uc* of *Drosophila melanogaster*

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ABSTRACT

A derivative of the unstable *X* chromosome, *Uc*, isolated in 1978 is still unstable and exhibits most of the genetic properties characteristic of the original *Uc*. This derivative, *Df(1)cm-In*, contains an inversion of the genes between bands 6F1-2 and 3D3-5 and a lethal deficiency between 6D5-7 and 6F1-2. This chromosome generated *Notch* mutations at a rate of 3.47 ± 0.32% during seven consecutive generations. Cytological analysis of 50 *Notch* mutations of independent origin in the *Df(1)cm-In* chromosome showed that all of the 50 had an apparently identical deletion involving the region between 3D3-5 and 3C7-8 of the *X* chromosome. The results of *in situ* hybridization indicated that the extent of deletion in all of the 20 *Notch* deficiencies sampled from the 50 mentioned above involves about 10 kb of the sequences from the 3′ end of the *Notch* locus. In addition to hypermutability and the accumulation of site-specific chromosome breaks, the *Df(1)cm-In* chromosome reinverts its inversion to the normal sequence and exhibits use of the existing chromosome breakpoints to generate new rearrangements.

THE unstable *X* chromosome *Uc* was discovered in February of 1978 among ethyl methanesulfonate-treated *X* chromosomes of a long time laboratory strain of *Drosophila melanogaster* (LIM 1979). Many different derivatives of the *Uc* are now available: Most carry one or more recessive lethal mutations (*Uc-l*), but some are lethal-free due to reversion of these mutations; these latter chromosomes are symbolized *Uc-l*^{r}. Still other derivatives of *Uc* carry a secondary lethal mutation which occurred after the reversion of the primary lethal mutation. Finally, some *Uc* derivatives carry mutations in visible *X*-linked loci.

Many of these *Uc*-derived *X* chromosomes have shown peculiar genetic properties, including hypermutability, accumulation of structural aberrations confined to the *X* chromosome, reinversion of existing inversions, generation of attached-*X* chromosomes in the male germline cells, involvement of an existing chromosome breakpoint to generate a more complex rearrangement, and destabilization of an ordinarily stable *X* chromosome which had been paired with *Uc*. The mutations and breaks on these chromosomes show site specificity since most of the rearrangements have a break in bands 6F1-2 and many mutations are in and around the 6F1-2 doublet and the *cut* locus (LIM 1979, 1980, 1981; LAVERTY and LIM 1982; LIM *et al.* 1983; LIM and CROWE 1984).

When the phenotype of an inherited trait is chromosome instability, a drastic change in the approach to the study of the trait is essential. One approach is to focus on a particular chromosome which produces stable mutations and to analyze these mutations. We selected a derivative of *Uc-l* designated as *Df(1)cm-In*, which contains an inversion of the region between 6F1-2 and 3D3-5 and a lethal deficiency between 6D5-7 and 6F1-2, and studied the occurrence of *Notch* mutations in this chromosome. We show that the *Df(1)cm-In* chromosome produces *Notch* mutations at a high rate and that these mutations are invariably deletions in the *Notch* locus. In addition to this site-specific hypermutability, the *Df(1)cm-In* chromosome exhibits many genetic properties characteristic of the original Uc.

MATERIALS AND METHODS

Flies were reared on standard cornmeal-molasses brewers' yeast-agar medium at 25°. A description of the chromosomes used in this study is summarized in Table 1. Additional information on mutant symbols can be found in LINDSLEY and GRELL (1968).

Isolation of *Notch* mutations: The *X* chromosomes used in this study can be traced to a lethal mutation detected in the unstable *X* chromosome, *Uc-l*, designated as *IJD15* isolated in February 1978 (LIM 1979). Because of its lethal mutation, the *X* chromosome was maintained in heterozygous condition with an *FM6 X* chromosome in females, and the heterozygous females were mated to *FM6/Y* males. This cross produces only two types of fertile flies: *Uc-l/FM6* females and *FM6/Y* males. *FM6/FM6* females are sterile and the zygotes of the constitution *Uc-l/Y* males are inviable due to the lethal mutation.

Over the years, this *Uc-l* chromosome acquired a number of rearrangements resulting in the emergence of several

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TABLE 1

Chromosomes used in this study

Abbreviation	Description
Uc-1^{IJD15}	Unstable *X* chromosome (*Uc*) with a *Uc*-generated lethal mutation (*IJD15*) and the recessive markers *y^{59b}*, *z*, *w^{i}*, *ct^{6}*, and *f*
In(1)3D;6F	A paracentric inversion recurring in many *Uc-1* chromosomes. A line of *Uc-1^{IJD15}* with this inversion gave rise to *In-Df(1)ct* and *Df(1)cm-In*
In-Df(1)ct	*In(1)3D;6F* with a *ct* deficiency, *Df(1)6F;7C*, derived from *Uc-1^{IJD15}*. This chromosome is maintained as stock no. 42 in the Eau Claire stock collection
Df(1)cm-In	*In(1)3D;6D* with a *cm* deficiency, *Df(1)6D;6F*, derived from *Uc-1^{IJD15}*. This chromosome is maintained as stock no. 24 in the Eau Claire stock collection
Df(1)N, *Df(1)cm-In*	*Df(1)3C7;3D* generated in the *Df(1)cm-In* chromosome. A full description of the rearrangements for this chromosome is *Df(1)3C7;3D*, *Df(1)6D;6F*, *In(1)3D;6D*. The markers in the chromosome are *y^{59b}*, *z*, *w^{i}*, *N^{-}*, *cm^{-}*, *ct^{6}*, and *f*
Fm6	First multiple inversions 6; carries multiple inversions and *y^{31d}*, *sc^{8}*, *dm*, and *B* mutant markers. Homozygous females are viable but sterile because of *dm* but males are viable and fertile
FM6K	First multiple inversions 6, Kidd; carries the multiple inversions and *y^{31d}*, *w^{i}*, *sc^{8}*, *dm^{+}*, and *B* mutant markers; homozygous females are viable and fertile
C(1)DX, yf	An attached *X* chromosome homozygous for *y* and *f*; used in maintaining males carrying the *Uc X* chromosome patroclinously
y^{+}ct^{+}·Y	A *Y* chromosome with two segments of the *X* chromosome: one containing *y^{+}* and a 6D to 7D segment containing *ct^{+}*

derivatives. Among the earlier rearrangements detected in the *IJD15* line was *In(1)3D;6F*. The *Df(1)cm-In* chromosome used in this study carries *In(1)3D;6F* with a deletion extending from 6F1-2 to 6D5-7 in the inverted region.

The mating scheme used for detecting of *Notch* mutations arising in the *Df(1)cm-In X* chromosome is shown in Figure 1. Initially, 29 females of genetic constitution *FM6/Df(1)cm-In* (*FM6/Uc-l* in Figure 1) were mated to *FM6/Y* males and the offspring from each of the mated females were tallied. The flies used in this study were from stock no. 24 of the Eau Claire stock collection and were represented by G_0 females in Figure 1. Nine of the 29 *FM6/Df(1)cm-In* females produced three types of G_1 daughters: *FM6/FM6* females homozygous for *B*; *Df(1)cm-In/FM6* females, heterozygous for *B* and like their mother; and females heterozygous for *B* but with *Notch* wings, *N,Df(1)cm-In/FM6*. The remaining 20 females produced only two types of G_1 daughters: *FM6/FM6* and *Df(1)cm-In/FM6* females.

The normal-winged sisters, *Df(1)cm-In/FM6*, of *Notch*-winged females, *N,Df(1)cm-In/FM6*, in the nine vials were designated as class A females. The normal-winged carrier females, *Df(1)cm-In/FM6*, in the remaining 20 vials (vials without *Notch* females) were designated as class B females. The practice of designating the normal-winged sisters of *Notch* females as class A and the normal-winged females in the vials without *Notch* wing females as class B was used for the subsequent five generations (G_2–G_6). Because of the instability associated with the *X* chromosome, a female of either class may produce normal-winged daughters of either class, as shown in Figure 1.

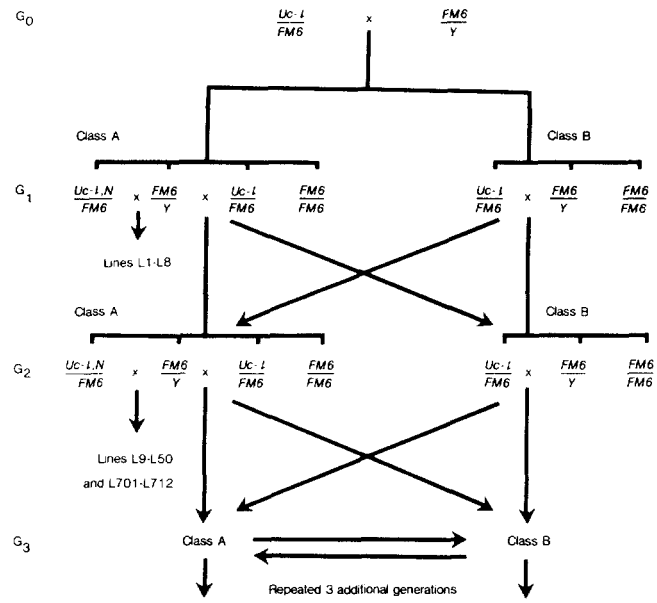

FIGURE 1.—The mating scheme for detection of *Notch* mutations in the *Df(1)cm-In X* chromosome, *Uc-l*.

Throughout the testing, the inseminated carrier females were placed individually in vials and their *B/B^{+}* daughters (carriers) were tallied according to wing phenotype up to the 18th day from the date of establishing the cultures.

Cytological analysis: The *Notch* females, *N,Df(1)cm-In/FM6*, detected were mated with *In-Df(1)ct/y^{+}ct^{+}·Y* males and female larvae with light brown mouth parts, *N,Df(1)cm-In/In-Df(1)ct*, were selected for analysis of polytene chromosomes. The *Df(1)cm-In* and *In-Df(1)ct* chromosomes share the same inversion since both of these *X* chromosomes were derived from *Uc-l* line *IJD15* (see Table 1). Therefore, heterozygotes for *Df(1)N*, *Df(1)cm-In* and *In-Df*(1)*ct*, are free of an inversion loop, but have two prominent "buckles," one for each of the deletions as shown in panel a, Figure 2. We found that the combination of these two *X* chromosomes makes it easier to analyze deletions around the *Notch* locus.

The technique for chromosome squash preparations for cytological analysis has been described (LIM and SNYDER 1968). All cytological preparations were made permanent by mounting with Euparal. With a few exceptions, at least 10–15 nuclei per slide were examined for cytological analysis. In addition to the traditional cytological analysis using orcein-stained slides, unstained slides prepared for *in situ* hybridization were examined with phase contrast microscope for gross structural aberrations. The 50 *Notch* mutants used for cytological analysis were taken from the mutants detected in the G_1 and G_2 progeny, as indicated in Figure 1. One to three larvae were examined cytologically for each stock.

In addition, *FM6/Df(1)N*, *Df(1)cm-In* females were mated to *FM6K/Y* males and the female larvae with colorless Malpighian tubules, *FM6K/Df(1)N*, *Df(1)cm-In*, were selected to prepare polytene chromosome squashes. In these preparations, the proximal half of the *Df(1)N*, *Df(1)cm-In X* chromosome tends to be asynaptic. This situation was exploited whenever it was desirable to examine asynapsed *Df(1)N*, *Df(1)cm-In X* chromosomes.

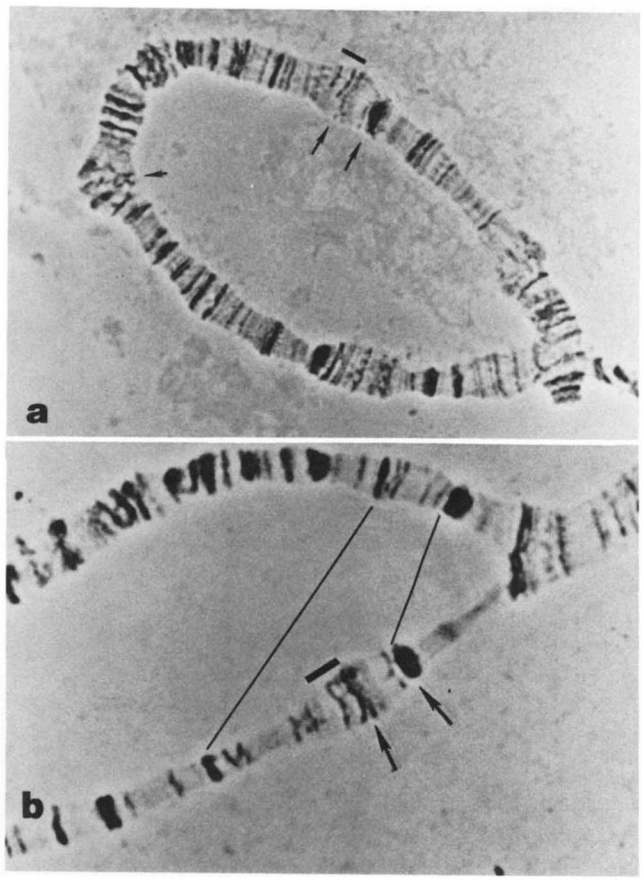

FIGURE 2.—The polytene chromosomes stained with lacto-acetic arcein for cytological analysis. a, The polytene chromosomes of a female heterozygous for *Df(1)cm-In* and *In-Df(1)ct X* chromosomes. The chromosome segment indicated by a bar at the upper center (6D to 6F including the *cm* locus) is deleted in the *Df(1)cm-In* chromosome (lower one). A small arrow at the left indicates *Df(1)6F;7C* designated as *Df(1)ct* in the *In-Df(1)ct X* chromosome. Two larger arrows at the upper center indicate the region between 3D and 3C7 still intact in the *Df(1)cm-In X* chromosome. b, The polytene chromosomes of a female heterozygous for *Df(1)N*, *Df(1)cm-In* and *In-Df(1)ct*. The longer of the two thin lines connecting the two *X* chromosomes indicates 6A1-2 and the shorter one indicates the approximate position of 3C7 in the 3C1-7 complex. A bar associated with the *In-Df(1)ct* chromosome (lower one) indicates the region corresponding to *Df(1)cm* in the *Df(1)N*, *Df(1)cm-In* chromosome (upper one) and two arrows indicate the region deleted to generate *Df(1)N* in the *Df(1)N*, *Df(1)cm-In X* chromosome.

A total of 95 slides of *Df(1)cm-In/In-Df(1)ct* female larvae were prepared to represent the *Df(1)cm-In X* chromosome without *Notch* mutation. These slides were also made permanent, and more than one nucleus per slide were analyzed.

***In situ* hybridization of polytene chromosomes:** Larvae were dissected in 45% acetic acid, and the salivary glands were transferred to a fresh drop of 45% acetic acid to remove the membrane enclosing the gland cells. The gland cells were placed in a fixative composed of lactic acid (1 part), water (2 parts) and glacial acetic acid (3 parts) for three to four minutes, and then squashed in the fixative. The slides with chromosome squashes were kept at room temperature for 10–15 hr, followed by storage in 95% ethanol at −80° for 2–4 days before removing the cover glass. For all slides, the salivary glands from a single larva were squashed for each slide.

For removing the cover glasses, the slides were placed in 95% ethanol at −80° for 1–2 hr; then the cover glasses were pried off with a razor blade, and the slides were returned to 95% ethanol at −80°. The slides were then allowed to return to room temperature gradually by placing the staining dish with the cold ethanol and slides at room temperature for a few hours; after this, the slides were kept in 95% ethanol for an additional 10–20 hr, and then air-dried. The air-dried slides were placed in 2 × SSC, 20 mM Pipes (pH 7.0) and incubated at 65° for 1 hr for heat treatment. Heat-treated slides were then dehydrated by passing three times through 70% ethanol for 10 min each, twice through 95% ethanol for several minutes each, and then air dried.

Chromosomal DNA was denatured by placing the slides in 0.07 M NaOH in 2 × SSC for 3 min, and the nick-translated probe DNA ($3–8 \times 10^7$ dpm/μg) was denatured by heating at 95–100° for 4 min and then quenched in an ice bath. A procedure similar to the one described by BINGHAM and JUDD (1981) was used for hybridization, and the probe DNA was nick translated according to the protocol of MANIATIS, FRITSCH and SAMBROOK (1982).

After about 20 hr of hybridization at room temperature, the cover glasses were gently floated off in 2 × SSC, and the slides were incubated in a dish of ribonuclease-A (10 μg/ml) for 1 hr in 2 × SSC prewarmed to 37°. The slides were then dehydrated by passing through 70% ethanol three times and 95% ethanol twice (about 10 min each), and air dried.

The hybridized slides were dipped in Kodak NTB-2 emulsion (1:1 dilution with deionized-distilled water) and were dried at room temperature for several hours before placing them in a dark box with desiccant. The exposure period was several to 10 days depending upon the material. The developed and fixed slides were stained in 5% Giemsa stain in 50 mM phosphate buffer (pH 7.0) for 20 min, washed in water and dried overnight at room temperature. Euparal was used to make the slides permanent and the slides were observed with bright field optics.

A set of four overlapping clones spanning the entire *Notch* locus (*NL35*, *NL22*, *N2* and *NR311*) described by KIDD, LOCKETT and YOUNG (1983), obtained from Dr. M. W. YOUNG, was used as the probes in the initial *in situ* hybridization experiments. The four *Eco*RI fragments of *N2* (4.9, 1.3, 2.2 and 6.2 kb) subcloned into pBR322 and maintained in the *Escherichia coli* strain C600, were used for a sharper definition of the deletion breakpoint [see MANIATIS, FRITSCH and SAMBROOK (1982) for subcloning protocol].

The *Notch* locus is among the most extensively analysed genetic loci in *Drosophila*. For the physical map and the overlapping recombinant phages containing the locus, our discussion will conform to the terminology and symbols in Figure 2 of KIDD, LOCKETT and YOUNG (1983). For molecular alterations within the locus and a correlation between physical, genetic, and exonic region maps of the locus, the readers are referred to Figure 1 of GRIMWADE *et al.* (1985) and Figure 1 of WHARTON *et al.* (1985). The overlapping *Notch* clones of KIDD, LOCKETT and YOUNG (1983) in relation to the physical, genetic and exonic region maps are shown in Figure 3.

RESULTS

***Notch* mutations arising in the *Df(1)cm-In* chromosomes:** The results of breeding experiments spanning seven consecutive generations for detection of *Notch* mutations arising in the *Df(1)cm-In/FM6* fe-

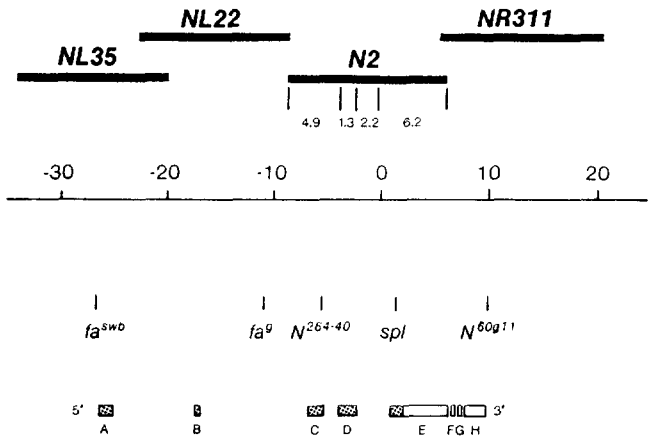

FIGURE 3.—Genetic, physical and exonic region maps of *Notch* locus in relation to the four overlapping *Notch* clones used in the study. Thick bars at the top represent approximate position of four overlapping *Notch* clones of KIDD, LOCKETT and YOUNG (1983) in relation to the scale in kb (−30 to 20), the distribution of five selected *Notch* alleles and relative position of *Notch* exonic regions (A–H). The four sections associated with *N2* clone are juxtaposed *Eco*RI fragments which has been subcloned. The stippled exonic regions (A–D and the 5′ end of E) are the parts of the *Notch* RNA corresponding to the 36 tandemly arranged 40 amino acid repeats. [Modified from KIDD, LOCKETT and YOUNG (1983) and WHARTON *et al.* (1985).]

males are summarized in Table 2. Of the 1316 *Df(1)cm-In/FM6* females (carrier females), 252 produced one or more daughters with *Notch* wings. The number of females which produced more than one *Notch* daughter is shown in parentheses in the third column of the table; thus 9 (7) in G_0 generation means seven of the nine females with *Notch* daughters produced more than one *Notch* fly. Progeny number in the fifth column represents the average number of carrier daughters (including *Notch* flies) for each class. Most of the *Notch* mutations originated premeiotically; 154 of the 252 females with *Notch* daughters produced more than one *Notch* daughter. The mutation rates were estimated by the unweighted formula of ENGELS (1979b).

The rate of *Notch* mutation by class A females ranged from a high of 7.96 ± 2.41% in G_1 to a low of 0.41 ± 0.24% in G_5. The range of the rates observed for the class B females was between a high value of 4.37 ± 0.87% in G_1 to a low one of 1.80 ± 0.58% in G_4. The 485 class A females in six consecutive generations (G_1–G_6) produced *Notch* mutations at a rate of 4.15 ± 0.61%; the rate for 802 class B females for this same period was 3.00 ± 0.36%. These results indicate no obvious difference between the two classes of carrier females in regard to the output of *Notch* mutations. A rank sum test in which each generation was tested as an independent experiment also showed no significant difference between the two classes of females. Our results seem to show a trend for decrease in *Notch* mutation production from G_0 to G_6. Kendall's *Tau* test for correlation between generation and mutation rate, however, indicates no significant difference.

The mutants detected were maintained by mating *Df(1)N, Df(1)cm-In/FM6* females with *FM6/Y* males. Viability and fertility of the females were, however, greatly reduced. Keeping duplicate copies and constant attention were therefore, necessary to maintain these *Notch* mutants.

When a *Df(1)cm-In* culture is established with a large number of carrier females (20 or more females to a vial), *Notch* mutations can hardly be detected. In individually mated cultures, such as the ones reported in this study, however, the output of *Notch* mutations is very high; about one in every five carrier females will produce at least one *Notch* daughter among her progeny. We feel that this negative correlation between population density and *Notch* mutation output apparently reflects larval competition resulting in selective elimination of *Notch*-deficiency-carrying larvae.

Cytological analysis: The *Uc-l* line selected for the study of *Notch* mutation production was known initially to have only one rearrangement, *In(1)3D;6F*. Examination of several larvae at the commencement of the study showed that the inversion had a small deletion adjacent to the left break of *In(1)3D;6F*, so that the *X* chromosome now had two rearrangements: *Df(1)6D;6F* and *In(1)3D;6D*. This *X* chromosome was designated *Df(1)cm-In*, since the deleted region includes the *cm* locus. *Notch* mutations arising in the *Df(1)cm-In* chromosome are documented in this study. Panel a in Figure 2 shows the *X* chromosomes from a larva heterozygous for *Df(1)cm-In* and *In-Df(1)ct*. The figure clearly shows that the region of the *X* chromosome from 3C1-7 to 3D3-5 is present in both of the *X* chromosomes.

A total of 253 larvae heterozygous for *N, Df(1)cm-In* and *In-Df(1)ct* representing 50 *Notch* mutations of independent origin were examined cytologically. The salivary gland chromosomes of 143 of the 253 were stained with lacto-acetic-orcein for cytological study. The unstained chromosomes of the remaining 110 larvae were prepared for *in situ* hybridization, but these unstained chromosomes were also analyzed for structural aberrations before proceeding with the hybridization. At least two to as many as eight larvae from each of the 50 *Notch* mutant lines were examined. All of the 253 slides showed an apparently identical deletion, *Df(1)3C7-8;3D3-5*, associated with the *Notch* mutations. Because the *Notch* deletion had occurred immediately adjacent to the existing deficiency of *Df(1)cm-In*, the new sequence of the *X* chromosome carrying the *Notch* deletion is tip to 3C7/6D to 3D/6F to base. This *X* chromosome was designated as *Df(1)N, Df(1)cm-In*. Panel b of Figure 2 shows the *X* chromosome from the right end of region 2 to the

TABLE 2

***Notch* mutations arising in the *Df(1)cm-In X* chromosomes**

Generation	No. of females: Tested	No. of females: With *Notch* daughters	Range of *Notch* cluster size	Progeny nos. mean ± SE (in %)	Mutation rate mean ± SE (in %)
G_0	29	9 (7)	9.1–52.9[a]	21.9 ± 7.4	4.63 ± 2.16
G_1, Class A	66	28 (18)	1.3–100	39.4 ± 18.8	7.96 ± 2.41
Class B	146	42 (33)	21.9–52.4	32.8 ± 16.1	4.37 ± 0.87
G_2, Class A	102	27 (16)	3.7–37.5	13.9 ± 8.1	4.88 ± 1.21
Class B	152	29 (14)	3.8–55.5	13.4 ± 6.8	2.79 ± 0.69
G_3, Class A	91	17 (13)	3.6–100	16.5 ± 12.6	6.15 ± 2.08
Class B	136	17 (9)	2.3–100	19.0 ± 14.7	3.18 ± 1.07
G_4, Class A	67	10 (5)	4.5–51.1	11.9 ± 6.8	2.92 ± 1.23
Class B	105	16 (7)	4.3–42.1	11.6 ± 8.5	1.80 ± 0.58
G_5, Class A	76	4 (1)	1.7–13.3	21.9 ± 12.8	0.41 ± 0.24
Class B	123	15 (6)	2.7–100	18.4 ± 11.8	2.99 ± 1.26
G_6, Class A	83	14 (9)	2.9–60.8	25.5 ± 10.1	2.47 ± 0.95
Class B	140	24 (16)	3.6–38.9	19.2 ± 8.3	2.59 ± 0.65

[a] No. of *Notch* daughters divided by total number of females carrying the *Df(1)cm-In* chromosome (including the *Notch* daughters) in percent.

beginning of region 7 including *In(1)3D;6D* and the double deletions.

The 3C1-7 bands are in a difficult region to stretch for cytological study. Among the cytological preparations, however, several contained well stretched chromosomes showing the deleted regions. The 3C7 band can clearly be detected in every one of these favorably stretched chromosomes. Whether or not the band 3C7 has been reduced in size cannot be determined objectively.

Likewise, 13 of the 95 larvae representing the control group (N^+ chromosomes) carrying the *Df(1)cm-In* chromosome were examined cytologically. These 95 larvae represent 31 sublines (cultures established by pair matings) of the *Df(1)cm-In X* chromosome. The chromosomes of 85 of the 95 larvae stained with lacto-acetic-orcein and unstained chromosomes prepared from 10 larvae for *in situ* hybridization were examined. A deletion of the region between 3C7-8 and 3D3-5 was observed in one of the 95 N^+ larvae; however, this region was not deleted in the remaining 94 control larvae.

As was expected of any *Uc* derivative, additional rearrangements were observed among both the 253 *Df(1)N,Df(1)cm-In* chromosomes and the 95 *Df(1)cm-In* chromosomes (N^+). They included the generation of an additional inversion, *In(1)3D;4E* in some lines, and precise reversion of the original *In(1)3D;6D* in others. Reinversion of preexisting inversions in the *Uc X* chromosomes has been observed previously (LIM 1979, 1980). Because of reduced fertility in *Notch* mutants, the larvae used for chromosome squashes were produced by a few carrier females reared together in a vial. For this reason, the timing of events associated with the generation of a new rearrangement cannot be determined. It is clear however, that

TABLE 3

Number of larvae with additional rearrangements

Chromosomes	Lines	*In(1)3D;4E*	Reinv.[b]	Total no. for the line
Df(1)N,Df(1)cm-In (253)[a]	N^{L26}	3		4
	N^{L27}		2	3
	N^{L42}		2	2
Df(1)cm-In (95)[a]	*7*	1		7
	32		1	1
	406	2		2
	430	2		2
	442	1		1
	469	2		2
	470	2		2
	H		1	7
	S		1	1

[a] Total number of larvae examined.
[b] Reinv. = reinversion of *In(1)3D;6D* to the normal sequence.

the additional rearrangements and structural changes, like the *Df(1)N* in the *Df(1)N,Df(1)cm-In* chromosome, were new rearrangements generated by involvement of one or two preexisting breakpoints [see leapfrogging of LIM (1979)].

The above cytological observations led us to conclude that: (1) all of the 50 *Notch* mutations of independent origin in the *Df(1)cm-In* chromosomes sampled for cytological studies were associated with an apparently identical deletion, *Df(1)3C7;3D*, and (2) in addition to the output of *Notch* deletions, some of the *Df(1)cm-In X* chromosome were generating new rearrangements by leapfrogging of the existing breakpoints at a high rate. The instability of breakpoints in the *Df(1)cm-In X* chromosome reflected in the leapfrogging is summarized in Table 3.

***In situ* hybridization:** Our strategy for the deter-

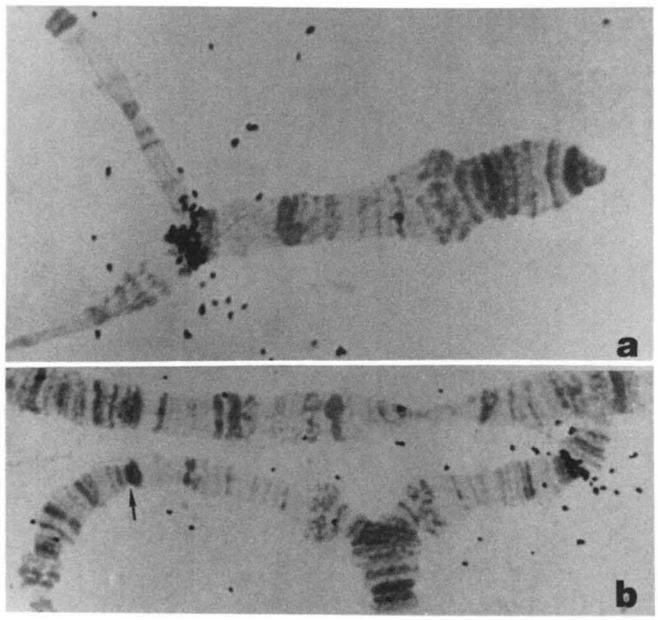

FIGURE 4.—*In situ* hybridized polytene chromosomes. a, The tip region of the *X* chromosomes in a female heterozygous for the *Df(1)N, Df(1)cm-In* chromosome (running toward the upper left corner) and the *In-Df(1)ct* chromosome (extending toward the lower left corner) hybridized with nick-translated *N2* clone. Note the more heavily labeled 3C7 region of the *In-Df(1)ct* chromosome. b, The tip region of the *X* chromosomes in a female heterozygous for the *In-Df(1)ct* (extending to the right) and *Df(1)N, Df(1)cm-In* chromosomes (extending to the left) hybridized with nick-translated *NR311* clone. An arrow to the left indicates the deficiency breakpoint free of silver grains. The 3C7 region in the *In-Df(1)ct* chromosome is heavily labeled.

mination of the extent of these *Notch* deletions was to probe the *Df(1)N, Df(1)cm-In X* chromosomes *in situ* with the 3′-most *Notch* sequence (*NR311*) and commence toward the 5′ end of the *Notch* locus. This strategy is the obvious one since our cytological data on the *Notch* mutants indicated that the region between *3D3-5* to *3C7-8* has been deleted. A total of 25 *Notch* mutants among the 50 we have analyzed cytologically were sampled for hybridization with the 3′-most *Notch* clone, *NR311*. Very clear results emerged from the study; the *Df(1)N, Df(1)cm-In X* chromosomes of all of the 25 mutants failed to hybridize with the probe, *NR311*. In contrast, a strong label was observed in the 3C7 band of the *In-Df(1)ct* chromosome in the same cell (see panel b of Figure 4). The result was quite consistent; all of the *In-Df(1)ct X* chromosomes showed a strong label over the 3C7 band, but none of the *Df(1)N, Df(1)cm-In X* chromosomes did.

The result of *in situ* hybridization with the next 3′-most *Notch* clone, *N2*, was quite different. A definitely weaker label over the 3C7 band of the *Df(1)N, Df(1)cm-In X* chromosomes, compared to the 3C7 label in the *Df(1)cm-In X* chromosomes, was observed in all nuclei in all slides representing the same set of 25 *Notch* mutants probed previously with *NR311* (see panel a, Figure 4). The number of silver grains in the *Df(1)N, Df(1)cm-In* chromosome divided by the number in the *In-Df(1)ct* chromosome gave the estimated mean value of 46.1 ± 17.5%. Based on the assumption that the number of grains is linearly related to the length of DNA, this value represents about 6.7 kb of the total *N2* clone. This was interpreted to mean that about 7.9 kb of the 14.6-kb *N2* clone were deleted in the *Notch* mutants. This would put the breakpoint within the 2.2-kb *Eco*RI fragment of the *N2* clone, which corresponds to the region between $N^{264\text{-}40}$ and *spl* in the genetic map (see Figure 3). In relation to the exonic region map, the breakage hotspots are in the region between exonic regions D and E.

As shown by KIDD, LOCKETT and YOUNG (1983), the recombinant phage *N2* yields 4 *Eco*RI fragments. These fragments, in the order from 3′ end to 5′ end, are 6.2, 2.2, 1.3, and 4.9 kb. None of the *X* chromosomes of 20 *Notch* mutants, of the 25 mutants probed with *NR311* and *N2*, hybridized with the 6.2-kb subclone of the *N2*. However, all of the *X* chromosomes of the same 20 *Notch* mutants, hybridized with the 2.2-kb subclone of *N2*, although the label in the *Df(1)N, Df(1)cm-In* chromosomes was decidedly weaker than that in the *In-Df(1)ct* homologues. These subclone hybridization data confirm our previous conclusion based on the silver grain counts in the *N2*-hybridized-slides.

A sample of 10, among the 20 *Notch* mutants mentioned above, were hybridized with the 1.3- and 4.9-kb *Eco*RI fragments of the *N2* clone. As was expected, both of these *N2* subclones hybridized strongly to the *Notch* locus of the *Df(1)N, Df(1)cm-In* chromosomes.

All of the *in situ* hybridization data mentioned above are consistent with our conclusion that the 50 *Notch* mutations arising in the *Df(1)N, Df(1)cm-In X* chromosomes have a break near the 3C7 band and that the breakpoint for 20 of those which were studied in more detail is within the 2.2-kb *Eco*RI fragment of the *N2* clone.

DISCUSSION

***Notch* deficiencies in the *Df(1)cm-In* chromosomes:** Recurrence of an apparently identical chromosome aberration in different stocks is one of the hallmarks of the *Uc X* chromosomes and it has been well documented (LIM 1979, 1980). The *Notch* deficiencies detected in the *Df(1)cm-In* chromosomes fit this pattern. This chromosome also shows another genetic property of the *Uc X* chromosome—involvement of existing chromosome breakpoints for generation of new rearrangements. *Uc-*l^{IJD15}, the ancestral lethal line from which *Df(1)cm-In* was derived, had no detectable aberrations. The very first aberration detected in the lethal line was *In(1)6F;10F* (LIM 1979), but other aberrations were observed in later genera-

tions. *In(1)3D;6F* detected in this line gave rise to at least two derivatives through leapfrogging: this inversion with *Df(1)6D;6F* which we designated as *Df(1)cm-In*, and this inversion with *Df(1)6F;7C*, designated as *In-Df(1)ct*. As shown in this study, the *Df(1)cm-In* chromosome generates *Notch* deficiencies, *Df(1)3D3-5;3C7-8*, at a rate of 3.47 ± 0.32%. In addition, *In(1)3D;6D* reinverts back to the normal sequence. *In(1)3D;4E*, which was not selected in the genetic screen employed here, also occurred a number of times in the *Df(1)cm-In* chromosome.

The *Notch* locus, because of its many faceted nature (WELSHONS 1965; WELSHONS and WELSHONS 1985, 1986), is among the most thoroughly studied genes in Drosophila (WELSHONS 1974; WELSHONS and KEPPY 1975, 1981; KEPPY and WELSHONS 1977, 1980). The entire locus encompassing about 40 kb of DNA sequences has been cloned (ARTAVANIS-TSAKONAS, MUSKAVITCH and YEDVOBNICK 1983; KIDD, LOCKETT and YOUNG 1983) the cDNA of its transcript has been isolated, the nucleotide and amino acid sequences of its transcript have been determined (WHARTON *et al.* 1985) and the role of this gene in neurogenesis has been established (LEHMANN *et al.* 1983).

The cytological position of the *Notch* locus, as determined by SLIZYNSKA (1938) and DEMEREC (1939), was band 3C7. More detailed and thorough cytogenetic and recombinational analysis (WELSHONS 1974; WELSHONS and KEPPY 1975, 1981) placed the locus to include the interband region immediately to the right of 3C7. Our results support this placement. The break position of the *Notch* deficiency in the *Df(1)cm-In* chromosome is within the 2.2-kb *Eco*RI fragment of the *N2* clone (KIDD, LOCKETT and YOUNG, 1983) which corresponds to the intronic region between exons D and E of the gene; however, band 3C7 was clearly still present in chromosomes with this deficiency.

Chromosome instability in the *Uc*: Features associated with chromosome rearrangements in the *Df(1)cm-In* and other *Uc* derivatives are quite distinct from the *P*-induced rearrangements reported by ENGELS and PRESTON (1984). The analysis of multiple-point *P*-induced rearrangements, especially three-point rearrangements, suggests that these multibreak rearrangements were generated from a single multibreak event followed by random rejoining of chromosome segments, as opposed to a sequence of two-break events. In contrast, the leapfrogging of chromosome breakpoints in the *Df(1)cm-In* chromosomes and in other derivatives of the *Uc* chromosome requires a series of two-point rearrangements taking place at different times and utilizing at least one existing breakpoint. We note that the rearrangements studied by ENGELS and PRESTON (1984) occurred in a single generation; the follow-up studies of their *hdp* inversions, however, indicate that many of these *hdp* inversions can reinvert to the normal sequence. This observation indicates that *P*-induced breakpoints are also capable of leapfrogging. Furthermore, the ENGELS-PRESTON model for the origin of multiple-break rearrangements in the *P-M* system of hybrid dysgenesis rules out the hypothesis of rearrangement formation that involve cointegrate structure or homologous recombination. In contrast, the leapfrogging phenomenon in the *Uc X* chromosome is compatible with a mechanism involving homologous recombination.

All of the chromosome rearrangements observed in the *Uc X* chromosomes are confined to the *X* chromosome; essentially no translocations have been observed [LIM (1979, 1980) and J. K. LIM, unpublished data]. The *P* element induced rearrangements that have been observed to date are likewise largely confined to single chromosomes, since a large scale genetic screen for translocation gave negative results (ENGELS 1979a). Perhaps the topological isolation of chromosome arms in the nucleus (MATHOG *et al.* 1984) or the preferential segregation of the chromosomes involved in translocations (adjacent *vs.* alternate segregation) or other still unknown mechanisms may be responsible for the absence of interchromosomal rearrangements.

Another noteworthy feature of chromosome rearrangements in the *Uc X* chromosome is the restoration of the normal sequence in an inverted chromosome. The results of ENGELS and PRESTON (1984) on *P*-element-induced reinversion indicate that those inversions with *P* elements at the both breakpoints can reinvert at a higher rate than those without *P* elements or an element at only one of the breakpoints. This observation suggests intrachromosomal recombination between homologous endpoints. Our preliminary *in situ* hybridization studies indicate that neither *P* elements [*pπ25.1* of O'HARE and RUBIN (1983)] nor I factor [*pI407* of BUCHETON *et al.* (1984)] are involved in chromosome breakage events observed in the *Uc X* chromosomes. A retrovirus-like transposon, *gypsy* [$\lambda bx^{34e}\cdot 6a2$ of MODOLELL, BENDER and MELSELSON (1983)] was shown to be responsible for instability of the *cut* locus in a number of *Uc* derivatives and in chromosomes destabilized by *Uc* derivatives (LIM *et al.* 1983; JACK 1985). Our *in situ* hybridization data, however, indicate that *gypsy* is not associated with any of the chromosome breakpoints in the *Uc-l* chromosomes.

The foldback (FB) family of transposable elements (POTTER 1982) in Drosophila is known to generate chromosome rearrangements at high frequencies (TRUETT, JONES and POTTER 1981; COLLINS and RUBIN 1982, 1984; GRIMWADE *et al.* 1985). The most extensive studies on the FB element-associated chro-

mosome rearrangements are those done by COLLINS and RUBIN (1982, 1984) on the white-crimson mutation and its derivatives documented by GREEN (1967). Compared to only one copy of precise inverted repeats of 31 base pairs (bp) at the termini of *P* elements (O'HARE and RUBIN 1983), the FB elements have at least 17 copies of 31-bp tandem repeats within each of the inverted repeats (POTTER 1982). This periodicity within the inverted repeats as well as the sequence homology between the 10-, 20- and 31-bp repeats in the inverted repeats of the FB elements suggest that the FB elements have structural features suitable for recombination involving homologous sequences. In order to check the possible involvement of FB elements to generate bizarre chromosome aberrations observed in the *Uc X* chromosomes, the *FB4* of POTTER *et al.* (1980) has been tested through *in situ* hybridization. The results show that none of the breakpoints of seven different chromosome rearrangements found in the *Uc-l X* chromosomes hybridizes with *FB4*.

A specific cross between M strain females and P strain males is necessary to produce the dysgenic hybrids. The site-specific *Notch* deficiencies reported in this study do not require an outcross. The *Df(1)cm-In* chromosome was detected among the *Uc-l X* chromosomes which have been balanced with *FM6* and were maintained by sib-mating them to the *FM6/Y* males for more than 7 yr (1968–1985). Therefore, a substantial degree of homozygosity is expected in these lines.

It is known that females heterozygous for *FM4* or *FM6* may generate *Notch-Minute* daughters and *Confluens*-associated progeny through a rare double cross over involving the 3C-4F region transposed into 11F in this balancer *X* chromosome [see p. 406 of LINDSLEY and GRELL (1968)]. Two separate lines of observation preclude such a possibility here: (1) most of the *Notch* deficiencies (more than 60% of the cases) detected in this study were produced by a premeiotic event, and (2) a random sample of 50 *Notch* deficiencies examined cytologically were deletions involving the region between 3C7-8 and 3D3-5 (not 3C-4F region) in the *Df(1)cm-In* chromosome.

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LITERATURE CITED

ARTAVANIS-TSAKONAS, S. A., M. A. T. MUSKAVITCH and B. YEDVOBNICK, 1983 Molecular cloning of *Notch*, a locus affecting neurogenesis in *Drosophila melanogaster*. Proc. Natl. Acad. Sci. USA **80:** 1977–1981.

BINGHAM, P. M. and B. H. JUDD, 1981 A copy of the *copia* transposable element is very tightly linked to the w^a allele at the *white* locus of *D. melanogaster*. Cell **25:** 705–711.

BUCHETON, A., R. PARO, H. M. SANG, A. PELISSON and D. J. FINNEGAN, 1984 The molecular basis of I-R hybrid dysgenesis in *Drosophila melanogaster*; identification, cloning and properties of the I factor. Cell **38:** 153–163.

COLLINS, M. and G. M. RUBIN, 1982 Structure of the Drosophila mutable allele, white-crimson, and its white-ivory and wild-type derivatives. Cell **30:** 71–79.

COLLINS, M. and G. M. RUBIN, 1984 Structure of chromosomal rearrangements induced by the FB transposable element in *Drosophila*. Nature **308:** 323–327.

DEMEREC, M., 1941 The nature of changes in the White-Notch region of the X chromosome of *Drosophila melanogaster*. Proc. 7th Int. Congr. Genet. 1939 (Edimb. Camb: Issued as supplement vol. to J. Genet.), pp. 99–103.

ENGELS, W. R., 1979a Germ line aberrations associated with a case of hybrid dysgenesis in *Drosophila melanogaster* males. Genet. Res. **33:** 137–146.

ENGELS, W. R., 1979b The estimation of mutation rates when premeiotic events are involved. Environ. Mutagen. **1:** 37–43.

ENGELS, W. R. and C. R. PRESTON, 1984 Formation of chromosome rearrangements by P factors in *Drosophila*. Genetics **107:** 657–678.

GREEN, M. M., 1967 The genetics of a mutable gene at the white locus of *Drosophila melanogaster*. Genetics **56:** 467–482.

GRIMWADE, B. G., M. A. T. MUSKAVITCH, W. J. WELSHONS, B. YEDVOBNICK and S. ARTAVANIS-TSAKONAS, 1985 The molecular genetics of the *Notch* locus in *Drosophila melanogaster*. Dev. Biol. **107:** 503–519.

JACK, J. W., 1985 Molecular organization of the *cut* locus of *Drosophila melanogaster*. Cell **42:** 869–876.

KEPPY, D. O. and W. J. WELSHONS, 1977 The cytogenetics of a recessive visible mutant associated with a deficiency adjacent to the *Notch* locus in *Drosophila melanogaster*. Genetics **85:** 497–506.

KEPPY, D. O. and W. J. WELSHONS, 1980 The synthesis of compound bands in *Drosophila melanogaster* salivary gland chromosomes. Chromosoma **76:** 191–200.

KIDD, S., T. J. LOCKETT and M. W. YOUNG, 1983 The *Notch* locus of *Drosophila melanogaster*. Cell **34:** 421–433.

LAVERTY, T. R. and J. K. LIM, 1982 Site-specific instability in *Drosophila melanogaster*: evidence for transposition of a destabilizing element. Genetics **101:** 461–476.

LEHMANN, R., F. JIMENEZ, U. DIETRICH and J. A. CAMPOS-ORTEGA, 1983 On the phenotype and development of mutants of early neurogenesis in *Drosophila melanogaster*. Wilhelm Roux's Arch. Dev. Biol. **192:** 62–74.

LIM, J. K., 1979 Site-specific instability in *Drosophila melanogaster*: the origin of the mutation and cytogenetic evidence for site specificity. Genetics **93:** 681–701.

LIM, J. K., 1980 Site-specific intrachromosomal rearrangements in *Drosophila melanogaster*: cytogenetic evidence for transposable elements. Cold Spring Harbor Symp. Quant. Biol. **45:** 553–560.

LIM, J. K., 1981 Patroclinous attached-X females in *Drosophila melanogaster*. Genetics Supplement **97:** s66 (abstract).

LIM, J. K. and D. J. CROWE, 1984 The origin of the destabilized *Drosophila* X chromosome is in the germ cells of certain hybrid females. Genetics **107** (Suppl): s63.

LIM, J. K. and L. A. SNYDER, 1968 The mutagenic effects of two monofunctional alkylating chemicals on mature spermatozoa of *Drosophila*. Mutat. Res. **6:** 127–137.

LIM, J. K., M. J. SIMMONS, J. D. RAYMOND, N. M. COX, R. F. DOLL and T. P. CULBERT, 1983 Homologue destabilization by a

putative transposable element in *Drosophila melanogaster*. Proc. Natl. Acad. Sci. USA **80:** 6624–6627.

LINDSLEY, D. L. and E. H. GRELL, 1968 Genetic variations of *Drosophila melanogaster*. Carnegie Inst. Wash. Publ. 627.

MANIATIS, T., E. F. FRITSCH and J. SAMBROOK, 1982 *Molecular Cloning: A Laboratory Manual*. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.

MATHOG, D., M. HOCHSTRASSER, Y. GRUENBAUM, H. SAUMWEBER and J. SEDAT, 1984 Characteristic folding pattern of polytene chromosomes in *Drosophila* salivary gland nuclei. Nature **308:** 414–421.

MODOLELL, J., W. BENDER and M. MELSELSON, 1983 *Drosophila melanogaster* mutations suppressible by the suppressor of Hairy-wing are insertions of a 7.3 kilobase mobile genetic element. Proc. Natl. Acad. Sci. USA **80:** 1678–1682.

O'HARE, K. and G. M. RUBIN, 1983 Structure of P transposable elements and their sites of insertion and excision in the *Drosophila melanogaster* genome. Cell **34:** 25–35.

POTTER, S. S., 1982 DNA sequence of a foldback transposable element in *Drosophila*. Nature **297:** 201–204.

POTTER, S., M. TRUETT, M. PHILLIPS and A. MAHER, 1980 Eucaryotic transposable genetic elements with inverted terminal repeats. Cell **20:** 639–647.

SLIZYNSKA, H., 1938 Salivary chromosome analysis of the white-facet region of *Drosophila melanogaster*. Genetics **23:** 291–299.

TRUETT, M. A., R. S. JONES and S. S. POTTER. 1981 Unusual structure of the FB family of transposable elements in *Drosophila*. Cell **24:** 753–763.

WELSHONS, W. J., 1965 Analysis of a gene in *Drosophila*. Science **15:** 1122–1129.

WELSHONS, W. J., 1974 The cytogenetic analysis of a fractured gene in *Drosophila*. Genetics **76:** 775–794.

WELSHONS, W. J. and D. O. KEPPY, 1975 Intragenic deletions and salivary band relationships in *Drosophila*. Genetics **80:** 143–155.

WELSHONS, W. J. and D. O. KEPPY, 1981 The recombinational analysis of aberrations and the position of the *Notch* locus on the polytene chromosome of *Drosophila*. Mol. Gen. Genet. **181:** 319–324.

WELSHONS, W. J. and H. J. WELSHONS, 1985 Suppression of the *facet-strawberry* position effect in *Drosophila* by lesions adjacent to *Notch*. Genetics **110:** 465–477.

WELSHONS, W. J. and H. J. WELSHONS, 1986 Enhancement and suppression of a euchromatic position effect at *Notch* in Drosophila. Genetics **113:** 337–354.

WHARTON, K. A., K. M. JOHANSEN, T. XU and S. ARTAVANIS-TSAKONAS, 1985 Nucleotide sequence from the neurogenic locus *Notch* implies a gene product that shares homology with proteins containing EGF-like repeats. Cell **43:** 567–581.

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