Molecular Analysis of Diepoxybutane-Induced Mutations at the rosy Locus of Drosophila melanogaster

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ABSTRACT

We have analyzed at the molecular level diepoxybutane-induced mutants determined to have lesions affecting expression of the ry locus. Of the 21 mutants analyzed here, genetic analysis suggested that five were putative deficiencies involving ry and adjacent lethal loci. However, molecular analysis confirmed that only two of these five putative deficiencies were in fact deletions detectable by the methods used in the analysis. The remaining 16 mutants were viable as homozygotes, suggesting that their lesions were confined to the ry locus. Seven of these 16 intragenic mutants were determined to be deletions of genetic material as evidenced by altered restriction patterns relative to the wild type patterns. Thus, nine of 21 (43%) diepoxybutane-induced mutants are due to deletions ranging in size from approximately 50 base pairs to more than 8 kilobase pairs. Most of the deletions (seven of nine or 78%) are intragenic and less than 250 base pairs in size; it seems that most, if not all, affect coding rather than regulatory sequences.

DIEPOXYBUTANE (DEB) is a potent chemical mutagen in a variety of biological systems (reviewed in EHRENBerg and HUSSAIN 1981). This di-functional alkylating agent is capable of producing alkali-labile sites in DNA and of forming inter- and intrastrand cross-links. Exactly how this compound acts and the precise end results of its premutagenic lesions are not clear, but there is evidence that this compound is mutagenic in Drosophila melanogaster (GRAF et al. 1984), is an efficient chromosome breaker (WATSON 1966; ZIMMERING 1983) and causes a high percentage of multilocus deletions in Drosophila (SHUKLA and AUERBACH 1980; OLSen and GREEN 1982).

We chose to analyze DEB-induced mutations at the rosy (ry) locus of D. melanogaster on a molecular level since this type of analysis could allow us to classify intragenic mutations unambiguously as deletions. Recent studies by COTé et al. (1986) have demonstrated the utility of this system for the analysis of spontaneous and X-ray-induced mutations. A similar type of analysis has also been reported for X-ray-induced mutants at the Adh locus of Drosophila (KELLey et al. 1985).

Forward mutations at the ry locus are easily detected by a phenotypic change in the eye color from the dull red wild type to a reddish brown. The ry locus and its gene product have been well characterized on a genetic, cytogenetic and biochemical level (CHOVNICK, GELBART and McCARRON 1977; CHOVNICK et al. 1978) and the ry gene has recently been cloned and mapped to a specific polytene band (BENDER, SPIERER and HOGENESS 1983; SPIERER et al. 1983). Functional tests have shown that the entire ry locus is contained within an 8.1-kb SalI restriction fragment (RUBIN and SPRADLING 1982). The ry transcriptional unit spans nearly the entire SalI fragment (W. Bender, unpublished data) so one would expect to find mutations in most regions of the fragment.

MATERIALS AND METHODS

Materials: Diepoxybutane was purchased from Aldrich Chemical Company. Restriction endonucleases were purchased from Pharmacia P-L Biochemicals, Bethesda Research Laboratories and Boehringer-Mannheim. Proteinase K was obtained from Sigma and RNase A from Sigma or Pharmacia P-L Biochemicals. Nitrocellulose and DEAE membrane were from Schleicher and Scheull. Radiochemicals were obtained from New England Nuclear. Nick translation materials and agarose were from Bethesda Research Laboratories.

Mutagenesis and stock maintenance: Mutagenesis was done on the specifically characterized ry/+ allele which exhibits normal levels of enzyme activity and whose gene product has a fast mobility relative to other isoalleles (CHOVNICK, GELBART and McCARRON 1977). Males of the genotype kar2 ry+/- were collected within 6 hr of eclosion and aged with females for a 2-day period. After the 2-day period, the males were separated from the females, aged an additional 24 hr, starved for 3 hr and fed 5 mM DEB for 24 hr in a 5% aqueous sucrose solution by the method of AARON, NARDIN and LEE (1977). Treated males were mated individually for 2 days with two females, y; cnY5; ry4. (For a description of symbols, see LINDSLEY and GRELL 1968.) In some experiments multiple broods were examined for the purpose of expanding the number of progeny. F1 progeny were screened for the occurrence of the ryosy phenotype. Such exceptional flies were mated with Df(3R) ry+/KRS flies. Df(3R) ry+/KRS is a deletion of genetic material spanning the entire ry locus and adjacent loci and is maintained as a heterozygote over the third chromosome balancer, Tp(3)KRS, M(3)S34 kar ry Sb. F2 progeny which exhibited...
the kar ry phenotype were inbred. If possible, the stocks were made homozygous; when not possible, the mutant chromosome was maintained as a heterozygote over the MKRS balancer chromosome.

DNA from the heterozygous DEB-induced mutants could not be easily analyzed as ryMKRS/MKRS because the restriction fragments generated by ry* on the balancer chromosome could not readily be distinguished from those fragments generated by the newly induced mutant allele. To circumvent this problem, females of the heterozygous DEB strains were mated with ry6* or other mutant strains known to carry large deletions in the rosy microregion. The modifications included treatment with RNase A (50 µg/ml, 37°C, 15 min) followed by treatment with Proteinase K (150 µg/ml, 37°C, 60 min) after removal of cellular debris. These enzymatic treatments were followed by three extractions with phenol and two extractions with chloroform:isoamyl alcohol (24:1). The DNA was recovered by ethanol precipitation.

**Probes:** The 8.1-kb SaI and the 4.6-kb EcoRI fragments from the Canton S wild-type ry allele were collected in a chromosomal walk of the rosy region (BENDER, SPIERER and HOGNESS 1983) and subcloned into the plasmid vector pBR322; both clones were obtained from W. BENDER and are referred to as pry8.1 and pry4.6 in this report. Additionally, it has recently been demonstrated that the IS12 locus is contained entirely within pry8.1 in the region 5' to the ry locus (Clark et al. 1986). Plasmid DNA was isolated from amplified bacterial cultures using an alkaline-SDS lysis procedure (ISH-HOROWITZ and BRUCE 1981).

Restriction fragments of pry8.1 and pry4.6 were recovered from agarose gels (0.8–1.2%) using NA-45 DEAE membrane as the binding medium. This procedure was carried out according to the manufacturer's directions. Plasmid DNA or DNA fragments were radiolabeled with [32P]dCTP (600 Ci/mmol) using a nick translation kit according to the manufacturer's directions. Labeled DNA was separated from unincorporated nucleotides by gel filtration through Sephadex G-50-150 in 150 mM NaCl, 10 mM Tris, 1 mM EDTA (pH 8). The typical yield was 25–80 x 10^6 cpm/µg DNA when plasmid DNA was nick translated and less than 3 x 10^6 cpm/µg DNA when a fragment was used in the reaction.

**Restriction digests and blot hybridizations:** Two to three micrograms of genomic DNA were digested to completion with a given restriction endonuclease(s) according to the manufacturer's directions. DNA was fractionated on 0.8–1.2% agarose gels using a Tris-borate buffer (0.089 M Tris, 0.089 M boric acid, 0.2 mM EDTA [pH 8]). pry8.1 was digested with various restriction endonucleases and used as molecular weight markers on the gels and resulting autoradiographs. Following electrophoresis, the gel was stained with ethidium bromide, illuminated with UV light and photographed using Polaroid type 665 film. Prior to blotting, the gel was soaked in a denaturing solution (1.5 M NaCl, 0.3 M NaOH) and then in a neutralizing solution (5 mM NaCl, 0.5 M Tris [pH 7]); each soaking was for one hour with gentle shaking. The DNA was transferred to nitrocellulose by standard capillary blotting techniques using 12 x SSC buffer (MANIATIS, FRITSCH and SAMBROOK 1982).

Prehybridizations were in 5 x SSC, 5 x Denhardt's, 0.1% SDS and 100 µg/ml sheared and sonicated calf thymus DNA, at 62°C for 2–4 hr. Hybridizations were in 5 x SSC, 5 x Denhardt's, 0.1% SDS and 2–6 x 10^6 cpm of radiolabeled probe, at 62°C for 15–20 hr. The filters were then washed four times for 30 min at 62°C in 3 x SSC, 5 x Denhardt's and 0.1% SDS. Air-dried filters were exposed to Kodak XAR-5 film at ~80°C with a du Pont Cronex Lightning Plus intensifying screen. After the appropriate exposure time, the film was developed according to the manufacturer's directions.

**RESULTS**

**Mutagenesis:** Fifty-one DEB-induced ry mutants were recovered from an examination of approximately 189,000 F1 test progeny (Table 1). In this report, these strains will be identified individually by isolation numbers, for example, 1-18 refers to a mutation isolated from vial number 18 of experiment 1. When strain numbers contain a lettered suffix after the experiment number, for example, 3A-118, it re-
fers to a specific brood in a multibrood mutagenesis experiment. When strain numbers contain a lettered suffix after the vial number, it indicates more than one mutant was recovered from the vial, for example, 3-20B refers to the second mutant recovered from vial 20 of experiment 3.

Of these 51 DEB-induced mutants, 23 involved germ-line mutations which were transmitted and 21 of these mutant strains were available for analysis in this research. A third strain, 1-81, was lost during this research but DNA had been extracted and was available for analysis. Of the 20 surviving strains, 15 are able for analysis. Of the 51 DEB-induced mutants, 23 involved germ-line mutations which were transmitted and 21 of these mutant strains were available for analysis in this research. A third strain, 1-81, was lost during this research but DNA had been extracted and was available for analysis. Of the 20 surviving strains, 15 are able for analysis. Of the 51 DEB-induced mutants, 23 involved germ-line mutations which were transmitted and 21 of these mutant strains were available for analysis in this research. A third strain, 1-81, was lost during this research but DNA had been extracted and was available for analysis. Of the 20 surviving strains, 15 are able for analysis.

**TABLE 1**

<table>
<thead>
<tr>
<th>F1 examined</th>
<th>No. of mutants</th>
<th>Frequency of mutants</th>
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<tbody>
<tr>
<td>189,263</td>
<td>51</td>
<td>23*</td>
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* Twenty-one available for analysis.

**TABLE 2**

<table>
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<tr>
<th>Deficiency analysis of heterozygous strains</th>
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<tr>
<td>Mutant strain</td>
</tr>
<tr>
<td>---------------</td>
</tr>
<tr>
<td>3-20A</td>
</tr>
<tr>
<td>3-20B</td>
</tr>
<tr>
<td>3B-65</td>
</tr>
<tr>
<td>5-97</td>
</tr>
<tr>
<td>3A-118</td>
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* + indicates that the mutant is viable over the deficiency. "-" indicates that the mutant is not viable over the deficiency. "NT" indicates "not tested."

**Restriction endonuclease analysis:** Twenty-one DEB-induced mutations at the ry locus were analyzed by comparing the restriction patterns of DNA from the mutant strains with the patterns observed in the DNA of wild type flies. It has been estimated that this method of analysis is sensitive enough to detect deletions of approximately 50 bp or larger (Zachar and Bingham, 1982) as well as smaller deletions or even single base substitutions which alter the recognition site of a restriction endonuclease used in the analysis. pry8.1 was the primary probe used in the analysis of control and mutant DNA. Additional probes were used in the analysis of strain 5-97, and these probes are described in the discussion of strain 5-97.

In this analysis, DNA from the mutant strains was compared with ry⁺¹ DNA, the paternal allele from which the mutants were derived and with ry⁻¹, the mutant allele used in the initial genetic selection procedure. Digested DNA from ry⁺¹ and ry⁻¹ flies were compared, and no differences were observed in the restriction fragments generated by PvuII, BglII and SstI (data not shown). These were the restriction endonucleases used initially to detect all of the mutations subsequently classified as deletions in this study. These data were used to eliminate the possibility that the same alteration observed in the mutant strains was present in either parental strain and also eliminated the need to use DNA from both parental strains as internal controls during this analysis.

**Analysis of the homozygous strains:** Of the 21 mutant strains examined in this analysis, 16 were maintained as homozygotes. Digestion with PvuII (Figures 2, A and B, and 3, A and B) and SstI (Figure 3C) and probing with pry8.1 gave normal (i.e., wild type) restriction patterns for eight of the strains (1-12, 1-75, 1-81, 2-81, 4-65, 5-18, 6-74 and 6-138). Continued analyses indicated that these eight strains also had normal restriction patterns with BglII and NruI digests (data not shown).

However, this preliminary analysis with PvuII and
SstI suggested that eight of the homozygous strains (1-18, 2-15, 2A-109, 3A-91, 3B-28, 4-96, 5-36 and 11B-115) involved deletions of genetic material. These strains were then subjected to further restriction analysis. The estimated size of the deletions is expressed as an average value since, in most cases, the size was derived from the data of multiple restriction analyses.

While 11B-115 was initially detected as a possible deletion in the 2.22-kb PvuII fragment, 3A-91 presented the same restriction pattern as $\gamma^{+/-}$ (Figure 2A, lanes 3 and 7). Subsequent restriction analysis indicated both mutations were due to deletions in the 1.51-kb SstI fragment (Figure 3C). Further analysis was accomplished with an SstI/XhoI double digest (Figure 4A, lanes 4-6) and with an EcoRI/SstI double digest (Figure 4B, lanes 4-6). Both XhoI and EcoRI cut the 1.51-kb SstI fragment into two unequal fragments, the 3' fragment being the smaller of the two (Figure 4C). While these 3' fragments resulting from either double digest are evident in $\gamma^{+/-}$ DNA (lane 5 of Figure 4, A and B), they can not be detected in either mutant. This is not meant to imply that the fragments were deleted entirely in the mutant strains, but rather that the fragments generated by the double digests coincide with the small fragments from other parts of the $\gamma$ locus and that the resulting doublets were not resolved. Taken together, these data indicate that both 11B-115 and 3A-91 are deletion mutants, the deletions being no greater than 50 bp and localized to the 3' end of the 1.51-kb SstI fragment.

Mutants 1-18, 2-15 and 5-36 were initially detected as possible deletions in the 2.22-kb PvuII fragment (Figure 2B, lanes 3 and 6; Figure 3A, lane 4). Additional restriction analysis with BglII and SstI (Figure 4C).
Gels transferred to nitrocellulose and hybridized with pry8.1. The strains and in lanes (4), (5), (6) are Ssfl/Xhol double digests. Restriction fragments in resulting autoradiographs are shown here. Arrowheads indicate the Ssfl and Ssfl/EcoRI digests of genomic DNA. Lanes (3) and (6) are SstI-Nru1 double digests. C. Restriction map of the 1.51-kb DNA (2-3 pg) was applied to each lane, separated on 1.2% agarose gels. Transferred to nitrocellulose and hybridized with pry8.1. The resulting autoradiographs are shown here. Arrowheads indicate altered restriction fragments. A. Comparison of BglII and SstI digests of genomic DNA. Lanes (1) and (4) are 3A-91, lanes (2) and (5) are ry*++, lanes (3) and (6) are 11B-115. DNA in lanes (1), (2), (3) are SstI digests and in lanes (4), (5), (6) are SstI/XhoI double digests. B. Comparison of SstI and SstI/EcoRI digests of genomic DNA. Lanes (1) and (4) are 3A-91, lanes (2) and (5) are ry*++, lanes (3) and (6) are 11B-115. DNA in lanes (1), (2), (3) are SstI digests and in lanes (4), (5), (6) are SstI/EcoRI double digests. C. Restriction map of the 1.51-kb SstI fragment showing the restriction sites pertinent to this analysis: (R) EcoRI, (T) SstI, (U) PvuII, (X) XhoI. The horizontal bar below the map indicates the 2.22-kb PvuII fragment.

5A) indicated deletions of 50–100 bp for 2-15 and 5-36 in the overlapping 3.45-kb BglII and 2.80-kb SstI fragments (Figure 5D), but 1-18 presented a normal wild-type restriction pattern. Subsequent analysis with NruI indicated a 50-bp deletion in the 0.58-kb NruI fragment for 2-15 (Figure 5B, lane 1); however, 1-18 (data not shown) and 5-36 (Figure 5B, lane 4) presented normal patterns when digested with NruI, suggesting that their alterations were in the 0.45-kb SstI-NruI fragment, contained in the 4.8-kb NruI fragment (Figure 5D). Additional analysis of 5-36 with various double digests (Figure 5C) indicated a 75 ± 25-bp deletion localized to the 0.45-kb SstI-NruI fragment. Mutant 1-18 was analyzed in a similar manner (data not shown), but it again presented a normal restriction pattern suggesting that its alteration is not due to a detectable deletion and that the apparent restriction fragment polymorphism observed with the PvuII digest (Figure 2B, lane 3) was due to anomalous migration of the DNA in the gel.

Mutant 4-96 was initially detected as a deletion mutant with a PvuII digest showing that the 2.22-kb and 0.94-kb fragments characteristic of ry*++ were not present in the mutant DNA, but were replaced with a single larger fragment sized at approximately 3.0 kb (Figures 3A, lane 8 and 6A, lanes 3 and 4). This indicated a deletion of about 160 bp spanning the PvuII site between the 2.22- and 0.94-kb fragments. The nearest restriction sites to this PvuII site are NruI on the 5’ side and BamHI on the 3’ side; the NruI-BamHI fragment is approximately 0.3 kb (Figure 6B). Additional restriction analysis (Figure 5B, lane 3 and Figure 6) indicate that the deletion in 4-96 is 185 ± 25 bp and that the deletion does not affect the NruI or BamHI restriction sites adjacent to the altered PvuII site.

Mutants 2A-109 and 3B-38 are the two strains with deletions initially localized to the 0.94-kb PvuII frag-
A

![Restriction analysis of 4-96.](image)

**FIGURE 6.**—Restriction analysis of 4-96. Digested DNA (2–3 µg) was applied to each lane, separated on a 1.0% agarose gel, transferred to nitrocellulose and hybridized with pry8.1. The resulting autoradiograph is shown here. Arrowheads indicate the altered restriction fragments. A. Analysis of 4-96. Lanes (1), (3), (5) are \( \text{ry}^{+/-} \) and lanes (2), (4), (6) are 4-96. DNA in lanes (1) and (2) was digested with \( \text{NruI} \), in lanes (3) and (4) with \( \text{PvuII} \) and lanes (5) and (6) are \( \text{BamHI/SalI} \) double digests. B. Restriction map of the 1.05-kb \( \text{NruI} \) fragment showing the restriction sites pertinent to this analysis: (A) \( \text{AvaI} \), (B) \( \text{BamHI} \), (N) \( \text{NruI} \), (U) \( \text{PvuII} \).

B

![Restriction map of the 1.05-kb NruI fragment showing the restriction sites pertinent to this analysis:](image)

**FIGURE 7.**—Preliminary restriction analysis of heterozygous strains. Digested DNA (2–3 µg) was applied to each lane, separated on 0.8% agarose gels, transferred to nitrocellulose and hybridized with pry8.1. The resulting autoradiographs are shown here. A. PryI analysis of \( \text{DEB/ry}^{50} \) heterozygotes: lane (1) \( \text{ry}^{+/-} \), (2) \( \text{ry}^{50} \), (3) \( \text{ry}^{+/-}/\text{ry}^{50} \), (4) 3-20A/\( \text{ry}^{50} \), (5) 3-20B/\( \text{ry}^{50} \), (6) \( \text{ry}^{+/-}/\text{ry}^{50} \), (7) 3B-65/\( \text{ry}^{50} \). B. PryII analysis of \( \text{DEB/ry}^{50} \) heterozygotes: lane (1) \( \text{ry}^{+/-} \), (2) \( \text{ry}^{50} \), (3) 3-20A/\( \text{ry}^{50} \), (4) 3-20B/\( \text{ry}^{50} \), (5) \( \text{ry}^{+/-} \), (6) 3A-118/\( \text{ry}^{50} \), (7) \( \text{ry}^{50} \), (8) 3B-65/\( \text{ry}^{50} \), (9) 5-97/\( \text{ry}^{50} \), (10) \( \text{ry}^{+/-} \). Arrowhead indicates the 2.1-kb fragment characteristic of 5-97. C. Restriction map of the \( \text{ry} \) locus showing the extent of the deletions of \( \text{ry}^{50} \) and \( \text{ry}^{506} \). (S) \( \text{SalI} \), (T) \( \text{SalI} \), (U) \( \text{PvuII} \).

ment (Figure 2A, lanes 1 and 4). Subsequent restriction analysis (data not shown) identified the 2A-109 alteration as a deletion of 155 ± 25 bp in the 5' region of the 0.94-kb \( \text{PvuII} \) fragment and the 3B-38 alteration as a 200 ± 20-bp deletion in the 3' region of this \( \text{PvuII} \) fragment.

**Analysis of the heterozygous strains:** Initially, various restriction endonucleases were used to analyze the five DEB-induced mutant chromosomes carried as heterozygotes over the \( \text{Tp(3)MKRS, M(3)S34 kar ry^2 Sb} \) chromosome. This balancer chromosome carries the \( \text{ry}^2 \) allele, a mutation due to a B104 transposable element insertion within the 3' end of the \( \text{ry} \) locus (COTÉ et al. 1986). It was not possible to discriminate between bands derived from the DEB-induced mutant allele and those generated by the \( \text{ry}^2 \) allele. To circumvent this problem, flies of the heterozygous strains were mated with \( \text{ry}^{+/-} \) or \( \text{ry}^{50} \) flies (both strains carry deletions as depicted in Figure 7C) and the DNA of F1 progeny bearing the DEB/\( \text{ry}^{50} \) phenotype was analyzed.

Digestion with \( \text{PvuII} \) gives normal restriction patterns for mutants 3-20A, 3-20B and 3B-65. When analyzed as heterozygotes over the \( \text{ry}^{50} \) deletion, these mutants show the five bands characteristic of \( \text{ry}^{+/-} \) and \( \text{ry}^{50} \) (Figure 7A). When analyzed as heterozygotes over the \( \text{ry}^{506} \) deletion, all three mutants again show the normal restriction patterns for \( \text{ry}^{+/-} \) and \( \text{ry}^{506} \) (Figure 7B).

\( \text{SalI/PvuII} \) double digests of 5-97/\( \text{ry}^{50} \) revealed a 2.1-kb fragment that is distinct from the bands observed in \( \text{ry}^{+/-} \) or \( \text{ry}^{50} \) (Figure 8A). Digestion of 5-97/\( \text{ry}^{50} \) DNA and probing with pry4.6 (Figure 8G) also revealed the presence of this 2.1 kb fragment (Figure 8B), suggesting that the fragment is located within the 3' region of the \( \text{ry} \) gene.

\( \text{SalI} \) digests of \( \text{ry}^{+/-} \) DNA typically generate six bands. Four of these bands are common to \( \text{ry}^{+/-} \) and \( \text{ry}^{50} \), but the 2.8- and 0.57-kb fragments found in \( \text{ry}^{+/-} \)
were applied to each lane, separated on 0.8% agarose gels, transferred to nitrocellulose and hybridized as indicated below. The resulting autoradiographs are characteristic of 5-97 hybridized with pry8.1. Lane (1) pry8.1, (2) pry8.1 digests of genomic DNA; they differ only in the Sall restriction map of the 5-97/ry6" deletion mutant 3A-118/ry6" failed to show any detectable bands other than those derived from ry6" (data not shown). When a PvuII digest of 3A-118/ry6" was analyzed, only the bands characteristic of ry6" were observed (Figure 7B, lane 6). These data indicate that the 3A-118 mutation is due to a deletion spanning the entire ry locus and this conclusion is consistent with the results of the genetic analysis.

**DISCUSSION**

The DEB-induced mutants analyzed here were all initially isolated over the intragenic ry4" mutation which is viable over the Df(3R)ry1608 chromosome, thus allowing for the recovery of deletions, both large and small. Two of the DEB mutant alleles (3A-118 and 5-97) are associated with large chromosomal aberrations. Although we have not rigorously demonstrated that pic is affected by a continuous deletion extending from ry to pic, the molecular analysis clearly indicates that the 3A-118 and 5-97 deletions extend to the IS12 locus on the proximal side. The 3A-118 allele lacks DNA which hybridizes with pry8.1 and since this plasmid also contains the entire IS12 locus, the 3A-118 deficiency must delete IS12 as well as ry. The 5-97 allele retains approximately 4.1 kb of DNA which hybridizes with pry8.1 and further molecular analysis localized this 4.1 kb fragment to the 3' end of the ry locus; thus, the 5-97 allele fails to hybridize with that portion of pry8.1 which contains the IS12 locus. Thus, 3A-118 and 5-97 are intergenic mutations resulting from large continuous deletions of genetic material.

Seven of the DEB-induced mutations (2-15, 2A-109, 3A-91, 3B-38, 4-96, 5-36 and 11B-115) are due to small (i.e., less than 250 bp) deletions within the ry locus. The remaining 12 strains carry intragenic alterations which may be either missense or nonsense mutations caused by base substitution, frameshift mutations or deletions too small to be detected by the
methods used in this analysis. Thus, 43% (9/21) of the DEB-induced mutations at the ry locus are caused by deletions ranging in size from approximately 50 bp to more than 8 kb.

The classification of only two of the 21 DEB-induced mutations as large intergenic deletions is in marked contrast to earlier reports indicating that at least one-third of DEB-induced mutations at the y and w loci are large, intergenic deletions (Olsen and Green 1982) or that at least two-thirds of DEB-induced mutations at the w, sn and m loci are intergenic deletions large enough to include the locus of the visible mutation as well as a sex-linked lethal locus (Shukla and Auerbach 1980). Both of these earlier reports are predicated upon the assumption that the observed mutations were due to a single mutagenic event, a continuous deletion extending to adjacent loci, and were not due to a cluster of point mutations in the adjacent loci. Since neither of the genetic tests used to detect the deletions were confirmed by cyogenetic or molecular analyses which would confirm that the mutations were due to deletions, the assumption of a single continuous deletion must be considered as not proven.

The demonstration that DEB causes translocations in Drosophila (Watson 1966) indicates that DEB is capable of multiple hits during a single exposure to the mutagen. Such a multiple hit event could explain a possible clustering of point mutations leading to observations of a high frequency of deletions among DEB-induced mutations on the X chromosome and would explain the observed results for three of the DEB-induced mutations at the ry locus. The three heterozygous strains (3-20A, 3-20B and 3B-65) which are not viable as homozygotes but do not exhibit detectable deletions within the ry locus and which are viable over the Df(3R)ry1608 chromosome are probably due to a point mutation (or very small deletion) within the ry locus and a second site mutation away from the ry region.

The seven intragenic DEB-induced deletions were mapped to the two internal PvuII fragments (2.22 and 0.94 kb); the exception is 3A-91 which may be located within the 2.22-kb fragment or just 5' to the fragment. This is consistent with the results of Coté et al. (1986) who localized 15 of 16 ry mutations identifiable by genomic blotting experiments to the two internal PvuII fragments. Fine structure recombination analysis has defined the ry locus in terms of the genetic map positions of ry mutants and ry structural element variant alleles. ry23 and ry606 form the left boundary and ry2 and ry7 form the right boundary of the XDH structural element (Gelbart, McCarron and Chovnick 1976). When this genetic map is "superimposed" on the restriction map of the ry locus, the genetic boundaries of the structural element coincide closely with the two internal PvuII fragments (Coté et al. 1986).

Of the 21 DEB-induced mutations, the alterations of eight could be localized to a specific intragenic restriction fragment (Figure 9); 3A-118 is excluded from this discussion since it involves a total loss of the ry locus. These deletions are localized to the DNA regions that are reportedly within the defined boundaries of the XDH structural element. Thus, it would seem that the DEB-induced deletions cause the mutant phenotype by alteration of coding rather than regulatory sequences.

We are grateful to Dr. W. Bender for providing pry8.1 and the plasmid containing the 4.6-kb EcoRI ry fragment as well as the initial molecular analysis of the ry locus. This work was supported by Environmental Protection Agency grant R810390.

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