MUTAGENESIS IN *CAENORHABDITIS ELEGANS*. II. A SPECTRUM OF MUTATIONAL EVENTS INDUCED WITH 1500 R OF γ-RADIATION

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ABSTRACT

We previously established a γ-ray dose-response curve for recessive lethal events (lethals) captured over the *eT1* balancer. In this paper we analyze the nature of lethal events produced, with a frequency of 0.04 per *eT1* region, at a dose of 1500 r. To do so, we developed a protocol that, in the absence of cytogenetics, allows balanced lethals to be analyzed for associated chromosomal rearrangements. A set of 35 lethal strains was chosen for the analysis. Although the dosage was relatively low, a large number of multiple-break events were observed. The fraction of lethals associated with rearrangements was found to be 0.76. Currently most X- and γ-ray dosages used for mutagenesis in *C. elegans* are 6000–8000 r. From our data we conservatively estimated that 43% of rearrangements induced with 8000 r would be accompanied by additional chromosome breaks in the genome. With 1500 r the value was 5%.—The 35 lethals studied were derived from 875 screened F_1's. Among these lethals there were (1) at least two *unc-36* duplications, (2) at least four translocations, (3) at least six deficiencies of chromosome *V* (these delete about 90% of the *unc-60* to *unc-42* region) and (4) several unanalyzed rearrangements. Thus, it is possible to recover desired rearrangements at reasonable rates with a dose of only 1500 r.—We suggest that the levels of ionizing radiation employed in most published *C. elegans* studies are excessive and efforts should be made to use reduced levels in the future.

IONIZING radiation (X or γ ray) is commonly used in the nematode *Caenorhabditis elegans* to produce specific chromosome rearrangements. In order that these rearrangements might be readily amenable to analysis, it is desirable to use as low a mutagen dose as possible. The induction of too many chromosomal breaks is liable to produce complex events whose correct analysis is difficult. This is especially true for mutagenesis in *C. elegans*. Here, in contrast to *Drosophila melanogaster*, the detailed cytological monitoring of chromosome structure is not possible. The analyses are, therefore, almost entirely dependent on indirect genetic interpretation. Most of the doses currently used for *C. elegans* are in the range of 6000 to 8000 r (GREENWALD and HORVITZ 1980; HERMAN, MADL and KARI 1979; MENEELY and WOOD 1984; ROSE, BAILLIE and CURRAN 1984). Since these levels are higher than those generally used for Drosophila, we decided to estimate the amount of genetic damage they produce and are therefore studying the efficacy of γ-ray mutagenesis in *C. elegans*.

The first quantitative data concerning the effect of γ rays in *C. elegans* was provided previously in the form of a dose-response curve (ROSENBLUTH, CUDDEFORD and BAILLIE 1983). However, the "response" in that study measured only the frequency of at least one putative recessive lethal mutation in a 40-map unit (mu) interval; no attempt was made to determine whether any of these recessive lethal mutations were associated with chromosomal breaks. The present study analyzes a set of 35 lethal-bearing strains, selected after treatment with 1500 r of γ-irradiation, and seeks to determine the frequency of lethals that are associated with detectable chromosomal rearrangements. To do so, a protocol was developed that analyzes balanced lethal mutations for association with chromosomal rearrangements, without the aid of cytogenetics. Our findings suggest that with doses of 6000–8000 r there is an exceedingly high probability of producing multiple-break events.

MATERIALS AND METHODS

General: The nomenclature in this paper follows the uniform system adopted for *C. elegans* (HORVITZ *et al.*, 1979). Nematodes were cultured on Petri plates containing nematode growth medium streaked with *Escherichia coli*, strain OP50 (BRENNER 1974). The wild-type strain, N2, and strains carrying the following mutations of *C. elegans* var. Bristol were originally obtained from the Medical Research Council stock collection in Cambridge, England, or from the Caenorhabditis Genetics Center at the University of Missouri, Columbia, Missouri. Linkage group I (LGI): *unc-13(e450)*; LGII: *unc-4(e120)*; LGIII: *dpy-1(e1), unc-36(e251), sma-2(e502), unc-32(e189), dpy-18(e364), unc-64(e246)*; LGIV: *unc-31(e169)*; LGV: *unc-60(e677), unc-46(e177), dpy-11(e224), unc-23(e25), unc-42(e270), sma-1(e30)*; LGX: *unc-6(e78)*; and the reciprocal translocation *eT1(III;V)*, which carries the recessive *unc-36* defect *e873*. The characteristics of *eT1(III;V)* are described in RESULTS.

The relative positions of the genes on LGIII, LGV and eT1 are shown in Figure 1. The following mutations were induced with ethyl methanesulfonate in the course of an earlier study (ROSENBLUTH and BAILLIE 1981). On *eT1(III)*: *dpy-11(s287)*; on *eT1(V)*: *sma-2(s262), bli-5(s277)*; on LGV: *let-326(s238), let-327(s247)*. In addition, two spontaneous LGV mutations were used: *let-328(s472)* and *let-329(s473)*. The four lethal mutations arose in *dpy-18/eT1(III); unc-46/eT1(V)* hermaphrodites and were *cis* linked to *unc-46*. Their positions were established by recombination mapping. Appropriate complementation tests showed that they identified separate genes. The position of *let-326* with respect to *unc-60* is still ambiguous. Although three of 103 viable Unc-46 recombinants from *let-326 unc-46/unc-60 dpy-11* hermaphrodites failed to carry *unc-60*, *let-326* could still be to the right of *unc-60* if these recombinants were due to double crossover events. *let-329*, which mapped about 1 mu to the right of *unc-46*, failed to complement *sDf26* and was, therefore, positioned to the left of *dpy-11* (see RESULTS).

Recombination mapping of lethal mutations: The distances of lethal mutations from either *dpy-18(III)* or *unc-46(V)* were determined as described previously (ROSENBLUTH and BAILLIE 1981). Left-right positioning of LGV lethals with respect to *unc-60*, *unc-46* and *dpy-11* was done by progeny testing Unc-46 recombinants from *unc-46 let-(sx)/unc-60 dpy-11* hermaphrodites.

unc-36 *duplications: testing linkage group III genes for coverage*: For the *unc-36 (+)* duplications, *sDp3* and *sDp4* (see RESULTS), male strains were constructed that were *sDp3; eT1/eT1* and *sDp4 eT1(III)/eT1(III);eT1(V)/eT1(V)*, respectively. Males from these strains were crossed to *m eT1* hermaphrodites, where "m" represents a mutant allele of the gene being tested. The absence of mutant non-Unc-36 F_2 individuals among the progeny of self-fertilizing wild-type F_1 hermaphrodites suggested that the duplication carried *m(+)*. This was subsequently confirmed by testing individual wild-type F_2 hermaphrodites to determine whether any produced only mutant Unc-36 and wild-type progeny (*i.e.*, no Unc-36's). These were homozygous for *m eT1*, with the duplication present and carrying the *m(+)* allele.

Tests for dominant crossover suppression by lethal mutations: LGIII lethals: *let-(sx) dpy-18/sma-2 unc-64* hermaphrodites (lacking the *unc-46* marker) were constructed and allowed to "self." For lethals

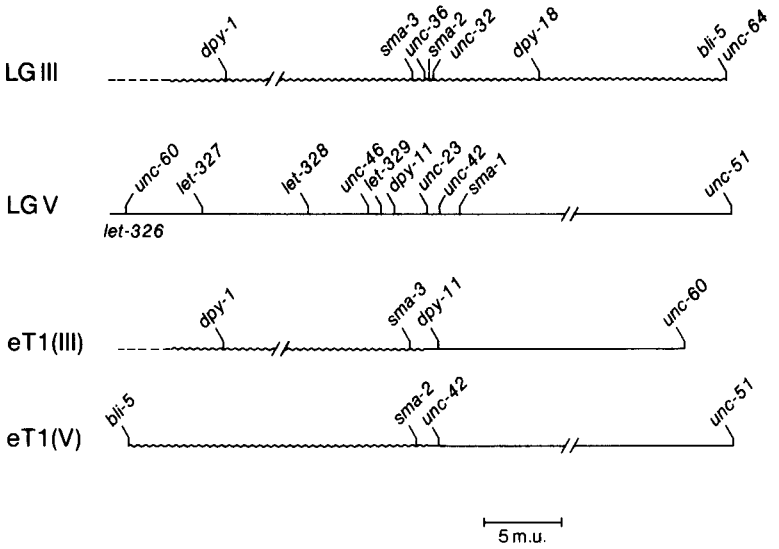

FIGURE 1.—Relative positions of genes on LGIII, LGV, *eT1(III)* and *eT1(V)*.

mapping close to *dpy-18*, the frequency of recombination, p, between *sma-2* and *unc-64* was calculated from the progeny as $p = 1 - \sqrt{0.996 - 3(U)}$, where U was the frequency of the Unc-64 recombinants. A frequency significantly lower than 0.20 indicated a dominant crossover suppressor associated with the *dpy-18* chromosome. LGV lethals: the frequency of recombination, p, between *unc-60* and *dpy-11* in a *let-(sx) unc-46/unc-60 dpy-11* hermaphrodite (lacking the *dpy-18* marker) was calculated from the progeny as follows. For lethals near *unc-60*, $p = 1 - \sqrt{1 - 3(U)}$ and for lethals near *unc-46*, $p = 1 - \sqrt{1 - 3(D)}$, where U and D were the frequencies of Unc-60 and Dpy-11 recombinants, respectively. Frequencies significantly lower than 0.18 indicated the presence of a crossover suppressor.

Testing of crossover suppressors for association with a translocation (pseudolinkage tests): For BC 891, hermaphrodites retained from the crossover suppression test, which carried the lethal-associated dominant crossover suppressor in the *trans*-configuration to *sma-2 unc-64*, were crossed with N2 males. Individual progeny males (Po's) were crossed to homozygous *sma-2;unc-m* hermaphrodites, where *unc-m* was either *unc-13(I), unc-4(II), unc-31(IV)* or *unc-6(X)*. Individual wild-type F_1 hermaphrodites from the males that had been crossover suppressor/+ (*i.e.*, gave no Sma's), were allowed to "self" and the F_2 progeny were scored. Those F_1's that had received the "+" chromosome were expected to produce a normal number (200–300) of progeny and show no pseudolinkage between *sma-2* and the *unc-m* marker. The F_1's receiving the crossover suppressor were expected to produce fewer than normal numbers of offspring if the suppressor was associated with a translocation. Furthermore, if the *unc-m* chromosome being tested was homologous to part of that translocation, fewer Sma's than Sma Unc's were anticipated. In the case of a possible *III;X* translocation two possibilities were considered, assuming that only balanced zygotes survive. If a lethal mutation associated with the crossover suppressor was in an *X* chromosome gene, none of the Po males should have carried the translocation (that is, all of the Po males should have been *sma-2 unc-64/+ +*). Alternatively, if there was no lethal associated with the *X*, all of the selected F_1 hermaphrodites were expected to carry the translocation and thus give fewer than normal progeny.

For BC 1042 similar tests were performed using *dpy-11;unc-m* double mutants. The crossover suppressors in BC 1031 and BC 1033 were tested only for *III;V* translocations using the double mutant *unc-32;dpy-11*. Wild-type hermaphrodites from these strains were crossed to *unc-46/+* males. The resulting F_1 Unc-46 hermaphrodites were then crossed to *unc-32/eT1(III);dpy-11/eT1(V)* males and individual wild-type F_2 hermaphrodites were selfed. The progeny of Dpy Unc-32 producing F_2's were scored to determine whether those that failed to produce Unc-46's showed pseudolinkage between *unc-32* and *dpy-11*. The occurrence of such progeny indicated that the lethal *unc-46* chromosome was associated with an *unc-36(+) (III;V)* translocation.

Complementation tests for lethal mutations: Complementation between *let-(sx)* and *let-(sy)* was tested by constructing *dpy-18/eT1(III); unc-46 let- (sx)/eT1(V)* males and crossing them with *dpy-18/eT1(III);unc-46 let-(sy)/eT1(V)* hermaphrodites. The presence of Dpy Unc males and fertile Dpy Unc hermaphrodites among the progeny indicated that the mutations complemented.

RESULTS

Strains, putatively heterozygous for at least one 1500-r-induced recessive lethal mutation balanced over *eT1(III;V)*, were analyzed for the presence of chromosomal rearrangements.

Characteristics of the translocation, eT1(III;V)

To follow the analyses an understanding of *eT1(III;V)* is essential. This translocation was described previously (ROSENBLUTH and BAILLIE 1981). Its characteristics are briefly as follows. The translocation *eT1* consists of two half-translocations: *eT1(III)*, segregating from the normal LGIII, and *eT1(V)*, segregating from the normal LGV. The LGIII breakpoint is either in or very close to *unc-36* (see Figure 1). As a heterozygote with normal chromosomes, *eT1* suppresses recombination over an interval of 40 mu (*unc-36* to *unc-64* on LGIII; *unc-60* to approximately 2 mu to the right of *dpy-11* on LGV). As a homozygote it is viable and exhibits an Unc-36 phenotype due to an associated recessive *unc-36* mutation. Since only euploid zygotes survive to adulthood, an *eT1* heterozygote will show pseudolinkage between a marker on its normal LGIII and one on its normal LGV (that is, for any markers that lie within the crossover suppressed regions). Thus, the phenotypic ratios produced by a wild-type *dpy-18/eT1(III); unc-46/eT1(V)* hermaphrodite are one Unc-36: four Wild: one Dpy-18 Unc-46. The total number of progeny from such a heterozygote is only about 100 (36% of that from a "normal" individual).

For the sake of brevity, we have omitted the symbols III and V from many of the genotypes in the remainder of this work.

Source of the lethal strains

The lethal-bearing chromosomes had been recovered in a previous study (ROSENBLUTH, CUDDEFORD and BAILLIE 1983) after the following screening protocol. Phenotypically wild-type Po hermaphrodites, *dpy-18/eT1;unc-46/eT1*, after treatment with 1500 r, were allowed to self. A gamete carrying a recessive lethal mutation, *cis* linked to either *dpy-18* or *unc-46* and within the crossover suppressed region, was expected to give rise to a wild-type F_1 whose F_2 progeny would lack Dpy-18 Unc-46's. Thus, F_2 progeny from individual wild-type F_1's were screened for the absence of mature Dpy Unc's. Thirty-five strains recovered on this basis were chosen for the present study. They were selected randomly and were maintained by picking wild-type hermaphrodites.

A flow diagram (Figure 2) describes the manner in which the mutants were analyzed.

Initial characterization of the strains

Unless indicated otherwise, all strains segregated wild-type and Unc-36 phenotypes but no Dpy Unc-46's. As a first approximation it was assumed that the

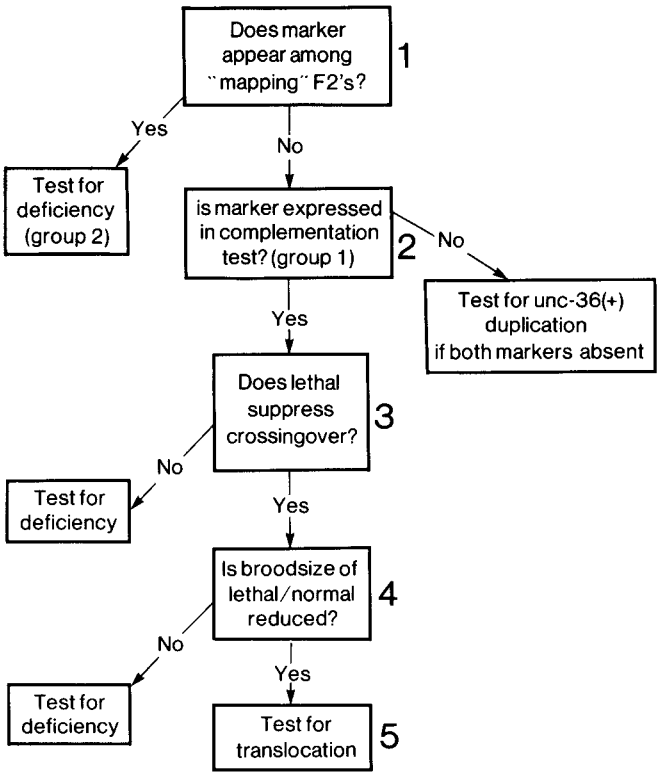

FIGURE 2.—Flow diagram of mutant characterization. Marker is either *dpy-18(III)* or *unc-46(V)*. Step 1 ("mapping" experiment) refers to the marker whose F_2 frequency was not normal.

wild types were *dpy-18/eT1; unc-46/eT1* and that the lack of Dpy Unc's was the result of a recessive lethal mutation *cis* linked to either the *dpy-18* or *unc-46* marker (that is to say within the crossover suppressed region on LGIII or LGV). However, the lack of Dpy Unc's could also have resulted from the presence of a chromosomal rearrangement that prevented the phenotypic expression of the *dpy-18(e364)* or *unc-46(e177)* mutation without necessarily causing a lethal effect. Such strains could have been derived from homozygous *eT1* F_1's carrying an *unc-36(+)* duplication or heterozygous *eT1* F_1's carrying the wild *dpy-18(+)* or *unc-46(+)* allele on one of the non-*eT1* homologues. The latter could have been the result of a *cis*-linked duplication or a γ-ray-induced recombination event. The initial experiments, therefore, attempted to map the presumed lethal mutations to one of the two marked chromosomes (*III* or *V*) and tested the strains for complementation with *dpy-18* and *unc-46* mutations.

"Mapping" experiments: (Figure 2, step 1). Wild-type hermaphrodites from each strain were crossed to either N2 or *unc-36(e251) sma-2/++* males. The F_2 progeny from several individual wild-type F_1's were scored and the *dpy-18* and *unc-46* markers, now no longer balanced over *eT1*, were expected to segregate independently. Table 1, columns 3 and 4, show the Dpy/Wild and Unc-46/Wild F_2 ratios, respectively. The ratios were ranked as either "normal," "low" or "zero."

TABLE 1

Initial characterization of lethal strains

Strain (group):	Mapping experiments			Complementation tests for markers: ratios of F_1 males		Final name of lethal[a]
	Brood/F_1 ♂	F_2 ratios D:W	F_2 ratios U46:W	D:W	U46:W	
(1a)						
BC 891	112	Zero	Normal	21:55		*sT1(III;X)*
BC 1049	66	Zero	Normal	31:95		
BC 1056	Variable	Zero	Normal	?		
(1b)						
BC 881	274	Normal	Zero		38:95	*sDf27(V)*
BC 893	193	Normal	Zero		0:154	
BC 1021	203	Normal	Zero		28:60	*sDf26(V)*
BC 1037[b]	210	Normal	Zero		?	
BC 1040	Variable	Normal	Zero		39:105	*sDf28(V)*
BC 1042	103	Normal	Zero		22:78	*sT2(IV;V)*
BC 1058	233	Normal	Zero		41:128	*s723(V)*
(1c)						
BC 1020[b]	93	Zero	Zero	0:75	0:49	
BC 1031	166	Zero	Zero	0:124	33:168	*sT3(III)*
BC 1033	82	Zero	Zero	0:190	39:161	*sT4(III)*
BC 1035	40	Zero	Zero	0:206	0:162	*sDp3(III)*
BC 1043	47	Zero	Zero	0:85	0:68	
BC 1051	63	Zero	Zero	Variable	0:192	
BC 1052	42	Zero	Zero	0:212	0:201	*sDp4(III)*
BC 1053[b]	85	Zero	Zero	25:107	?	
BC 1057[b]	109	Zero	Zero	57:202	?	
(2a)						
BC 886	188	Low	Normal	41:131		
BC 1044	87	Low	Normal			
BC 1050	256	Low	Normal			
BC 1054	224	Low	Normal			
(2b)						
BC 889	183	Normal	Low		24:73	*sDf20(V)*
BC 1022	251	Normal	Low			*s741(V)*[d]
BC 1036	271	Normal	Low			*s742(V)*
BC 1039	283	Normal	Low			*s739(V)*
BC 1045	145	Normal	Low		29:132	*sDf29(V)*
BC 1048	262	Normal	Low		73:153	*s738(V)*
BC 1055	258	Normal	Low		28:131	*sDf30(V)*
(2c)						
BC 888[b,c]	237	Low	Low	17:326	96:308	
BC 892	Variable	Variable	Low	38:314	22:94	
(3)						
BC 1041[b]				0:199	?	
BC 1046[b]				?	0:144	
BC 1047				0:47	6:32	

D, U46 and W = Dpy, Unc-46 and wild-type phenotypes, respectively. ? = confusing male phenotypes.

[a] Name assigned to lethal mutation after analysis.

[b] The strain produces either sick or no Unc-36 phenotypes.

[c] The strain carries a *sma-2(+)* duplication that does not cover *unc-36*.

[d] *s741* is linked to a LGV crossover suppressor.

Normal was the ratio expected in the absence of a *cis*-linked lethal; low indicated a *cis*-linked lethal recombinationally separable from the marker; zero indicated that the marker phenotype had not appeared among the F_2 progeny. A zero value meant that the strain carried either a tightly linked lethal or a rearrangement that prevented the expression of the marker. Three of the strains were not analyzed in this way, since no outcrossed hermaphrodites were detected. The remaining strains could be placed into two major groups. For the 19 strains in group 1 at least one of the F_2 ratios, Dpy/Wild or Unc-46/Wild, was zero, that is, at least one of the marker phenotypes had not appeared among the F_2's. For the 13 strains in group 2 none of the ratios were zero and at least one ratio in each case was low. This meant that (1) these strains carried both of the marker mutations (*dpy-18* and *unc-46*), (2) selection of these strains had, in fact, been the result of a *cis*-linked lethal being present and (3) the lethal could be recombinationally separated from its linked marker when no longer balanced over *eT1*.

Complementation tests for markers: (Figure 2, step 2). To determine whether the F_2 zero mapping ratios of group *one* strains represented tightly linked lethals or chromosomal rearrangements suppressing the marker phenotypes, the strains were complementation tested by crossing their wild-type hermaphrodites with *dpy-18*/+ or *unc-46*/+ males. Columns 5 and 6 of Table 1 give the resulting phenotypic male ratios. A value greater than 0 indicated that the marker mutation was expressible in the absence of a homozygous *cis*-linked lethal. These strains presumably each carried a lethal tightly linked to the marker. Thus, in group 1a two strains carried a *dpy-18* linked lethal; in *1b* five strains carried an *unc-46* linked lethal; in *1c* two strains carried *dpy-18* and two carried *unc-46* linked lethals. This tight linkage represented either a small map distance between marker and lethal or a chromosomal rearrangement (associated with the marker) that acted as a dominant crossover suppressor. A value of 0 for the complementation ratios suggested the presence of a chromosomal rearrangement that prevented the expression of the marker phenotype. Eight strains gave such a 0 value for at least one of the two markers: BC 893 and seven strains in group 1c. Four of the latter expressed neither the Dpy-18 nor Unc-46 phenotypes.

Analyses for new chromosomal rearrangements

Based on their initial characterizations, 17 strains were now tested for the presence of new chromosomal rearrangements. The final names assigned to their mutations are shown in the last column of Table 1.

Tests for unc-36(+) *duplications*: Strains BC 1035 and BC 1052 (from group 1c) expressed neither Dpy-18 nor Unc-46 in either the mapping or complementation experiments. Progeny from wild-type hermaphrodites of these strains always included Unc-36's. The hermaphrodites were, therefore, either (1) heterozygous *eT1's* with unmarked homologues that carried a recessive lethal mutation or (2) homozygous *eT1's* carrying a homozygous lethal *unc-36(+)* duplication derived from the normal LGIII chromosome. To distinguish between these two possibilities wild-type hermaphrodites from each strain were crossed with *unc-36(e251) sma-2(e502)*/++ males. The progeny of those wild-type F_1's that carried the closely linked *unc-36 sma-2* mutations were scored and their

Unc-36 frequencies were examined. From an *unc-36 sma-2* +/+ + *let* F_1 the frequency was expected to be 0.007, whereas from an *unc-36 sma-2/eT1; +/eT1; Dp unc-36(+)* F_1 it would be approximately 0.28. The actual Unc-36 frequencies among the F_2's from BC 1035 and BC 1052 were 0.26 and 0.04, respectively, suggesting that both strains carried an *unc-36(+)* duplication. That of BC 1035 appeared not to be linked to *eT1*. The 0.04 frequency of BC 1052 could be explained by postulating a duplication that was linked to one of the *eT1* chromosomes, but outside of the crossover suppressed region. It should be noted that an *unc-36 sma-2* +/+ + *let* F_1 with an LGIII-linked *sma-2(+)* duplication, that did not include *unc-36(+)*, could also have accounted for the BC 1052 frequency. However, this interpretation was ruled out by subsequent experiments.

Linkage of the *unc-36(+)* duplications to *eT1* was also tested with marked *eT1* chromosomes. Wild-type hermaphrodites from each of the two strains were crossed with *m eT1*/+ + males where *m eT1* was either *dpy-11(s287)eT1(III);eT1(V)* or *eT1(III);sma-2(s262)eT1(V)*. The F_2 progeny from individual *m eT1*-bearing wild-type F_1's were scored (Table 2). In the case of BC 1052 the presence of Unc-36 F_2's from the *dpy-11 (s287) eT1*/+ + males ruled out the previously discussed duplication (that is, one which covered *sma-2* but not *unc-36*). The first five rows of Table 2 present the predicted phenotypic ratios for the three possibilities: *Dp unc-36(+)* was either on *eT1(III)* (tightly linked to the *dpy-11* gene) or on *eT1(V)* (linked to the *sma-2* gene) or on neither of these two chromosomes. The results, shown in the last two rows, confirmed that *Dp unc-36(+)* of strain BC 1035 was not linked to either *eT1* chromosome and that the BC 1052 duplication was, in fact, linked to *eT1(III)*. The duplications were now named *sDp3* (in BC 1035) and *sDp4* (in BC 1052), respectively.

To estimate the minimum number of chromosomal breaks represented by these duplications, we measured the extent of each. Coverage of LGIII genes was tested by scoring the progeny from duplication-bearing hermaphrodites that were also *m eT1*/+ *eT1* (where *m* was the gene to be tested; see MATERIALS AND METHODS).

In the case of *sDp3*, it was already evident from Table 2 that to the right of *unc-36, sma-2* was not covered. To the left of *unc-36*, the progeny from *dpy-1 eT1*/+ *eT1;sDp3* hermaphrodites showed that *sDp3* covered *dpy-1*. No Dpy's appeared among 1272 offspring, and 21/75 progeny wild types produced only Dpy Unc-36's and Wilds, but no Unc-36, proving that these were *dpy-1 eT1/dpy-1 eT1; sDp3*, with *dpy-1(+)* on the duplication. Thus, if *sDp3* carried all of the genes on the left end of LGIII and if it was not inserted into the middle of another chromosome, it could have been created by only two breaks: one occurring between *unc-36* and *sma-2* on the *dpy-18* marked LGIII and the second occurring on some other linkage group to provide a telomeric cap for stability (see DISCUSSION).

On the other hand, *sDp4* represented more than two breaks. Shown at the bottom of Table 2, on the left, are the progeny from wild-type *dpy-11(s287) eT1/sDp4* + *eT1* hermaphrodites. The Dpy's and Unc-36's represent crossovers between the *unc-36(+)* duplication and the *dpy-11* gene. Since their frequencies were low and since *dpy-11* is close to the eT1(III) breakpoint (Figure 1), *sDp4*

TABLE 2

Phenotypic F_2 ratios from linkage tests for unc-36 *duplications*

	Parental male							
	dpy-11 eT1(III)/++;eT1(V)/+				*eT1(III)/+;sma-2 eT1(V)/++*			
	W	D	U36	DU	W	S	U36	SU
If duplication is	Predicted ratios							
a. not linked to *eT1(III;V)* and								
(i) covers *sma-2*	6	2	3	1	8	0	3	1
(ii) does not cover *sma-2*	6	2	3	1	6	2	3	1
b. linked to *dpy-11* on *eT1(III)* and								
(i) covers *sma-2*	2	0	0	1	8	0	3	1
(ii) does not cover *sma-2*	2	0	0	1	6	2	3	1
c. linked to *sma-2* and carried on the *eT1(V)* chromosome	6	2	3	1	2	0	0	1
Source of parental hermaphrodite	Observed ratios							
BC1035	3.9 (115)	1.2 (36)	3 (89)	1 (29)	4.7 (283)	1.1 (67)	4.3 (258)	1 (60)
BC1052	1.7 (338)	0.01 (2)	0.02 (4)	1 (199)	6.4 (205)	0 (0)	2.9 (92)	1 (32)

W, U36, DU, S and SU = wild-type, Unc-36, Dpy Unc, Sma and Sma Unc, respectively.

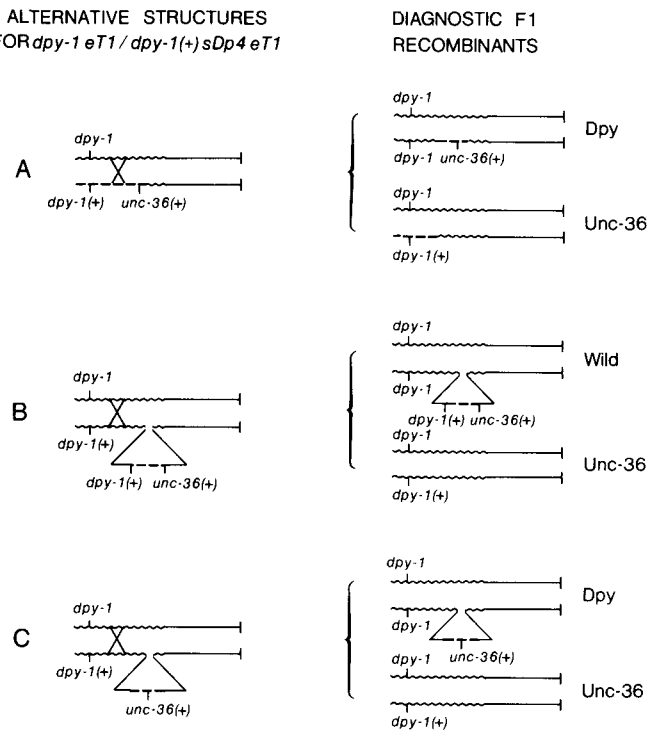

FIGURE 3.—Three alternative structures for *dpy-1 eT1(III)/+ sDp4 eT1(III)* and the diagnostic F_1 recombinants. The dashed lines represent *sDp4*. The relative positions of *dpy-1(+)* and *unc-36(+)* on *sDp4* are arbitrary.

had to be joined to *eT1(III)* near that breakpoint. Its left-right position with respect to the latter could be determined from the progeny of the previously mentioned *unc-36(e251)sma-2(e502)/sDp4 eT1; +/eT1* heterozygotes. The progeny had included Unc-36 recombinants which had to be products of recombination, occurring between the *eT1(III)* breakpoint and *sDp4* but outside of the crossover suppressed region. *sDp4* was, therefore, positioned just to the left of the breakpoint. Three possibilities now existed regarding the structure of the *sDp4 eT1(III)* chromosome. Either *sDp4* made up the whole left end of the new chromosome or it was inserted in the middle. In the latter case it either did or did not carry a *dpy-1(+)* allele. Progeny from *dpy-1 eT1/+sDp4 eT1* hermaphrodites were examined to determine which was the correct structure. Figure 3 diagrams the three possible heterozygotes and the expected diagnostic F_1 recombinants from each. The actual F_1 progeny were 975 Wild, 296 Unc-36, 243 DpyUnc-36 and no Dpy's. The Unc-36's had to be one of the recombinants from crossovers between *dpy-1* and *unc-36(+)*. The absence of Dpy's suggested that the reciprocal recombinant, *dpy-1 eT1/dpy-1 sDp4 eT1*, carried a *dpy-1(+)* allele. That this was so is shown by the fact that nine of 40 of the F_1 wild types produced Wild and Dpy Unc-36's but no Unc-36's in the next generation. Consequently, the structure of the *sDp4* chromosome had to be that shown in heterozygote "B" (Figure 3). If it is assumed that only broken chromosome ends can fuse, this new

TABLE 3

Tests for crossover suppression and pseudolinkage effects

Source of lethal		Recombination frequency, *p*, between markers in *trans* with *lethal*			
Group	Strain	*let/sma-2 unc-64* (normal $p = 0.20$)	*let/unc-60 dpy-11* (normal $p = 0.18$)	Markers showing pseudolinkage	Name
1a	BC 891	0.01		*sma-2;unc-6*	*sT1(X;III)*
1b	BC 881		0.07	ND	
1b	BC 1021		0.014	ND	
1b	BC 1040		ND		
1b	BC 1042		0	*unc-31;dpy-11*	*sT2(IV;V)*
1b	BC 1058		0.21		
1c	BC 1031		0	*unc-32;dpy-11*	*sT3(III)*
1c	BC 1033		0	*unc-32;dpy-11*	*sT4(III)*

ND = not tested.

chromosome was, therefore, the product of at least one break on *eT1(III)* and two breaks on LG(III). One break on LG(III) had to be left of *dpy-1* and the other to the right of *sma-2* (Table 2 had shown that *sDp4* covered *sma-2*). Consistent with this, progeny from *sDp4 eT1/eT1; bli-5(s277) eT1/+ eT1* showed that *sDp4* did not carry *bli-5(+)* (the right end of LGIII). Thus, the formation of *sDp4 eT1(III)* appears to have been the result of at least three breaks induced by 1500 r.

Tests for new dominant crossover suppressors and translocations: (Figure 2, steps 3–5). Strains failing to segregate one of the marker phenotypes (Dpy-18 or Unc-46) among the "mapping" F_2's (Table 1, group 1), but expressing the marker in complementation tests, apparently carried a lethal mutation that was tightly linked to the marker. This tight linkage suggested that the marker may have been associated with a new dominant crossover suppressor. To test this, a heterozygote, carrying the marked lethal chromosome over a normal, doubly marked homologue, was constructed and the progeny scored for recombination (MATERIALS AND METHODS). Eight lethal chromosomes were chosen for testing and the results from seven are shown in Table 3. All, except that in BC 1058, suppressed recombination. The lethal chromosome of BC 1040 was not tested because it produced an Unc-60 phenotype when heterozygous with *unc-60 dpy-11*.

Dominant crossover suppression is often associated with a translocation heterozygote that produces a low number of viable zygotes and causes pseudolinkage between genes on nonhomologous chromosomes (ROBERTS 1976). Four of the suppressors, when hetrozygous with normal chromosomes, produced reduced brood sizes (Table 1, column 2). They were, therefore, putative translocations, and tests were performed to determine whether they caused any pseudolinkage effects.

The suppressor to be tested was placed into various heterozygotes, each carrying two marked normal chromosomes, one of which was a homologue of

the suppressor. The progeny of these heterozygotes were then scored for pseudolinkage of the two markers (MATERIALS AND METHODS). Table 3, column 5, shows that the four tested suppressors each caused pseudolinkage between at least one pair of markers and were, therefore, associated with translocations. New names were assigned to the translocations as shown in the last column of this table. The suppressors of BC 881 and BC 1021 were not tested because in the absence of *eT1* they each gave normal numbers of self-progeny. Therefore, they showed no signs of lethality due to unbalanced zygotes produced by translocations.

BC 891 carried *sT1(III;X)* whose *X* chromosome break apparently was not in an essential gene, since males carrying the translocation were viable and fertile. As expected (see MATERIALS AND METHODS), all tested wild-type hermaphrodites produced by these males gave fewer than 120 self-progeny.

The lethal *unc-46* chromosomes from BC's 1031 and 1033 were associated with *III;V* translocations. It was possible that half of each translocation was one of the original *eT1* chromosomes, and that in each case only one new chromosome, *sTx(?)* [carrying *unc-36(+), unc-46(e177)* and a lethal] had been created. Thus, the wild types of these strains could have been *sTx(III)/eT1(III);eT1(V)/eT1(V)* or *eT1(III)/eT1(III); sTx(V)/eT1(V)*. Consistent with this was the fact that the average number of progeny from the wild-type hermaphrodites was relatively large: 154 Wild: 82 Unc-36 (236 total) and 116 Wild: 64 Unc-36 (180 total) for BC 1031 and BC 1033, respectively. (The average total progeny from *eT1/* normal heterozygotes is 103.) To determine whether the presumed *sTx* chromosome [carrying *unc-36(+)*] segregated from *eT1(III)* or *eT1(V)*, wild-type hermaphrodites from each strain were mated to *"m"eT1/+* males [where *"m"eT1* was either *dpy-11(s287)eT1(III);eT1(V)*, or *eT1(III);sma-2(s262)eT1(V)*]. The F_2 progeny from *"m"eT1*-carrying wild-type F_1 hermaphrodites were scored. If *sTx* segregated from the marked *eT1* chromsome only very few Unc-36's were expected; if it segregated independently about ¼ of the F_2's would be Unc-36. The average progeny from BC 1031-derived heterozygotes carrying *dpy-11(s287)eT1(III)* were 149 Wild: 0 Dpy: 0 Unc-36: 105 Dpy Unc-36. For those carrying *sma-2(s262)eT1(V)* there were 147 Wild: 0 Sma; 55 Unc-36: 50 Sma Unc-36. The absence of Unc-36 F_2's among the first progeny and their presence among the second indicated that the *sTx* chromosome segregated from *eT1(III)*. The absence of Sma-2's indicated that this new chromosome, now called *sT3(III)*, also carried a *sma-2(+)* allele. This was supported by the fact that three of 12 wild-type F_2's from the *sma-2 eT1(V)* heterozygote produced only wild-type and Sma Unc progeny [*i.e.*, they were *sT3(III)/eT1(III);sma-2 eT1(V)/sma-2 eT1(V)*]. These latter wild-type hermaphrodites were shown to carry an *unc-46* mutation by complementation testing. It can, therefore, be assumed that the new *sT3(III)* chromosome had been created as a result of two breaks: one between *sma-2(+)* and *dpy-18(e364)* on LGIII and the other on the LGV-*unc-46*-bearing chromosome. [BC 1031 had no expressed *dpy-18(e364)* in the initial complementation experiments.] Since the *unc-36(+)* allele of BC 1033 also segregated from *eT1(III)*, the half-translocation from this strain was named *sT4(III)*.

Tests for deficiencies: For 11 strains, whose lethal mutations mapped to LGV,

TABLE 4

Complementation tests between LGV mutations

let 326																				
+	*unc 60*																			
+		*let 327*																		
+	+	+	*let 328*																	
	+			*s 723*																
				+	*s 738*															
				+	−	*s 739*														
	+			+	+	+	*let 329*													
+	+		+	+	+	+	+	*dpy 11*												
	+		+					+	*s 742*											
					+	+		+	+	*unc 23*										
								+			*unc 42*									
								+			+	*sma 1*								
+	+	+	+					+					*s 741*							
−	−	−	+					+	+				+	*sDf 28*						
+	+	+	−	+	+	+	+	+	+				+	−	*sDf 27*					
+	+		−	−	−	−	−	+					+	−	−	*sDf 26*				
				+	−	−	−	+		+					+	−	*sDf 30*			
			+	+	−	−	−	−	+	+	+		+	+	+	−	−	*sDf 20*		
	+				+	+		+	+	−	−	+	+		+		+	+	*sDf 29*	

complementation tests were carried out, both *inter se* and with alleles of established genes (Table 4). The γ-induced mutations were assigned allele or deficiency names. (Their origin may be found by referring to Table 1.) For the other mutations, the gene name is shown. A deficiency was considered demonstrated if it failed to complement two complementing alleles. Six such deficiencies were found and their extents are diagrammed in Figure 4. Together they provide at least one deletion for almost every known LGV gene balanced by *eT1*. No deletion was found for the genes that lie in a small region betwen *sDf20* and

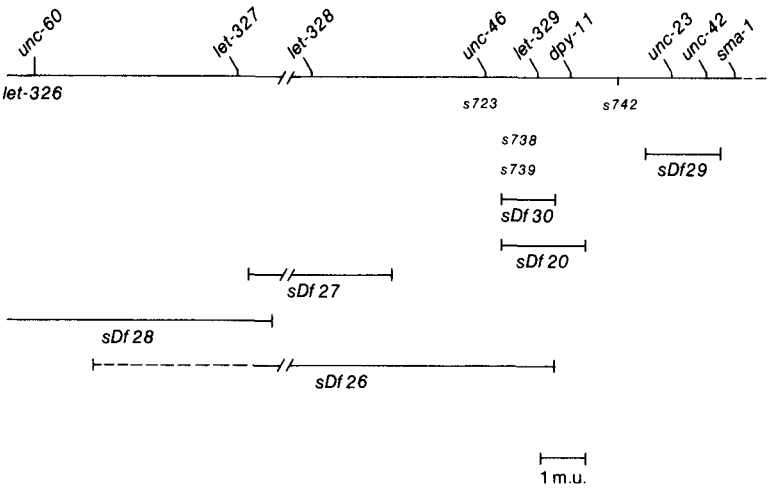

FIGURE 4.—Positions of lethal mutations and deficiencies induced with 1500 r on LGV. Established genes are shown above the LGV line.

sDf29. The extent of *sDf28* to the right was surprising. The mapping experiments had not produced a single recombinant between *sDf28* and *unc-46* among 525 progeny from *dpy-18/+; sDf28 unc-46/++* hermaphrodites. It was, therefore, expected that *sDf28* extended to *unc-46*. However, *let-328(s472)*, which maps 4 mu to the left of *unc-46*, complemented *sDf28*. Thus, the deficiency appeared to act as a dominant crossover suppressor. Brood sizes from individual *sDf28* heterozygous hermaphrodites (not bearing *eT1*) were variable, ranging from 60 to 223 progeny. It is possible that *sDf28* was associated with another γ-ray-induced event which was the cause of the variable brood sizes. The crossover suppression may be explained by assuming that the deficiency removed a pairing site of the kind proposed by HAWLEY (1980), resulting in reduced recombination on this portion of LGV. ROSENBLUTH and BAILLIE (1981) and ROSE, BAILLIE and CURRAN (1984) have proposed pairing regions of this type in *C. elegans*.

No deficiencies were associated with the lethals of five strains. These lethals were putative point mutations or, as yet undetected, small deletions. Recombination and deficiency mapping positioned *s723, s742, s738* and *s739* as shown. The latter two failed to complement each other and have not been left-right positioned with respect to *let-329*. The mapping of *s741* indicated that it was to the left of *unc-46* and associated with a crossover suppressor.

s741 *is associated with a crossover suppressor*: Based on initial two-factor mapping experiments, the strain BC 1022 carried the lethal mutation, *s741*, on LGV, approximately 10 mu from *unc-46*. Since it had to be in the region balanced by *eT1* and since *unc-60* is about 16 mu from *unc-46, s741* was positioned putatively between *unc-60* and *unc-46*. The progeny from *s741 unc-46/unc-60 dpy-11* hermaphrodites confirmed the position of *s741* to the left of *unc-46*: Progeny testing the Unc-46 recombinants showed that 42 of 43 were *s741 unc-46/unc-60 unc-46*, whereas one was *s741 unc-46/+ unc-46*. Surprisingly, each of the overlapping deficiencies, *sDf26, sDf27* and *sDf28*, complemented *s741* (see Table 4).

TABLE 5

Summary of categories represented by the 17 analyzed lethal events

unc-36(+) duplications	Translocations	LGV deficiencies	Crossover suppressor	LGV point mutations
sDp3	*sT1(III;X)*	*sDf20*	*s741(V)*	*s723*
sDp4[a]	*sT2(IV;V)*	*sDf26*		*s738*
	sT3(III)	*sDf27*		*s739*
	sT4(III)	*sDf28*		*s742*
		sDf29		
		sDf30		

[a] Linked to *eT1(III)*.

However, it was also found that *s741* partially suppressed recombination between *unc-60* and *dpy-11*. The frequency of Unc-60 recombinants (52/815) gave a map distance of 10 mu rather than the normal 18 mu. Since the average brood size from *s741* heterozygotes was normal for a strain bearing a lethal mutation, the crossover suppression was probably not due to a translocation. We offer two alternative explanations for the results. (1) *s741* is a recessive lethal mutation mapping to the left of *sDf28*. It is linked to (but separable from) an intrachromosomal rearrangement (inversion?) that partially suppresses crossing over on the left arm of LGV. (2) *s741* is a hypermorph; for example, it may be a duplicational insertion between *unc-60* and *dpy-11*. This insertion partially inhibits recombination in the region and is homozygous lethal due to gene dosage imbalance. The *s741 unc-46/+ unc-46* recombinant would then be the result of a double crossover.

Calculations

To estimate the frequency of rearrangements induced by γ-irradiation, we have made the following calculations. The set of 35 lethals, picked at random for study, had been induced by 1500 r with a frequency of 0.04 per eT1 region. Table 5 shows that at least 13 of 17 analyzed lethals (*i.e.*, 76%) were associated with a rearrangement. Based on the initial characterizations (Table 1), we assumed that among the remaining 18 lethals the proportion of rearrangements was similar. The strains bearing these 18 lethals included the following putative rearrangements: *unc-36(+)* duplications (BC 1020 and BC 1043); an *unc-46(+)* duplication (BC 893); translocations (BC 1049 and BC 1044); half-translocations (BC 1051, BC 1053 and BC 1057); and probably some deficiencies among the remaining ten strains. Thus, the frequency of recovered lethal rearrangements, induced by 1500 r was about 0.03 per eT1 region. For intrachromosomal rearrangements the frequency was 0.012, whereas for interchromosomal ones (translocations and duplications) including *sDf28* and *s741*, it was 0.018. (These are underestimates since deficiencies of *unc-36* would not have been recovered from the screen.) By assuming that eT1 represents about 1/6 of the haploid genome (or the length of an average chromosome), we estimated that there would be six times as many intrachromosomal and three times as many interchromosomal rearrangements per haploid genome as were recovered in the eT1 balanced region. Thus, 1500 r, would induce rearrangements associated with

lethals, with a total frequency of about 0.12 per gamete. Based on a linear extrapolation, 6000 and 8000 r would induce rearrangements (each representing at least two breaks) with frequencies of 0.48 and 0.65, respectively, per gamete. (A linear extrapolation is a conservative one since our previous data suggested that the induction of lethal mutations increases exponentially with dosage.)

DISCUSSION

The impetus for embarking on this study was our belief that most ionizing radiation doses (6000–8000 r) currently used to induce chromosomal rearrangements in *C. elegans* are excessive. In choosing a mutagen dose, one needs to weigh the desirability of screening as small a population as possible (using a relatively high dose) against the desirability of limiting the frequency of secondary events associated with the selected mutant (using a low dose). Since no quantitative data had been available regarding the induction of heritable rearrangements in *C. elegans* at lower levels of ionizing radiation, we attempted to obtain such data in order to provide a basis on which rational dosage choices could be make in the future.

Our previous report (ROSENBLUTH, CUDDEFORD and BAILLIE 1983) provided a dose-response curve for γ-ray induction of recessive lethals in *C. elegans*. We have now demonstrated that for the eT1-balanced region, at least 76% of the lethals induced with 1500 r were associated with rearrangements. From this result we have estimated that with 1500 r the frequency of lethal-associated chromosomal rearrangements would be about 0.12 per gamete, whereas with 6000 and 8000 r we conservatively estimated these frequencies to be about 0.48 and 0.65, respectively. To get a measure of the possible complexities caused by multiple breaks, we now estimate how many of the gametes detected as carrying one rearrangement (representing at least two breaks) would, in fact, carry more than one. Since the intercellular distribution of mutagenic events induced by sparsely ionizing radiation may be considered random (TRAUT 1979), we assume that the distribution of rearrangements among gametes is Poissonian. We estimate that with 1500 r 5% of the gametes would carry more than one rearrangement, whereas with 6000 and 8000 r it would be 34 and 43%, respectively. These are underestimates since they are based on the frequency of rearrangements associated with lethality. Obviously, not all rearrangements are of this type. Indeed, the *eT1* translocation is viable as a homozygote, and in Drosophila, LEFEVRE (1981) found that 70% of cytologically identified rearrangements, induced with 2000 r in ⅓ of the *X* chromosome, were in nonlethal chromosomes. Thus, our results support our belief that doses as high as 6000–8000 r are excessive, by which we mean that they have a high probability of inducing events too complex to be analyzed or secondary events that may remain undetected and lead to incorrect analyses. If a secondary event is linked to the selected rearrangement by an induced crossover suppressor or by pseudolinkage, outcrossing will not remove it. Furthermore, if the desired rearrangement is associated with a secondary event causing dominant sterility or lethality, it will never be recovered. There is evidence, both from our own experience (R. E. ROSENBLUTH, C. CUDDEFORD and D. L. BAILLIE, unpublished results) and others

(HERMAN 1978; MENEELY and HERMAN 1979) that the high doses do cause a great deal of sterility among the F_1 generation. We, therefore, conclude that efforts should be made to use reduced levels of ionizing radiation in the future.

In the analysis of *sDp3* we assumed that broken chromosomes must have telomeric caps in order to be genetically stable. This was based on conclusions drawn from Drosophila studies (ROBERTS 1976). It has been suggested that *C. elegans* has holocentric chromosomes and that the ends of these do not need to be capped (HERMAN, KARI and HARTMAN 1982). The suggestion was based, in part, on the ability to recover stable fragments from different, nonoverlapping, regions of the same chromosome (either *X* or *III*), which were 'free." However, in the absence of detailed cytological inspection one cannot rule out the possibility that these fragments carried small centromeric and telomeric regions from other chromosomes. This is a particularly strong possibility for free duplications generated with high doses of ionizing radiation. Consequently, we assume, until proven otherwise, that *C. elegans* chromosomes require telomeric ends for stability. We point out this issue because it demonstrates some of the doubts associated with conclusions based on radiation-induced mutations and, consequently, demonstrates the need to use as low a dose as possible.

In view of this, we now ask: How easily is a specific rearrangement recovered using the lower dose of 1500 r? The 35 lethal strains, picked at random for this study, had been induced with a frequency of 0.04 per eT1 region and, therefore, represented 875 screened F_1's. Among these there were the following: For every known gene on the left arm of LGV, except those lying in a small interval between *sDf20* and *sDf29*, there was at least one deficiency. For the genes that lie in the regions of overlapping deficiencies two or three independent deficiencies were recovered. For LGIII and LGV each, there was one translocation and two half-translocations that acted as dominant crossover suppressors. For LGV there was a crossover suppressor whose structure remains to be analyzed. The screening protocol also selected for *unc-36* duplications. Two of these were recovered. (We did not analyze for LGIII deficiencies.) The number of recovered rearrangements is underestimated since there must have been additional translocations, crossover suppressors and *unc-36* duplications among the 18 strains that were not analyzed in detail. These results suggest that, by screening 2000 F_1's treated with 1500 r, it should be possible to recover at least one deficiency and one duplication for the average gene and several crossover suppressors for most chromosome regions. It must be pointed out that the present data refer to rearrangements recovered from self-fertilizing hermaphrodites. Preliminary evidence indicates that the frequency of γ-ray-induced lethals in male sperm is about the same as that in the average hermaphrodite gamete. We expect that the frequencies of rearrangements will also be similar, but this awaits future research.

From these studies, we conclude that it is possible to induce desired chromosome rearrangements in *C. elegans* at reasonable frequencies with four- to five-fold lower doses of ionizing radiation than are currently used. We stress the importance of minimizing the chance of undetected events, since they may lead to incorrect analyses, not only at the chromosomal level but also at the molecular

level. In Drosophila, recombinant DNA libraries, constructed from strains carrying rearrangements, have been used to isolate breakpoint fusion fragments for chromosomal "jumping" (BENDER, SPIERER and HOGNESS 1983). Analogous studies will soon be carried out with *C. elegans* strains. In particular, we have begun a detailed analysis of the left arm of LGV, in which we plan to use chromosomal rearrangements induced with 500–1500 r (including those reported here) to isolate LGV DNA fragments. In the absence of cytological monitoring, it is particularly important that no secondary breakpoints be present. We hope that this study will encourage the use of lower ionizing radiation doses in the future.

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