ADONIS 0027510791001264

MUT 04978

Most ultraviolet irradiation induced mutations in the nematode *Caenorhabditis elegans* are chromosomal rearrangements

Helen I. Stewart, Raja E. Rosenbluth and David L. Baillie

Institute of Molecular Biology and Biochemistry, Department of Biological Sciences, Simon Fraser University, Burnaby, B.C., V5A 1S6 (Canada)

(Received 17 August 1990)
(Revision received 3 December 1990)
(Accepted 4 December 1990)

Keywords: *Caenorhabditis elegans*; Chromosome rearrangement

Summary

In this study we have determined the utility of 254-nm ultraviolet light (UV) as a mutagenic tool in *C. elegans*. We have demonstrated that irradiation of adult hermaphrodites provides a simple method for the induction of heritable chromosomal rearrangements. A screening protocol was employed that identifies either recessive lethal mutations in the 40 map unit region balanced by the translocation *eT1(III;V)*, or *unc-36(III)* duplications. Mutations were recovered in 3% of the chromosomes screened after a dose of 120 J/m^2. This rate resembles that for 1500 R γ-ray-induced mutations selected in a similar manner. The mutations were classified either as lethals [mapping to Linkage Group (LG)III or LGV] or as putative *unc-36* duplications. In contrast to the majority of UV-induced mutations analysed in microorganisms, we found that a large fraction of the *C. elegans* UV-induced mutations are not simple intragenic lesions, but are deficiencies for more than one adjacent gene or more complex events. Preliminary evidence for this conclusion came from the high frequency of mutations that had a dominant effect causing reduced numbers of adult progeny. Subsequently 6 out of 9 analysed LGV mutations were found to be deficiencies. Other specific rearrangements also identified were: one translocation, *sT5(II;III)*, and two *unc-36* duplications, *sDp8* and *sDp9*. It was concluded that UV irradiation can easily be used as an additional tool for the analysis of *C. elegans* chromosomes, and that *C. elegans* should prove to be a useful organism in which to study the mechanisms whereby UV acts as a mutagen in cells of complex eukaryotes.

Analysis of the *Caenorhabditis elegans* genome was begun by Brenner (1974) and is currently being pursued with great intensity in many laboratories (Wood, 1988). Over 800 genes as well as numerous deficiencies and duplications have been mapped genetically (Edgley and Riddle, 1990), and the correlation of the genetic and physical maps is well under way (Coulson et al., 1986, 1988). In addition, the functions of many genes are being elucidated using intragenic mutations, while sites regulating chromosome behaviour are being localized by means of chromosomal rearrangements (see Herman, 1988 and McKim et al., 1988 for reviews; Rosenbluth et al., 1990). Finally, Hartman and co-workers are investigating

Correspondence: Dr. David L. Baillie, Institute of Molecular Biology and Biochemistry, Department of Biological Sciences, Simon Fraser University, Burnaby, B.C., V5A 1 S6 (Canada).

the mechanisms responsible for the stability of the genome by identifying DNA-repair mechanisms in *C. elegans* (see Hartman et al., 1989 and earlier refs. cited therein). Essential for all these analyses is the availability of mutagens that have diverse targets and diverse modes of action. Currently, the mutagens commonly used as tools for *C. elegans* analyses are ethyl methanesulfonate (EMS), γ-radiation and formaldehyde. The first is used primarily for the induction of point mutations, while the latter two are used to induce deficiencies (Johnsen and Baillie, 1988) and, in the case of γ-rays, also more complex chromosomal rearrangements (Rosenbluth et al., 1985). In addition, for the purposes of gene cloning, mutations are induced by transposon tagging (Moerman and Waterston, 1989). The study presented here demonstrates the utility of ultraviolet light (UV) as an additional mutagenic tool for *C. elegans* and shows that the spectrum of induced mutations differs from those commonly reported in other systems.

The mutagenic property of UV irradiation has been extensively demonstrated in microorganisms as well as in some mammalian cell cultures (see, for example, Doudney, 1976). However, UV is seldom used to produce heritable mutations in metazoans, in spite of the fact that as early as the 1930s it was shown to cause mutations in *Drosophila melanogaster* (Altenburg, 1934) and in maize (Stadler and Sprague, 1936). The reluctance to use UV as a mutagen in complex organisms is presumably associated with the problem of conveniently administering the light to germ line cells without excessive attenuation due to overlying tissues. In Drosophila, recovery of mutant progeny requires irradiating either pole cells of embryos (Altenburg, 1934) or compressed abdomens of males (Mackenzie and Muller, 1940). We anticipated that gonadal shielding would not be a major problem in *C. elegans*, even in adults, since the organism is relatively small, having only about 1000 somatic cells (Sulston and Horvitz, 1977), and the oogonial nuclei lie at the periphery of the gonad. Indeed, a few UV-induced *C. elegans* mutations had already been recovered by others (Greenwald and Horvitz, 1980; D.C. Rein and H.F. Epstein, unpublished results cited in Zengel and Epstein, 1980).

The UV-induced mutations studied in microorganisms and cell cultures have generally been intragenic mutations rather than chromosomal rearrangements such as multi-gene deletions, duplications or translocations. To some extent this may be an artefact of the selection systems employed for the recovery of these mutations. To determine the efficacy of UV as a mutagen for *C. elegans*, we have used the *eT1*-screening system previously employed to measure the mutagenicity of EMS, γ-irradiation (Rosenbluth et al., 1983, 1985), formaldehyde (Johnsen and Baillie, 1988) and high and low LET (linear energy transfer) radiation (Nelson et al., 1989). The system predominantly recovers recessive lethal mutations in a large chromosomal target, comprising at least 10% of the *C. elegans* genome: the 40 map units (m.u.) recombinationally balanced by the *eT1(III;V)* translocation (Rosenbluth and Baillie, 1981). The lethal mutations are recovered as heterozygotes and can be analysed genetically to determine whether: (1) they are putative intragenic events or multi-gene deficiencies; and (2) they are associated with certain other chromosomal rearrangements. In addition to selecting for lethal mutations, the system also selects for duplications carrying the wild-type allele of the *unc-36* gene. The spectrum of mutational events selected depends on the mutagen used.

Preliminary tests (H.I. Stewart and D.L. Baillie, unpublished results cited in Coohill et al., 1988) established a dose at which UV-induced mutations were recovered with a high enough frequency to permit their analyses. Since then, Coohill et al. (1988) have produced UV dose-response curves for the frequency of *eT1*-balanced mutations in a wild-type strain and in strains carrying radiation-sensitive mutations. In those studies no attempt was made to analyse the nature of the mutations. We now present our initial findings and extend them to include an analysis of the recovered mutations. Our results lead us to conclude that a large fraction of UV-induced heritable mutations in *C. elegans* are associated with chromosomal rearrangements.

Materials and methods

General

The nematodes were handled and cultured on Nematode Growth Medium streaked with *Esche-*

richia coli OP50 as described by Brenner (1974). The nomenclature follows the uniform system adopted for *Caenorhabditis elegans* (Horvitz et al., 1979). Genotypes of genes and alleles are denoted in lower case [e.g., *unc-46(e177)*]; phenotypes are denoted with an upper case first letter (Unc-46). The lethal phenotype, as used in this study, refers to a worm that does not develop into a mature individual (i.e., a gravid hermaphrodite or a morphologically normal male).

Source of mutations

All mutations were derived from the wild-type N2 (var. Bristol) strain. The N2 strain and strains containing the following mutations were obtained either from S. Brenner's stock collection at the Medical Research Council in Cambridge, England, or from the Caenorhabditis Genetics Center at the University of Missouri, Columbia. Linkage Group(LG)I: *unc-13(e450)*; LGII: *unc-4(e120)*; LGIII: *dpy-1(e1), dpy-17(e164), dpy-18(e364), sma-2(e502), sma-3(e491), unc-64(e246)*; LGIV: *unc-31(e169)*; LGV: *dpy-11(e224), unc-23(e25), unc-46(e177), unc-60(m35)*; LGX: *unc-6(e78)* and the reciprocal translocation *eT1(III;V)* which carries the recessive *unc-36(III)* mutation *e873*. The mutation *lin-40(e2173)*, isolated by S.W. Emmons, was supplied by J. Hodgkin (MRC, Cambridge) and the deficiencies *mDf1* and *mDf3*, (Brown, 1984) were obtained from D.L. Riddle (Columbia, MO.). The deficiencies *nDf32* (Park and Horvitz, 1986), *nDf18* and *nDf31* originated in R.H. Horvitz's laboratory (MIT). All other mutations are denoted by an s prefix and originated in our laboratory. Among these are a set of LGV deficiencies and a set of lethal mutations most of which have been described in earlier publications (Johnsen and Baillie, 1988; Rosenbluth et al., 1988, 1990; Clark et al., 1990; Johnsen, 1990).

Characteristics of the translocation eT1(III; V) (abbreviated eT1)

The characteristics of *eT1* have been described previously (Rosenbluth and Baillie, 1981) and are briefly summarized here. The translocation is made up of two half-translocations, *eT1(III)*, segregating from the normal LGIII, and *eT1(V)*, segregating from the normal LGV. *eT1(III)* consists of the left halves of LGIII and LGV, while *eT1(V)* consists of the right halves of the two chromosomes (Fig. 1). The breakpoint on LGIII had been mapped close to *unc-36*. Since homozygous *eT1*

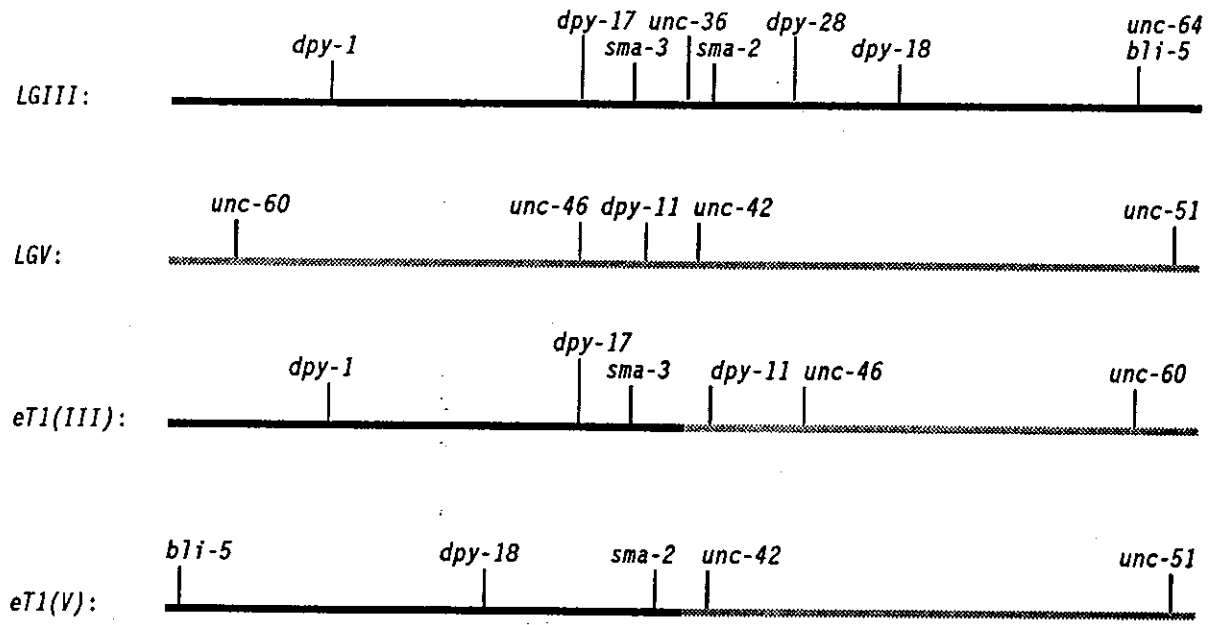

Fig. 1. Maps of LGIII, LGV and the translocation *eT1(III;V)*. Only genes relevant to this study are shown. Based on Edgley and Riddle (1990) and Rosenbluth et al. (1981).

has an Unc-36 phenotype, it is thought that the breakpoint is, in fact, in *unc-36*. The breakpoint on LGV is between *dpy-11* and *unc-42*, so that *dpy-11* lies on *eT1(III)*. Recombination appears to be normal in homozygous *eT1s*. However, as a heterozygote with normal chromosomes, *eT1* suppresses crossing over in the translocated regions: LGIII(right) and LGV(left). Because of the crossover suppression and because only euploid progeny survive, an *eT1* heterozygote shows pseudolinkage between markers on LGIII and LGV if they are in the respective crossover balanced regions. Furthermore, the total number of adult progeny from a heterozygous hermaphrodite is only 6/16 of that from a normal hermaphrodite; that is to say, 10/16 die as aneuploids.

The test strain BC2200, dpy-18/eT1(III); unc-46/eT1(V)

The test strain was the same as that used earlier by Johnsen and Baillie (1988). The markers, *dpy-18* and *unc-46*, are in the balanced regions of LGIII and LGV respectively. Thus wild-type hermaphrodites, *dpy-18/eT1;unc-46/eT1*, segregate *dpy-18;unc-46* homozygotes (Dpy Unc-46), parental wild-types and *eT1* homozygotes (Unc-36) in a ratio of 1:4:1. In the presence of a recessive lethal mutation on the normal chromosomes, anywhere in the balanced regions, the wild-types will fail to segregate fertile Dpy Unc-46s.

Ultraviolet mutagenesis

Using a 30-W G.E. UV germicidal lamp equilibrated for 15 min, worms were irradiated on agar in uncovered petri plates at a distance of 43 cm. The radiation was measured with a calibrated ultraviolet light meter (Model J225, Ultraviolet Products), and shown to be 2 W/sec/m^2. Thus, for example, irradiation for 60 sec gave a dosage of 120 J/m^2.

The dosage we adopted to measure UV mutagenesis was based on a preliminary experiment in which the effect of UV on hermaphrodite fecundity was tested. Wild-type hermaphrodites were subjected to a series of different UV doses and the number of progeny in sequential broods were counted. At a dose of 50 J/m^2, UV had no effect on fecundity, while with 250 J/m^2 the number of progeny produced in the first 4 h after irradiation was reduced to about 25% of the normal number. Based on these results we chose 120 J/m^2 as our standard dosage.

Young gravid P_0 adult *dpy-18/eT1;unc-46/eT1* (BC2200) hermaphrodites were treated with 120 J/m^2. Consistent with the protocol used for other mutagens in this laboratory (Rosenbluth et al., 1988; Johnsen and Baillie, 1988), the in utero embryos present at the time of mutagenesis were allowed to be expelled for 2 h (at 20°C) prior to collecting the F_1 progeny. After the 2 h, sequential F_1 broods were collected by transferring individual P_0s to fresh culture plates at given time intervals. A control set of untreated heterozygous hermaphrodites was also picked and transferred.

Screening for mutations

From the UV treated P_0s, all wild-type F_1s were placed individually on culture plates. The F_2 progenies were screened to determine the number of F_1s that (1) were sterile; or (2) failed to produce fertile Dpy Unc-46s. Strains were established from those F_1s that produced no fertile Dpy Unc-46s by picking a single wild-type F_2. Strains that continued to produce no fertile Dpy Unc-46s were maintained for further analysis by picking wild types in each generation.

Outcrossing and mapping to chromosome

Mapping the UV-induced mutations to one of the chromosomes (LGIII or LGV) required the removal of the *eT1* balancer. Wild-types from each strain were mated to N2 males. Individual wild-type F_1 hermaphrodites were allowed to self-fertilize at the standardized mapping temperature of 20°C (Rose and Baillie, 1979). Those F_1s that produced no Unc-36s carried the UV mutation over a normal '+' chromosome and their adult progeny were scored. In the absence of lethal mutations, a *dpy-18/+;unc-46/+* hermaphrodite would produce a phenotypic ratio of 9 wild-types: 3 Dpy-18s: 3 Unc-46s: 1 Dpy-18 Unc-46. Mutants that produced a significantly lower number of Dpy-18s than one-third of the wild-types, carried a lethal mutation on LGIII(right), *cis*-linked to *dpy-18*; while those giving an Unc-46: wild-type ratio of less than one-third carried a lethal on

LGV(left). The recombination distance between the lethal and its *cis*-linked marker was calculated, in map units, as $100p$, where $p = 1 - [1 - 3M/(W + M)]^{1/2}$, W = number of wild-types and M = number of Dpy-18s or Unc-46s. In some cases, the mutant strain appeared to carry a lethal mutation on both LGIII and LGV. If the recombination data suggested that one of the two lethals might be just outside the *eT1*-balanced region, an *eT1*-balanced heterozygote was constructed in which the chromosome carrying the other lethal was replaced by a marked chromosome (*dpy-18* or *unc-46*) that had not been exposed to UV. The appearance of some fertile Dpy-18 Unc-46 progeny then confirmed that the remaining lethal mutation was outside the *eT1*-balanced region.

Complementation mapping LGV lethals

Initially positioning of lethal mutations on LGV was carried out by complementation testing the lethals against a set of deficiencies whose breakpoints divide LGV(left) into a number of zones (Johnsen and Baillie, 1988; Johnsen, 1990). Once mapped to a zone(s), a mutation was tested for complementation with mutant alleles of genes located within that zone and, if necessary, with genes in neighbouring zones. Testing a lethal mutation against another lethal (or deficiency) was done as described by Rosenbluth et al. (1988). That is, advantage was taken of the fact that all lethals were balanced over *eT1* and pseudolinked to *dpy-18*. Thus two lethal mutations complemented each other if the cross-progeny of parents, each carrying one of the mutations, included mature Dpy-18 Unc-46 [or Dpy-18s in cases where the mutation tested was not linked to *unc-46(e177)*] males and hermaphrodites.

Determining developmental lethal phases

The terminal phenotypes of the lethals were determined by crossing wild-type hermaphrodites, from a given lethal strain, to *dpy-18/eT1;unc-46/eT1* males, picking F_1 Dpy18 Unc-46 hermaphrodites and allowing them to lay eggs for 6 h. The Dpy-18 Unc-46 progeny that were not homozygous for the lethal mutation grew faster and could be removed, leaving behind the homozygous lethal Dpy Uncs. Visual inspection classified their developmental blocking stages as either embryonic, early larval, late larval or sterile adult.

Testing for s1950 induced pseudolinkage

s1950 was tested to see if it caused pseudolinkage between *sma-2(e502)III* and markers on LGI, LGII, LGIV and LGX, according to the procedure described by Rosenbluth et al. (1985).

Testing for coverage of LGIII genes by sDp8 and sDp9

Males of the genotype *sDp8;eT1(III;V)* or *sDp9 eT1(III)/eT1(III); eT1(V)/eT1(V)* were crossed to marked *eT1(III;V)* hermaphrodites. The markers *dpy-1, dpy-17* and *sma-3* are on *eT1(III)*, while *bli-5(s277)* is on *eT1(V)*. The F_2 progeny from wild-type F_1 hermaphrodites were scored for the presence or absence of Unc-36 and marker phenotypes (see Results).

Results

Selection of UV-induced mutations

As described in Materials and Methods, *dpy-18/eT1;unc-46/eT1* P_0 hermaphrodites were treated with 120 J/m^2. Strains were established from wild-type F_1s no longer segregating fertile Dpy-18 Unc-46 F_2s (Fig. 2).

In the first instance, the mutations responsible for the absence of Dpy-18 Unc-46s were referred to as 'lethals'. The majority of the strains were expected to carry at least one recessive lethal mutation within the *eT1* balanced region, *cis*-linked to either *dpy-18(III)* or to *unc-46(V)*. The lethal mutation would affect at least one essential gene (*let*) and might be associated with a chromosomal rearrangement. Based on previous experience with γ ray-induced mutations (Rosenbluth et al., 1985), a small proportion of the strains, though initially classified as 'lethal strains', were expected to be homozygous *eT1s* that carried the wild-type allele of *unc-36* as a duplication.

Most of the irradiated P_0s were observed to be sickly. In the first experiment the progeny of 14 treated P_0s were examined. The average number of adult F_1 progeny per hermaphrodite was 80% of the number from untreated P_0s; the greatest reduction (to 59% of control) occurring 13–24 h post-treatment. 862 individual wild-type F_1s were

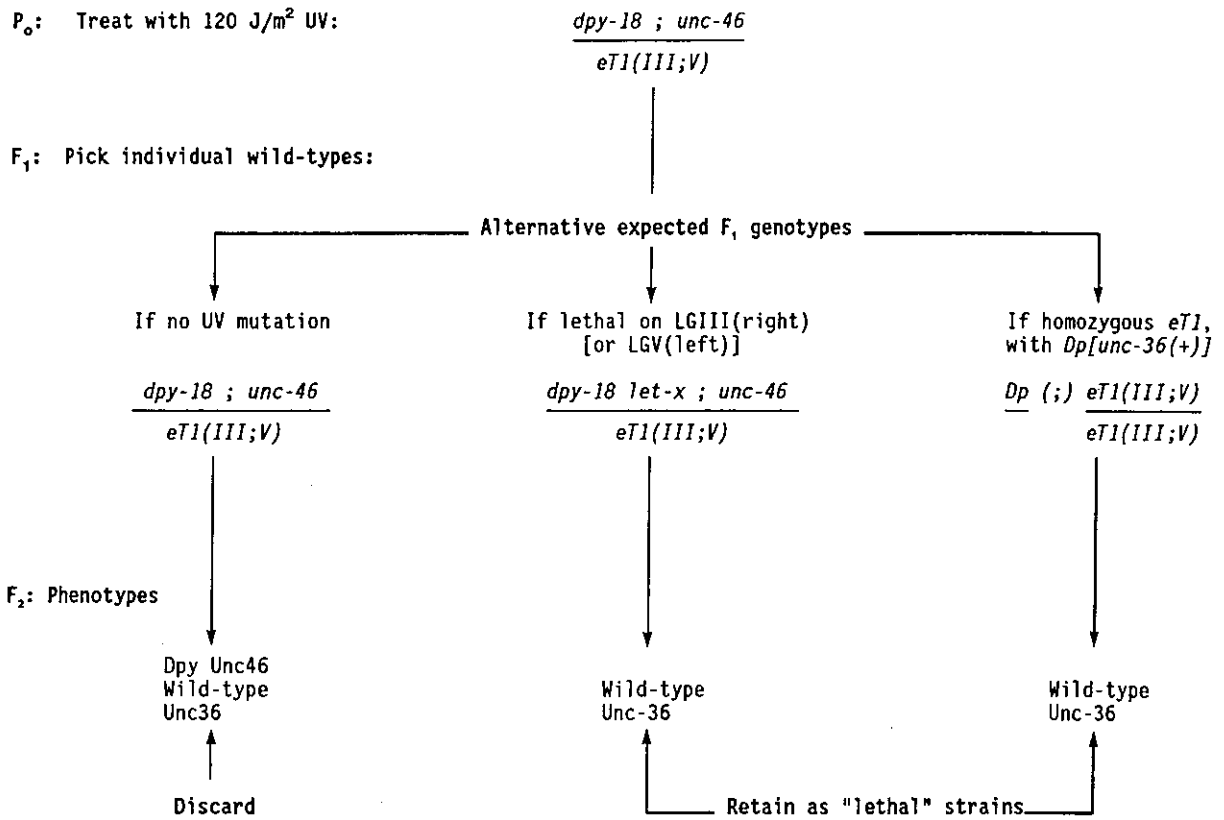

Fig. 2. Protocol for the recovery of *eT1*-balanced mutations.

picked and 49 of these (6%) proved to be sterile. Thus, while the P_0 fecundity and F_1 fertility were significantly affected after the treatment with 120 J/m^2, they provided an adequate supply of fertile F_1s (813) and permitted screening the F_2 progeny.

27 strains (3.1%) segregated no Dpy-18 Unc-46s and were retained as lethal strains. This is significantly higher than the spontaneous rate of 0.06% obtained previously (Rosenbluth et al., 1983). A second mutagenesis screen increased the number of lethals retained to 73.

Range of mutational events as assessed by brood sizes

As pointed out above, the screening protocol used is such that not only purely intragenic lethal mutations, but also chromosomal rearrangements may be recovered. The range of mutational events selected will depend on the mutagen used. Thus, for example, mutations selected after EMS treatment have been found to be predominantly intragenic mutations (Rosenbluth et al., 1988), while those selected after 1500 R γ-irradiation ranged from only a small percent of intragenic effects to predominantly multi-gene deficiencies and more complex chromosomal rearrangements (Rosenbluth et al., 1985). Johnsen and Baillie (1988), analysing formaldehyde induced lethals, suggested that the adult progeny brood size of a heterozygous putative lethal may be a crude indicator for the type of mutational event induced. That is, EMS-induced intragenic mutations generally do not have a dominant effect on brood size. In contrast, heterozygous lethals associated with large deficiencies, or more complex mutational events, often have significantly reduced brood sizes. Based on this, we initially assessed the range of mutations represented by the UV-induced lethals by analysing their progeny brood sizes.

We first determined the average total brood size (fecundity) for each of the 73 lethal mutations balanced over the *eT1* translocation. It must be

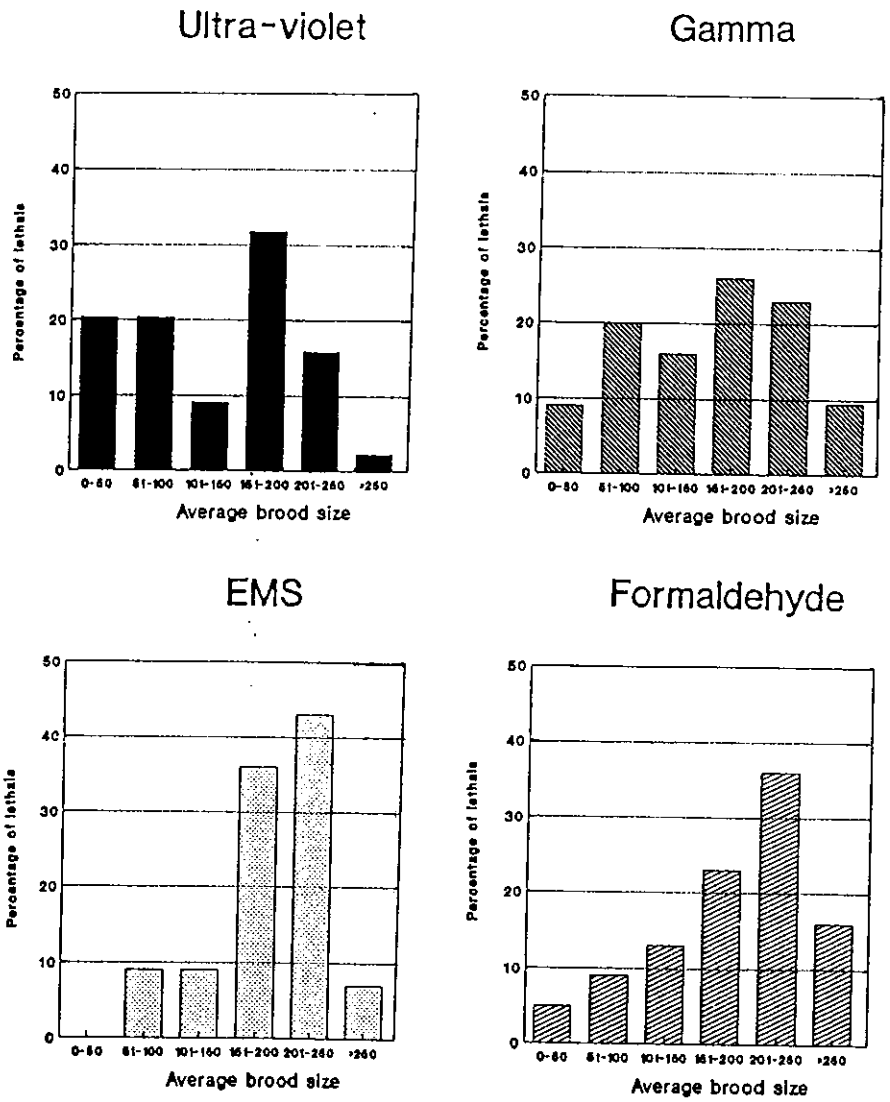

Fig. 3. Comparison of 'outcrossed' brood sizes for heterozygous mutations induced with either UV, γ irradiation, EMS or formaldehyde.

remembered that heterozygous *eT1*. hermaphrodites produce a large number of inviable aneuploid progeny. In the absence of any additional mutations, *dpy18 / eT1;unc46 / eT1* hermaphrodites have an average adult fecundity of 125. Wild-types from strains carrying a simple mutation in an essential gene were expected to produce about 104 progeny (5/6 of 125). The bulk of the *eT1* balanced strains had much lower brood sizes. 71% produced less than 50 progeny per hermaphrodite, suggesting that these carried chromosomal rearrangements.

We then measured the average brood sizes of 44 outcrossed lethals, no longer balanced over *eT1* (Materials and Methods). The average brood size of a *dpy-18/+;unc-46/+* hermaphrodite is 332. The presence of a recessive lethal on only one marked chromosome was expected to reduce this to about 250 progeny, while lethals on both chromosomes (i.e., LGIII and LGV) would reduce it to

187 (12/16 and 9/16 of 332 respectively). The distribution pattern of brood sizes for the outcrossed UV-induced lethals was compared with distribution patterns presented by Johnsen and Baillie (1988) for outcrossed lethals induced by 0.012 M EMS, 1500 R γ-radiation or 0.07–0.18% formaldehyde (Fig. 3). As can be seen, the UV pattern resembled more closely the one for gamma radiation than those for formaldehyde or EMS. The percentage of lethals producing < 150 progeny was 50% for UV and 44% for γ-ray-induced lethals; but it was only 25% for formaldehyde and 18% for EMS-induced ones.

Analyses of mutations

To determine whether chromosome rearrangements could be detected among the mutant strains, 44 strains were chosen at random for further study. The analyses essentially followed the protocol used for γ-radiation-induced mutations (Rosenbluth et al., 1985).

(1) Mapping to LGIII(right) or LGV(left). As a first step we attempted to map the putative lethal mutations to either normal LGIII(right) or normal LGV(left) (Materials and methods; Fig. 4). The F_2 ratios, Dpy-18/Wild and Unc-46/Wild established whether a lethal mutation was *cis*-linked to one of the markers and at what map distance. If one of these markers did not appear at all among the F_2s, the parent strain was crossed to either *dpy-18/+* or *unc-46/+* males to see if the strain still carried the marker. If marker progeny appeared from this cross, the strain did carry the

P_0: Wild-type hermaphrodite from mutant strain crossed to N2 male.

F_1: Pick Wild-type hermaphrodites.

F_2: From F_1s not segregating Unc-36s, score F_2 phenotypes and assign strain to a class (A - E).

	Types of UV mutations expected				
	Lethal on normal chromosome(s)			*unc-36(+)* duplication	
	On LGIII(right)	On LGV(left)	On each chromosome	Unlinked to *eT1*	Linked to *eT1*
F_1 genotype	*dpy-18 let ; unc-46* / + + / +	*dpy-18 ; unc-46 let* / + / + +	*dpy-18 let ; unc-46 let* / + + / + +	*Dp ; eT1 ; eT1* / / + / +	*Dp eT1 ; eT1* / + / +
F_2 ratios D18:Wild	Low or zero	Normal	Low or zero	Zero *	Zero *
F_2 ratios U46:Wild	Normal	Low or zero	Low or zero	Zero *	Zero *
				All F_1s give Unc-36s	
Class	A	B	C	D	E
Distribution of strains	12	16	4	1	3

Fig. 4. Protocol for mapping mutations to LGIII or LGV. When tested by crossing to *dpy-18/+* and *unc-46/+* males. See text for alternative genotype of Class E.

marker, but with a very tightly linked lethal mutation. We anticipated the five classes of strains shown in Fig. 4. Most strains were expected to fall into classes A to C (strains carrying lethal mutations that mapped to the normal chromosomes). Classes D and E represented strains that did not express the *dpy-18* and *unc-46* markers, neither in the mapping experiments nor in complementation tests. The simplest assumption was that these strains were homozygous *eT1*s carrying an *unc-36*(+) duplication. When crossed to N2 males they would give the F_1 genotypes shown in Fig. 4. In the case of class D the duplication would segregate independently of *eT1*, while in the case of class E it would be linked to one of the *eT1* chromosomes. Class E results could have been derived also from rare strains that were heterozygous *eT1*s, with a lethal mutation and the wild-type alleles of *dpy-18* and *unc-46* on the normal chromosomes. These alleles could have arisen from UV-induced crossover events or as tightly linked duplications. 36 of the 44 strains could be assigned to one of the anticipated classes (Fig. 4). 7 of the remaining 8 strains produced very small outcrossed brood sizes, while the eighth produced confusing phenotypes. Work on these 8 was discontinued.

32 strains carried lethals that mapped to the normal chromosomes. Of these, 12 strains carried lethals on LGIII, 16 carried lethals on LGV and 4 carried a lethal on each of the two chromosomes. This distribution of lethals (44% on LGIII and 56% on LGV) is significantly different (based on the Chi-square test) from the distribution found for EMS induced lethals, which are predominantly point mutations, and resembles that found for γ-irradiation lethals. For 898 strains carrying EMS induced lethal mutations on either LGIII(right) or LGV(left), Johnsen and Baillie (1988) reported that 65% mapped to LGIII and 34% to LGV. The distribution of 67 γ-irradiation induced lethals was 40% on LGIII and 60% on LGV [based on the combined results of Rosenbluth et al. (1985) and L.M. Addison, personal communication.] Johnsen and Baillie (1988) suggested that the difference in lethal distribution may be due to the preponderance of multi-gene deficiencies among the γ radiation-induced mutations. An LGIII deficiency deleting *unc-36* would not be picked up by the *eT1* screening protocol; it would be either in a wild-type or Unc-36 F_1. In addition, they point out that a deletion of a haplo-lethal region would result in a lethal F_1. Mains et al. (1990) have identified *mel-23(III)*, close to *dpy-28*, as a putative haploinsufficient gene. Thus deficiencies in either the *unc-36* or *mel-23* regions of LGIII would not have been recovered.

The results for the remaining four strains placed them in classes D and E, and suggested that these strains carried *unc-36*(+) duplications.

(2) Deficiencies on LGV(left). In the absence of cytological methods, detection of deficiencies in a given chromosomal region requires the availability of mutations in a large number of mapped genes. Since mutations in approximately 70% of the essential genes in LGV(left) have been mapped (Johnsen, 1990), we concentrated our search for deficiencies in this region. Recombination distances between the 16 LGV mutations and *unc-46* were calculated from the above mapping experiments. Using complementation mapping, it was possible to further position nine of the mutations on the maps previously established for LGV(left) (Johnsen and Baillie, 1988; Clark et al., 1990; Johnsen, 1990). Complementation tests with the remaining 7 mutations proved to be too difficult to carry out. 3 of these 7 produced average outcross brood sizes of less than 150 mature individuals, indicating that they probably carried complex mutations. Males, heterozygous for three others, did not mate well. The properties of the nine positioned LGV mutations are summarized in Table 1.

6 lethals failed to complement two or more adjacent genes and were designated as deficiencies *sDf70–sDf75* (Fig. 5). Unfortunately the only existing allele of the left-most marker on LGV, *let-450(s2160)*, has been lost, so that *sDfs 72, 73* and *74* could not be tested against it. One deficiency breakpoint was located in a region in which no other breakpoint had previously been located. The deficiency *sDf73* has a breakpoint that separates *let-448* from the *let-336, let-447, let-458* gene cluster. But, as yet, we do not know whether it is the left- or right-hand breakpoint and on which side of the cluster *let-448* is located.

It is of interest to note that, as heterozygotes, 4

TABLE 1

THE 9 LETHAL MUTATIONS POSITIONED ON LGV(LEFT)

Mutation name	Gene or deficiency	Recombination with *unc-46* in m.u. [a]	Average outcrossed brood size	Lethal arrest stage
s1963	*sDf70*	1.5 (0.6– 3.4)	206	Early larva
s1951	*sDf71*	1.8 (0.6– 4.3)	132	Embryo
s1966	*sDf72*	0.5 (0.4– 1.3)	110	Embryo
s1976	*sDf73*	0.0 (0.0– 1.3)	94	Early larva
s1939	*sDf74*	0.3 (0.1– 1.6)	201	Embryo
s1921	*sDf75*	8.5 (5.7–11.6)	178	Embryo
s1916	*lin-40*	6.6 (3.5–11.6)	165	Early larva
s1965	*let-349*	0.7 (0.1– 2.4)	171	Early larva
s1956	*let-462*	0.5 (0.1– 1.7)	213	Variable; embryo to early larva

[a] 95% confidence limits (in brackets) are based on the limits of the Unc-46 recombinants. These limits are taken from Table 1 of Crow and Gardner (1959).

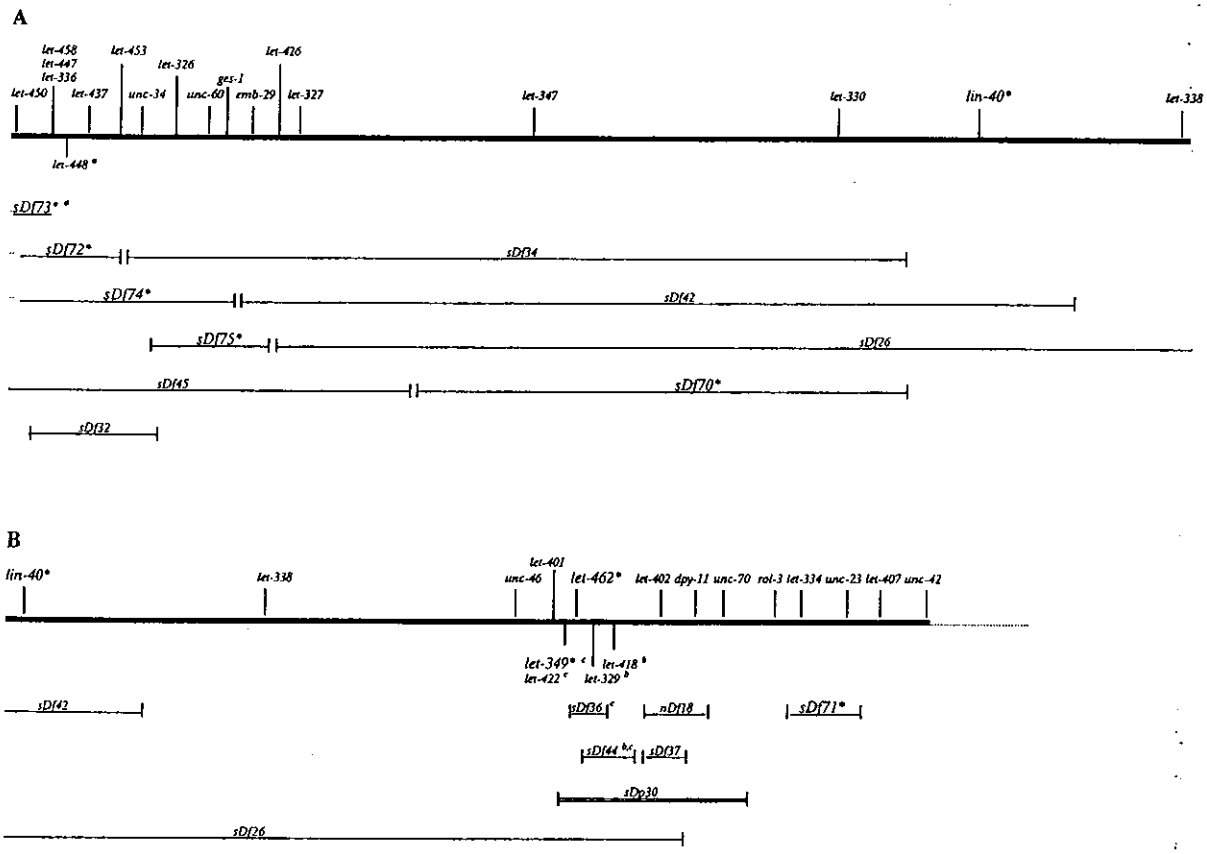

Fig. 5. Partial genetic map of LGV(left), simplified from that of Johnsen (1990). (A) Tip to *lin-40–let-338* region. (B) *lin-40–let-338* region to *unc-42*. UV deficiencies and genes with UV alleles are marked with an asterisk (*). The only other genes, deficiencies and duplications shown are those required to visualize the positions of UV induced mutations. (a) *let-448* is not deleted by *sDf73* and is either as shown or to the left of the deficiency. (b) The positions of *sDf44*, *let-329* and *let-418* are either as shown or to the left of *let-462*. (c) *let-349* and *let-422* are covered by *sDp30* and are deleted by *sDf26*, but are not deleted by the other 4 deficiencies in this region. These genes are either to the right or left of *sDfs36* and *44*. The distances between genes to the left of *unc-34* are not known.

of the 5 deficiencies mapping to the left of *unc-46(sDfs 70, 72, 73* and *74*) all severely inhibit recombination between themselves and *unc-46*. That is to say, all four lie to the left of *lin-40*, which is about 6.5 map units (m.u.) from *unc-46* (Rosenbluth et al., 1988), yet recombination data placed them significantly closer to *unc-46* than 6.5 m.u. (Table 1, column 3). Second site lethal mutations between the deficiencies and *unc-46* were ruled out by appropriate complementation tests with deficiencies spanning the regions. Similar effects on recombination have been observed with other LGV(left) deficiencies (Rosenbluth et al., 1990). The fifth deficiency, *sDf75*, lies to the left of *let-426* which is about 16 m.u. from *unc-46*. Recombination mapping placed *sDf75* only 8.5 m.u. from *unc-46*. Thus *sDf75* also inhibits recombination, but to a much lesser degree than the other 4 deficiencies.

The stages at which development of the homozygous deficiencies were arrested are shown in column 5 (Table 1). The embryonic blocking stages of *sDfs 71, 72, 74* and *75* indicate that these deficiencies either delete a gene whose expression is required for embryonic development or that the early arrests are due to cumulative effects caused by deleting several genes in their respective regions. In the region of *sDf75, emb-29* is known to be an 'embryonic lethal' (Cassada et al., 1981). The larval blocking stages of *sDfs 70* and *73* indicate that in these regions there are no embryonically expressed genes required for the completion of embryonic development. Three deficiencies had a dominant effect on the rate of development. That is, as heterozygotes, *sDfs 71, 72* and *74* retarded the rate of development.

Three LGV lethals each affected only one known gene. One, *s1956*, identified a gene not previously detected: *let-462*. The other two were mutations in previously identified genes: *let-349(s1965)* and *lin-40(s1916)*.

(3) A translocation among LGIII mutations. Four of the 16 lethals in Classes A and C, as heterozygotes over wild-type chromosomes, produced less than 150 progeny ('outcrossed brood size'). One of these, mapping to LGIII, had the characteristics of a putative translocation. The strain BC2748, carrying the lethal mutation *s1950*, produced no Dpy-18 F_2s in the mapping experiments, but still carried the *dpy-18* marker. To test whether the apparent tight linkage between *s1950* and *dpy-18* was due to an associated LGIII crossover suppressor, P_0 *dpy-18 let-(s1950)/sma-2 unc-64* hermaphrodites were constructed and their progeny scored for Sma-2 and Unc-64 recombinants. Among 470 F_1s there were 386 Wild-type, 0 Sma, 2 Unc-64 and 82 Sma Unc-64s, giving a recombination frequency of only 0.003 instead of the normal 0.20. The average brood size from each P_0 was only 97 (instead of about 250 from a simple heterozygous lethal). Thus *s1950* acted as a dominant crossover suppressor and caused a reduction in brood size. Dominant crossover suppressors in *D. melanogaster* and *C. elegans* are frequently associated with translocations [for *D.m.* see Roberts, 1970; for *C.e.* see McKim et al., 1988 and review by Herman (1988)]. If, as a heterozygote, such a translocation produces inviable aneuploid zygotes, then it will cause pseudolinkage between markers on the two normal chromosomes (as, for example, does *eT1*). Tests were carried out (Materials and Methods) which showed *s1950* to be associated with such a translocation, causing pseudolinkage between *unc-4(II)* and *sma-2(III)*. That is, *unc-4/T;sma-2/T* hermaphrodites produced 274 Wild-type, 0 Unc-4, 1 Sma-2 and 45 Sma-Uncs. The translocation was named *sT5 (II;III)*.

(4) Putative unc-36(+) duplications. (Classes D and E). Four strains expressed neither Dpy-18 nor Unc-46 phenotypes in the mapping experiments nor when complementation tested for these markers. All initially segregated wild-types and Unc-36s. The simplest assumption was that these strains were homozygous for *eT1* and heterozygous for the *unc-36(+)* allele. If so, the putative wild-type *unc-36* allele would necessarily have arisen from the normal LGIII chromosome and be carried in the form of a duplication, since the LGIII breakpoint of *eT1* is believed to be in *unc-36*. The mapping results described above suggested that the strain BC2755 carried a duplication segregating independently of the *eT1* chromosomes (Class D) while strains BC2719, BC2860 and BC2874 carried duplications linked to *eT1* (Class E). Another possible interpretation for the

ABLE 2

NALYSIS OF STRAINS BC2755, BC2719 AND BC2874 (CLASSES D AND E)

	F_2 ratios and brood sizes (from wild-type F_1 hermaphrodites)									
	If P_0 male is *dpy-11 eT1(III)/+ +;eT1(V)/+*					If P_0 male is *eT1(III)/+;sma-2 eT1(V)/+ +*				
	W	U36	D	DU	Brd	W	U36	S	SU	Brd
lternative genotypes for wild-type P_0 hermaphrodites from mutant strain	Predicted results									
) Heterozygous *eT1* with *dpy-18(+)*, *unc-46(+)* and a *let* on the normal chromosomes	4	0	0	1	< 100	4	0	0	1	< 100
) Homozygous *eT1*, − with *unc-36(+)* duplication										
(a) *cis*-linked to *dpy-11* on *eT1(III)*; does not cover *sma-2*	2	0	0	1	< 250	6	3	2	1	< 250
(b) *cis*-linked to *dpy-11* on *eT1(III)*; covers *sma-2*	2	0	0	1	< 250	8	3	0	1	< 250
(c) *cis*-linked to *sma-2* on *eT1(V)*	6	3	2	1	< 250	2	0	0	1	< 250
(d) Not linked to *eT1*; homozygous viable; does not cover *sma-2*	9	3	3	1	> 250	9	3	3	1	> 250
(e) Not linked to *eT1*; homozygous viable; covers *sma-2*	9	3	3	1	> 250	12	3	0	1	> 250
ɔurce of wild-type P_0 hermaphrodite	Observed results									
C2719 (Class E)	4.4 (427)	0 (1)	0 (0)	1 (97)	87	No *sma-2 eT1* bearing F_1s were produced				
C2874 (Class E)	2.2 (409)	0 (1)	0 (0)	1 (190)	200	8.6 (443)	3.3 (170)	0 (0)	1 (51)	223
C2755 (Class D)	8.4 (470)	2.1 (122)	2.7 (156)	1 (58)	269	7.7 (484)	2.1 (134)	2.3 (145)	1 (63)	276

', wild-type; U, Unc; D, Dpy; S, Sma. Brd, brood size. Numbers in brackets are individuals scored.

TABLE 3

TESTING LGIII GENETIC MARKERS FOR COVERAGE BY *unc-36*(+) DUPLICATIONS

Duplication	Marker tested	P_0 hermaphrodite	F_1 phenotypes				Covered
			W	U	M	MU	
sDp8	*dpy-1*	*sDp8;dpy-1 eT1/+ eT1;eT1/eT1*	529	177	197	65	No
	dpy-17	*sDp8;dpy-17 eT1/+ eT1;eT1/eT1*	495	173	131	67	No
	sma-3	*sDp8;sma-3 eT1/+ eT1;eT1/eT1*	429	93	0	18	Yes
	sma-2 *	*sDp8; eT1/eT1; sma-2 eT1/+ eT1*	484	134	145	63	No
sDp9	*dpy-1*	*sDp9 + eT1/dpy-1 eT1; eT1/eT1*	622	208	365 **		No
	dpy-17	*sDp9 + eT1/dpy-17 eT1;eT1/eT1*	700	21	0	279	Yes
	sma-2 *	*sDp9 eT1/eT1; + eT1/sma-2 eT1*	443	170	0	51	Yes
	bli-5	*sDp9 eT1/eT1; + eT1/bli-5 eT1*	All four phenotypes present				No

W, wild-type; U, Unc-36; M, marker phenotype.

* Data from Table 2.

** Non-Unc36 Dpys were present. Number given is for the combined Dpys plus Dpy Unc-36s.

Class E strains was that they were not homozygous *eT1*s but heterozygotes with normal LGIII and LGV chromosomes that now carried *dpy-18*(+) and *unc-46*(+) as well as a lethal mutation. The wild-type alleles of *dpy-18* and *unc-46* would have been derived from the *eT1* chromosomes (either as duplications or as UV-induced crossover products). Over a period of time, the class E strains continued to segregate wild-types and Unc-36s, but BC2755 eventually produced only wild-types. This suggested that the putative duplication of BC2755 had become homozygous.

In order to distinguish between the two proposed genotypes for class E strains, and to confirm the genotype for BC2755, wild-type hermaphrodites from each of the 4 strains were crossed to *dpy-11(s287)eT1(III)/+;eT1(V)/+* and *eT1(III)/+;sma2(s262)eT1(V)/+* males. The F_2 phenotypic ratios and average brood sizes ('Brd'), from wild-type F_1 hermaphrodites carrying the *dpy-11* or *sma-2* marker were scored for BC2755 and two of the class E strains (BC2719 and BC2874) (Table 2). The fourth strain, BC2860, produced confusing phenotypes and work with this strain was discontinued.

The first six rows of Table 2 present predictions based on the different genotypes considered in column one. Below that are the actual results. The data for BC2719 most closely matched prediction '1' and suggested that it is an *eT1* heterozygote, carrying a lethal and the wild-type alleles of *dpy-18* and *unc-46* on the normal chromosomes. The inability to obtain wild-type F_1s carrying the *sma-2 eT1* chromosome suggested that a *sma-2* mutation had been induced on LGIII. It is possible, therefore, that the lethal in BC2719 is a deficiency on the normal LGIII, deleting *sma-2* and at least one essential gene.

The data obtained with BC2755 and BC2874 were consistent with the assumption that these strains are homozygous *eT1*s carrying *unc-36*(+) duplications. The results for BC2755 matched most closely prediction 'd', confirming that its duplication segregates independently of *eT1* and is homozygous viable. The duplication does not cover *sma-2*. The results for BC2874 matched prediction 'b'. They confirmed that the duplication is on one of the *eT1* chromosomes [i.e., *eT1(III)*, close to the *dpy-11* gene], and indicated that it covers *sma-2* as well as *unc-36*. The duplications have been named *sDp8(III)* (in BC2755 and *sDp9(III)* (in BC2874).

The limits of *sDp8* and *sDp9* were determined by testing a number of LGIII genes for coverage by the duplications. Table 3 presents the progeny phenotypes from *sDp8+/+;eT1/m eT1* and *sDp9 eT1/m eT1* hermaphrodites (where *m* is a mutant allele of the tested gene). Presence of a non-Unc-36 marker phenotype (M) indicated that the duplication did not carry the wild-type allele of the tested gene. Conversely, absence of non-Unc-36 Ms indicated the duplication did carry the

TABLE 4

SUMMARY OF EVENTS REPRESENTED IN 44 STRAINS

Class of strain based on mapping experiments	Number of chromosome rearrangements				Number of intragenic events	Number of unknowns with brood [a] >150
	Identified			Putatives, i.e. with brood [a] <150		
	Translocations	Deficiencies	Duplications			
(A) Carry *let* on LGIII	1 [*sT5(II;III)*]			2		9
(B) Carry *let* on LGV		6 [*sDfs 70–75*]		3	3 [*lets 349* and *462* and *lin-40*]	4
(C) Carry *lets* on LGIII and LGV				1		3
(D) No Dpy-18 or Unc-46 markers expressed			1 [*sDp8*]			
(E) No Dpy-18 or Unc-46 markers expressed			1 [*sDp9*]	2		
(X) Unassignable				7		1
Totals	1	6	2	15	3	17

[a] 'Brood' refers to outcrossed brood sizes.

wild-type allele. Since *dpy-1* and *dpy-17* are both on *eT1(III)*, and therefore in *trans* with *sDp9*, these markers could be tested with *sDp9* only if recombination occurred between them and the duplication. The presence of non-M Unc-36 recombinants indicated that recombination did occur with both markers. The absence of Dpy-17 (the reciprocal recombinant) could therefore be taken to mean that *sDp9* covers *dpy-17*. Coverage of *dpy-18* could not be tested because the duplications presumably arose from *dpy-18(e364)* marked LGIIIs. The last column in Table 2 indicates which genes are covered. Neither duplication is a terminal duplication.

No attempt was made to determine whether *sDp8* is attached to another chromosome. However, in the course of constructing *sDp8*-bearing males, it was found that *sDp8* can be passed from male to male. It is therefore not attached to LGX. The insertion point of *sDp9* on *eT1(III)* was calculated using the frequencies of Unc-36 derived from the *dpy-11* (Table 2) and *dpy-17* (Table 3) experiments. Using the formula $p = 1 - [1 - 3(U36)]^{1/2}$ (where *U36* = frequency of Unc-36 and m.u. = $100p$), we calculate that *sDp9* is approximately 3 m.u. from *dpy-17* and 0.3 m.u. from *dpy-11*, thus placing it near *dpy-11* to the right of *dpy-17*.

Summary of results

Table 4 summarizes the results from the 44 mutations randomly chosen for analysis. 9 mutations were shown to be definite chromosomal rearrangements. 15 of the unknown mutations gave outcrossed brood sizes of less than 150 adults. Johnsen and Baillie (1988) have argued that such brood sizes are generally indicative of the presence of large deficiencies or more complex chromosomal rearrangements. We have therefore listed them as putative chromosomal rearrangements. It is likely that among the 17 unknown mutations giving brood sizes larger than 150 there are also a number of deficiencies, since out of 10 LGV mutations giving large brood sizes there were three deficiencies (*sDfs 70*, *74* and *75*; Table 1). Thus we estimate at least 29 of the 44 mutations to be chromosomal rearrangements.

Discussion

We have shown that germicidal (254 nm) UV irradiation of adult hermaphrodites provides a

simple method for the induction of heritable chromosomal rearrangements in *C. elegans*. With a dose of 120 J/m^2, mutations were recovered in 3% of the *eT1*-balanced chromosomes screened. Analysis of these lead to the conclusion that a large fraction are not simple intragenic lesions, but are deficiencies for several adjacent genes or more complex chromosomal rearrangements. Evidence for this conclusion came first from the fact that 50% of the hermaphrodites heterozygous for our mutations produced outcrossed brood sizes of less than 150 progeny. Many large deficiencies and other chromosomal rearrangements have dominant effects on the production of adult brood sizes. The large fraction of small brood sizes among the UV mutants is in sharp contrast to the 18% found among lethals recovered after treatment with EMS (Johnsen and Baillie, 1988), a mutagen that causes predominantly intragenic events. To confirm that chromosomal rearrangements had indeed been induced, we sought to identify: (1) deficiencies among the mutations mapping to LGV(left); and (2) interchromosomal events. The latter were sought by analysing strains that carried putative translocations or *unc-36* duplications. Six deficiencies, one translocation and two duplications were identified. We estimate that out of 44 mutations there could be as many as 29 chromosomal rearrangements.

The 6 deficiencies on LGV(left) will be useful tools for the continuing study of genome organization in that region. Deficiency and duplication breakpoints have divided the region into separate zones (Johnsen and Baillie, 1988; Johnsen, 1990). One of the UV-induced breakpoints provides further division of these zones. Three breakpoints, the left-hand ones of *sDfs 73*, *72* and *74*, are still undefined. Thus we do not know whether these deficiencies are 'terminal' or 'internal'. However, the fact that they each severely inhibit recombination in regions to their right may be a clue. In a previous study (Rosenbluth et al., 1990) a model for recombination pairing was proposed. The model was based on the difference with which different deficiencies affect recombination. It was proposed that deficiencies severely inhibiting recombination do not delete a pairing initiation site(s) to the left of *sDf32* that is deleted by deficiencies causing no (or only minor) inhibition. Based on this model we predict *sDfs 73*, *72* and *sDf74* will turn out to be 'internal' deficiencies.

With respect to the above cited model, *sDfs 73* and *75* provide additional data. Besides a site at which pairing for recombination initiates, the model also postulated the existence of 'alignment sites'. According to the model, the pairing process would spread from the initiation site by 'buttoning-up' the homologs at alignment sites which occur repeatedly along the chromosome and have a common nucleotide sequence. In a heterozygote for a deficiency that deletes one or more alignment sites, the sequential buttoning-up process would cause a misalignment to the right of the deficiency, so that heterologous regions would become aligned and recombination inhibited. A minimum of three alignment sites had been postulated for LGV(left). One of these was in the *sDf32* region. If there is only one site to the left of *let-326*, then the fact that *sDf73* caused severe inhibition of recombination would localize the site to the region deleted by both *sDf32* and *sDf73*. Conversely, the fact that *sDf75* did not cause severe inhibition, would suggest that no alignment sites lie within its region.

The translocation, *sT5(II;III)*, adds not only to the number of recombination balancers available for *C. elegans*, but also to the collection of chromosomal rearrangements used to identify homolog recognition sites (see McKim et al., 1988 for discussion). On LGIII, *sT5* suppresses recombination in the same region as do *eT1(III;V)*, *sT1(III;X)* (Rosenbluth et al., 1985) and *hT2(I;III)* (K. Peters and A.M. Rose, personal communication); that is, in the right half. The region of suppression on LGII was not established; all that is known is that the region between *unc-4* and the LGII breakpoint is suppressed. It is likely that *sT5* inhibits recombination in the same half of LGII as another translocation, *mnC2(II;X)* (Herman, 1978); that is, in the right half.

Finding that UV irradiation can lead to chromosomal rearrangements in germ cells has been documented in few previous studies. Slizynski (1942) cytologically analysed 21 UV-induced recessive lethal mutations in Drosophila and found 5 to be associated with deficiencies. Since then UV has rarely, if ever, been used to induce inherited rearrangements in Drosophila (Lindsley and Grell,

1967). Sadaie and Sadaie (1989) cytologically detected chromosome aberrations among *C. elegans* metaphase nuclei. The nuclei were in embryos derived from gametes treated with UV prior to fertilization. Whether these would lead to stable inherited chromosomal rearrangements was not determined. However, P.S. Hartman (personal communication) has recently isolated heterozygous UV-induced *unc-22* and *fem-3* mutations in *C. elegans*. 8 out of 15 of these are not viable as homozygotes and are likely to be deficiencies extending into at least one essential gene. If confirmed, these findings indicate that our results are not restricted to mutations isolated by the *eT1* screening system.

Comparing our UV data with those of two other mutagens known to induce chromosomal rearrangements in *C. elegans*, γ-rays and formaldehyde (at doses normally used in our laboratory), we find that the frequency of lethal induction by 120 J/m^2 UV is very similar to that by 1500 R γ-irradiation (Rosenbluth et al., 1985) and approximately twice that by 0.1–0.18% formaldehyde (Johnsen and Baillie, 1988). Regarding the type of mutations produced, Johnsen and Baillie (1988) presented data indicating that the mutagenic effects of 0.07–0.18% formaldehyde were less drastic than those of 1500 R γ-rays. The crude assessment of our UV-induced mutations, based on brood sizes and distribution between LGIII and LGV, suggests that the spectrum of UV events matched that of γ-ray events more closely than that of formaldehyde. The results for analysed LGV(left) mutations are consistent with this. The ratio of multi-gene deficiencies to apparent intragenic events is 6:3 for UV-, 7 : 4 for γ- and only 5 : 11 for formaldehyde-induced mutations. Whether the less drastic effect of the formaldehyde treatment is simply a function of dosage will require further analyses.

The apparent similarities between the genetic effects produced by γ-irradiation and UV are probably superficial, and only true at the levels analysed here. Biological damage by γ rays is due to high energy ionizing radiation while that by UV is due to direct DNA absorption. Based on molecular studies in other organisms, the potential premutational targets for DNA lesions are probably much fewer for UV than for γ irradiation. In terms of specific nucleotides, the major targets for damage by UV are adjacent pyrimidines (Friedberg, 1985). Recent DNA sequence analyses of a large number of UV-induced mutations in several genes found that the majority of mutations were, in fact, the results of lesions at such sites (Schaaper et al., 1987; Drobetsky et al., 1987; Vrieling et al., 1989; Romac et al., 1989). Damage by γ-irradiation probably has no nucleotide specificity. The types of the lesions induced by the two mutagens are likely to have different spectra and the mechanisms whereby the lesions result in stable mutations may be different. It would, therefore, be of interest to compare the genetic effects of UV and γ-irradiation further.

For example, γ irradiation of germline cells causes an increase in recombination ('map expansion'); but the increase is not uniform along the *C. elegans* chromosome (Kim and Rose, 1987). The increase is greatest in regions in which genes are clustered on the normal recombination map. One proposal to explain this phenomenon was that: (1) the normal rate of recombination per unit length DNA is not constant. That is, in regions of cluster normal recombination is suppressed; and (2) enhancement of recombination by γ-irradiation is due to the induction of DNA nicks which act as additional substrates for the recombination machinery in the suppressed regions. Since UV produces chromosome breaks in germ cells, a parallel experiment with UV could be run to see if it produces the same results. If the results are different, they might provide further insights into chromosome structure, recombination, and/or the mechanisms of radiation damage and DNA repair in *C. elegans*.

Finally, in regard to DNA repair, analysis of lethals induced by radiation in the presence of *rad* (radiation-sensitive) mutations may prove valuable. Hartman has identified *C. elegans rad* genes, 4 of which appear to be involved in DNA repair (Hartman, 1985). The functions of these genes are being analysed by studying the worm's response to irradiation damage in the presence of *rad* mutations. Thus, survival studies have shown that *rad-1* and *rad-2* mutations cause hypersensitivity to both UV and γ irradiation, while *rad-3* and *rad-7* mutations cause hypersensitivity to only UV (Hartman, 1985). Furthermore, analyses of DNA

from whole organisms have shown that mutations in *rad-3*, but not in the other three genes, diminish *C. elegans'* capacity to excise UV-induced pyrimidine dimers (Hartman et al., 1989). Testing the role of these genes for germ-line mutability, Coohill et al. (1988) have measured the effects of *rad* mutations on the frequency of *eT1*-balanced lethals induced with UV. They found *rad-3* mutants to be hypermutable, but *rad-1* and *rad-7* mutants were hypomutable. It would be interesting now to analyse UV lethals induced in the presence of *rad-3*, and determine whether *rad-3* causes a detectable change in the spectrum of mutational events.

In conclusion, this study has shown that UV irradiation produces stable heritable mutations in *C. elegans* that are predominantly chromosomal rearrangements and thus differ from the spectrum of UV induced mutations commonly reported in other systems. The ease with which UV radiation is applied to germ-line cells makes UV an additional tool for the analysis of *C. elegans* chromosomes. Furthermore, the ability to store mutants indefinitely in liquid nitrogen (Brenner, 1974), as well as the linking of genetic and physical maps (Coulson et al., 1986) should make *C. elegans* a useful organism in which to study the molecular bases whereby UV acts as a mutagen in cells of complex eukaryotes.

Acknowledgements

We are grateful to Robert C. Johnsen for providing many of the lethal mutations required for the mapping experiments, and for helpful discussions. We thank Phil S. Hartman for helpful comments regarding the manuscript. We also thank Lyndley Kemp for help in quantifying the UV dosage and Margaret Rogalski for preparing the worm medium. This work was supported by grants from the Natural Sciences and Engineering Council of Canada and the Muscular Dystrophy Association of Canada to D.L.B.; as well as an award from Simon Fraser University to H.I.S.

References

Altenburg, E. (1934) The artificial production of mutations by ultra-violet light, Am. Nat., 68, 491–506.

Brenner, S. (1974) The genetics of *Caenorhabditis elegans*, Genetics, 77, 71–94.

Brown, S.J. (1984) Genetic interactions affecting muscle organization in the nematode *C. elegans*, Ph.D. Thesis, University of Missouri, Columbia, MO.

Cassada, R., E. Isnenghi, M. Culotti and G. von Ehrenstein (1981) Genetic analysis of temperature-sensitive embryogenesis mutants in *Caenorhabditis elegans*, Dev. Biol., 84, 193–205.

Clark, D.V., R.C. Johnsen, K.S. McKim and D.L. Baillie (1990) Analysis of lethal mutations induced in a mutator strain that activates transposable elements in *Caenorhabditis elegans*, Genome, 33, 109–114.

Coohill, T., T. Marshall, W. Shubert and G. Nelson (1988) Ultraviolet mutagenesis of radiation-sensitive (rad) mutants of the nematode *Caenorhabditis elegans*, Mutation Res., 209, 99–106.

Coulson, A., J. Sulston, S. Brenner and J. Karn (1986) Toward a physical map of the genome of the nematode *Caenorhabditis elegans*, Proc. Natl. Acad. Sci. (U.S.A.), 83, 7821–7825.

Coulson, A., R. Waterston, J. Kiff, J. Sulston and Y. Kohara (1988) Genome linking with yeast artificial chromosome, Nature (London), 335, 184–186.

Crow, E.L., and R.S. Gardner (1959) Confidence intervals for the expectation of a poisson variable, Biometrika, 46, 441–453.

Doudney, C.O. (1976) Mutation in ultraviolet light – Damaged microorganisms, in: S.Y. Wang (Ed.), Photochemistry and Photobiology of Nucleic Acids, Vol. II, Academic Press, New York, pp. 309–374.

Drobetsky, E.A., A.J. Grosovsky and B.W. Glickman (1987) The specificity of UV-induced mutations at an endogenous locus in mammalian cells, Proc. Natl. Acad. Sci. (U.S.A.), 84, 9103–9107.

Edgley, M.L., and D.L. Riddle (1990) The nematode *Caenorhabditis elegans*, in: S.J. O'Brien (Ed.), Genetic Maps. Locus Maps of Complex Genomes, 5th edn., Book 3, Lower Eukaryotes, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, pp. 3.111–3.133.

Friedberg, E.C. (1985) DNA Repair, Freeman, New York, pp. 40–48.

Greenwald, I.S., and H.R. Horvitz (1980) *unc93(e1500)*: A behavioral mutant of *Caenorhabditis elegans* that defines a gene with a wild-type null phenotype, Genetics, 96, 147–164.

Hartman, P.S. (1985) Epistatic interactions of radiation-sensitive (*rad*) mutants of *Caenorhabditis elegans*, Genetics, 109, 81–93.

Hartman, P.S., J. Hevelone, V. Dwarakanath and D.L. Mitchell (1989) Excision repair of UV radiation-induced DNA damage in *Caenorhabditis elegans*, Genetics, 122, 379–385.

Herman, R.K. (1978) Crossover suppressors and balanced recessive lethals in *Caenorhabditis elegans*, Genetics, 88, 49–65.

Herman, R.K. (1988) Genetics, in: W. Wood (Ed.), The Nematode *Caenorhabditis elegans*, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, pp. 1–45.

Hodgkin, J., H.R. Horvitz and S. Brenner (1979) Nondisjunction mutants of the nematode *Caenorhabditis elegans*, Genetics, 91, 67–94.

Horvitz, H.R., S. Brenner, J. Hodgkin and R.K. Herman (1979) A uniform genetic nomenclature for the nematode *Caenorhabditis elegans*, Mol. Gen. Genet., 175, 129–133.

Johnsen, R.C. (1990) Genetic analysis of the left half of Linkage Group V in *Caenorhabditis elegans*, Ph.D. Thesis, Simon Fraser University, Burnaby, B.C., Canada.

Johnsen, R.C., and D.L. Baillie (1988) Formaldehyde mutagenesis of the *eT1*-balanced region in *Caenorhabditis elegans*: Dose-response curve and the analysis of mutational events, Mutation Res., 201, 137–147.

Kim, J.S., and A.M. Rose (1987) The effect of gamma radiation on recombination frequency in *Caenorhabditis elegans*, Genome, 29, 457–462.

Lindsley, D.H., and E.H. Grell (1967) Genetic Variations of *Drosophila melanogaster*, Carnegie Inst. of Washington.

MacKenzie, K. (1941) Mutation and lethal effects of ultra-violet irradiation on Drosophila, Proc. Roy. Soc. Edinburgh, 61B, 67–77.

McGhee, J.D., J.C. Birchall, M.A. Chung, D.A. Cottrell, L.G. Edgar, P.C. Svendsen and D.C. Ferrari (1990) Production of null mutations in the major intestinal esterase gene (*ges-1*) of the nematode *Caenorhabditis elegans*, Genetics, 125, 505–514.

McKim, K.S., A.M. Howell and A.M. Rose (1988) The effects of translocations on recombination frequency in *Caenorhabditis elegans*, Genetics, 120, 987–1001.

Mains, P.E., I.A. Sulston and W.B. Wood (1990) Dominant maternal-effect mutations causing embryonic lethality in *Caenorhabditis elegans*, Genetics, 125, 351–369.

Moerman, D.G., and R.H. Waterston (1989) Mobile elements in *Caenorhabditis elegans* and other nematodes, in: D. Berg and M. Howe (Eds.), Mobile DNA, American Society of Microbiology, Washington, D.C., pp. 537–556.

Nelson, G.A., W.W. Schubert, T.M. Marshall, E.R. Benton and E.V. Benton (1989) Radiation effects in *Caenorhabditis elegans*, mutagenesis by high and low LET ionizing radiation, Mutation Res., 212, 181–192.

Park, E.-C., and H.R. Horvitz (1986) *C. elegans unc-105* mutations affect muscle and are suppressed by other mutations that affect muscle, Genetics, 113, 853–867.

Roberts, P.A. (1970) Screening for X-ray induced crossover suppressors in *Drosophila melanogaster*: prevalence and effectiveness of translocations, Genetics, 65, 429–448.

Romac, S., P. Leong, H. Sockett and F. Hutchinson (1989) DNA base sequence changes induced by ultraviolet light mutagenesis of a gene on a chromosome in Chinese hamster ovary cells, J. Mol. Biol., 209, 195–204.

Rose, A.M., and D.L. Baillie (1979) The effect of temperature and parental age on recombination and nondisjunction in *Caenorhabditis elegans*, Genetics 92, 409–418.

Rosenbluth, R.E., and D.L. Baillie (1981) The genetic analysis of a reciprocal translocation *eT1(III,V)*, in *Caenorhabditis elegans*, Genetics, 99, 415–428.

Rosenbluth, R.E., C. Cuddeford and D.L. Baillie (1983) Mutagenesis in *Caenorhabditis elegans*, I. A rapid eukaryotic mutagen test system using the reciprocal translocation, *eT1(III,V)*, Mutation Res., 110, 39–48.

Rosenbluth, R.E., C. Cuddeford and D.L. Baillie (1985) Mutagenesis in *Caenorhabditis elegans*, II. A spectrum of mutational events induced with 1500 R gamma-radiation, Genetics, 109, 493–511.

Rosenbluth, R.E., T.M. Rogalski, R.C. Johnsen, L.M. Addison and D.L. Baillie (1988) Genomic organization in *Caenorhabditis elegans*: Deficiency mapping on linkage group V(left), Genet. Res., Camb., 52, 105–118.

Rosenbluth, R.E., R.C. Johnsen and D.L. Baillie (1990) Pairing for recombination in LGV of *Caenorhabditis elegans*: A model based on recombination in deficiency heterozygotes, Genetics, 124, 615–625.

Sadaie, T., and Y. Sadaie (1989) *Rad-2*-dependent repair of radiation-induced chromosomal aberrations in *Caenorhabditis elegans*, Mutation Res., 218, 25–31.

Schaaper, R.H., R.L. Dunn and B.W. Glickman (1987) Mechanisms of ultraviolet-mutation, Mutational spectra in the *Escherichia coli lacI* gene for a wild-type and an excision-repair-deficient strain, J. Mol. Biol., 198, 187–202.

Slizynski, B.M. (1942) Deficiency effects of ultra-violet light in *Drosophila melanogaster*, Proc. Roy. Soc. (Edinburgh), 61B, 297–315.

Stadler, L.J., and G.F. Sprague (1936) Genetic effects of ultraviolet radiation in maize, I. Unfiltered radiation, Proc. Natl. Acad. Sci. (U.S.A.), 22, 572–578.

Vrieling, H., M.L. van Rooijen, N.A. Groen, M.Z. Zdzienicka, J.W.I.M. Simons, P.H.M. Lohman and A.A. van Zeeland (1989) DNA strand specificity of UV-induced mutations in mammalian cells, Mol. Cell. Biol., 9, 1277–1283.

Wood, W.B. (Ed.) (1988) The nematode *Caenorhabditis elegans*, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

Zengel, J.M., and H.F. Epstein (1980) Identification of genetic elements associated with muscle structure in the nematode *Caenorhabditis elegans*, Cell Motility, 1, 73–97.