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Genetic analysis of a large autosomal region in *Caenorhabditis elegans* by the use of a free duplication

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Summary

In this paper we describe the use of a free duplication, *sDp2* (*I*;f), for the recovery, maintenance, and analysis of mutations defining essential genes in the left third of Linkage Group *I* of *Caenorhabditis elegans*. The lethals were induced in a strain of genotype (*sDp2*)+/*dpy-5*+*unc-13*/*dpy-5 unc-15*+, using either 12 mM ethylmethane sulphonate or 1500 r of gamma radiation. Lethal mutations linked to the *dpy-5 unc-13* chromosome were recognized by the absence of Dpy-5 Unc-13 individuals amongst the self progeny and were maintained by isolating Unc-13 hermaphrodites. These strains – which have two mutant alleles of the essential gene and a wild-type allele on the duplication – are balanced, since crossing-over does not occur between *sDp2* and the normal homologues. Using this sytem we have recovered 58 EMS-induced mutations. These have been characterized with regard to map position and complementation. Twenty-nine of the EMS-induced mutations lie to the left of *dpy-5* and define 20 complementation groups; 3 were inseparable from *dpy-5* and define 3 complementation groups; 21 were to the right and define 17 complementation groups. Among a set of 29 gamma radiation-induced lethal mutations, 17 appear to be single gene mutations or are very small deletions. We estimate that we have identified from one-sixth to one-half of the essential genes in the *sDp2* region.

1. Introduction

To facilitate genetic analysis in *Caenorhabditis elegans*, we are characterizing a large autosomal segment of linkage group (LG) *I*. In order to develop a system to allow analysis of a large segment of a chromosome, we have employed a free duplication. Based upon its genetic properties, Rose, Baillie & Curran (1984) suggested that *sDp2* might be useful for maintaining lethal mutations over a large portion of LG*I* left. This is the first report of the use of a free duplication as a genetic balancer for recovering and maintaining recessive lethal mutations. Several groups have recovered recessive lethal mutations over small autosomal regions in *C. elegans* (Herman & Meneely, 1979; Rose & Baillie, 1980; Rogalski, Moerman & Baillie, 1982; Anderson & Brenner, 1984; Rogalski & Baillie, 1985). Rosenbluth, Cuddeford & Baillie (1983) have used the well-characterized reciprocal translocation *eT1* (Rosenbluth & Baillie, 1981) to balance lethal mutations over two large autosomal regions. Sigurdson, Spanier & Herman (1984) have used the balancer, *mnC1*, to recover lethals over a large region of LG*II*. A disadvantage to these approaches is that the region under study is not precisely defined, since lethal mutations which are linked to and outside the boundaries of the cross-over-suppressed region will be recovered. These boundaries can be sharply defined by the use of a duplication or a deficiency. Deficiencies, however, cannot be used to maintain lethal mutations. Furthermore, deficiencies cannot generally be used to screen over large regions. For example, the deficiency (*sDf4*) of only approximately one-fifth of the *sDp2* region has low viability and could not be used practically in large-scale lethal screening experiments. A deficiency as large as *sDp2* is known not be viable (Rose, Baillie & Curran, 1984). Linked duplications have previously been used as balancers in *Drosophila melanogaster* (Judd, Shen & Kaufman, 1972) and in *C. elegans* (Meneely & Herman, 1979). A serious drawback to the previous use of a linked duplication in *C. elegans* was that lethal mutations on the other chromosome were also recovered. In the study by Meneely & Herman (1979), only 21/176 tested putative lethals were in the region of interest. A tremendous advantage to using a free duplication such as *sDp2* is that all the recovered lethal mutations are in a very precisely defined region on the linkage group of interest.

In this paper we report the isolation and characterization of an initial set of free duplication-rescued lethal mutations.

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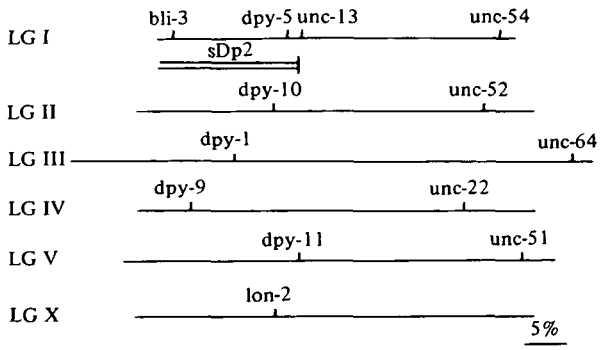

Fig. 1. Partial genetic map of *C. elegans* to show the extent of the region covered by the free duplication *sDp2*.

2. Materials and methods

(i) *General*

Wild-type and mutant strains were maintained and mated on petri plates containing nematode growth medium (NGM), streaked with *Escherichia coli* OP50 (see Brenner, 1974). The wild-type N2 strain and some mutant strains of *C. elegans* var. Bristol were obtained from D. Baillie, Simon Fraser University, Burnaby, Canada or the Caenorhabditis Genetics Center at the University of Missouri, Columbia. In addition to the new lethal mutations isolated in this study, the following mutations on LG *I* were used: *bli-4(e937)*, *dpy-5(e61)*, *dpy-14(e188)*, *him-1(e879)*, *let-75(s101)*, *let-76(s80)*, let-*77(s90)*, *let-78(s82)*, *unc-11(e47)*, *unc-13(e450)*, *unc-15(e73)*, *unc-87(e1459)*, *sDp2(I;f)* and *sDf4*. The deficiency, *sDf4*, which deletes the *unc-11 dpy-5* interval was generated (Rose, 1980) by treating wild-type male sperm with 0·07% formaldehyde as described by Rose & Baillie (1980). Strains heterozygous for *sDf4* have no obvious visible phenotype. It has been maintained in a strain heterozygous for *bli-4 dpy-14* by selecting phenotypically wild-type heterozygotes each generation.

(ii) *Construction of the strain for lethal screening*

In order to screen for lethal mutations of genes on the left third of linkage group *I*, the duplication *sDp2* was used. Figure 1 shows the region of the genome covered by *sDp2*. *sDp2* carries the wild-type alleles of *dpy-5* and *dpy-14* but not *unc-15* or *unc-13*. A strain was constructed in which each LG *I* homologue was differently marked. This construction involved N2 males crossed to *dpy-5 unc-15/ dpy-5 unc-15* hermaphrodites. Out-cross males of the genotype *dpy-5 unc-15/ + +* were crossed to duplication-carrying hermaphrodites of the genotype *+(sDp2)/ dpy-5 unc-13/ dpy-5 unc-13*. A single hermaphrodite of the genotype, *+(sDp2)/ dpy-5 + unc-13/ dpy-5 unc-15 +*, was used to establish the strain KR235.

Each of the segregating genotypes from KR235 was represented by a unique phenotype (Fig. 2). Dpy-5 worms are approximately one-half the length of Wt

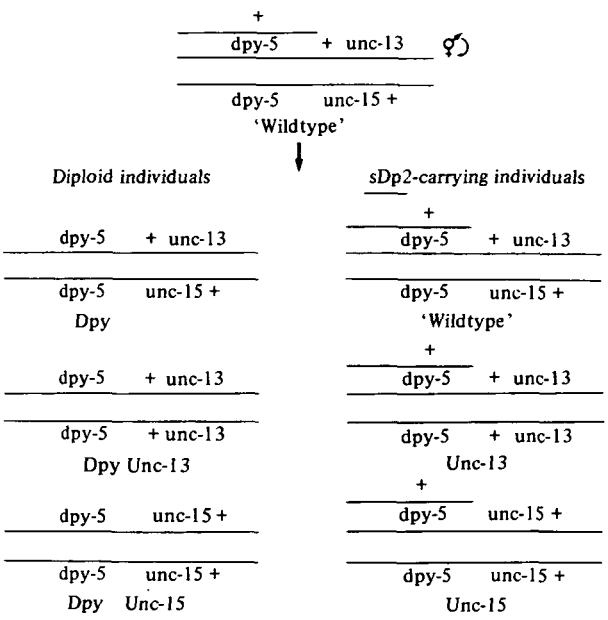

Fig. 2. Segregants from strain KR235. Individuals with two copies of *sDp2* are larval lethals and are not shown. The ratio of duplication-carrying to diploid progeny is 2:1. Approximately 8% of the progeny are Dpy Unc-13.

worms and are fatter. The two types of Uncs and Dpy Uncs that were present were phenotypically distinguishable from each other. Unc-13 animals are paralysed and contract when touched, while Unc-15 worms have a limp paralysed phenotype. Diploid progeny were either Dpy (*dpy-5 + unc-13 / dpy-5 unc-15 +*) or Dpy Unc (*dpy-5 unc-13* or *dpy-5 unc-15*). Duplication-carrying progeny were either Unc (*+(sDp2)/ dpy-5 unc-13 dpy-5 unc-13* or *+(sDp2)/dpy-5 unc-15/ dpy-5 unc-15*) or 'Wt' (*+(sDp2)/ dpy-5 unc-13 + / dpy-5 + unc-15*) ('Wt' = duplication-carrying non-Dpy, non-Unc, non-Dpy Unc). This strain was maintained by selecting the 'Wt' hermaphrodites and has been used for screening for LG *I* lethals.

(iii) *Induction and identification of lethal mutations*

Lethal mutations in genes present in the *sDp2* region were isolated. Individuals of the strain KR235 were treated either with 12 mM ethylmethane sulphonate (EMS) or with 1500 r of gamma radiation (Cobalt 60) (see Rosenbluth, Cuddeford & Baillie, 1983). Gravid 'Wts' were individually placed on 10 × 60 mm culture plates after treatment. Five days later gravid 'Wt' F_1s were placed individually on culture plates. Their progeny were screened for the absence of Dpy-5 Unc-13 individuals. If fewer than 2 Dpy-5 Unc-13s were observed, and if Unc-13s were present, a single Unc-13 was transfered to a fresh culture plate in order to confirm the existence of a lethal. A generation later the offspring of each Unc-13 strain were examined. Three types of strains were found: (1) both Uncs and Dpy Uncs were present (no lethal mutation); (2) no fertile Unc-13 could be recovered (a lethal not rescued by *sDp2*); and (3) the desired cases,

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Uncs and developmentally arrested Dpy Uncs were present. Thus lethal mutations were rescued by the presence of a wild-type allele provided by *sDp2*. Lethal mutations rescued in this way were maintained in strains with the genotype $++(sDp2)/$ (*let-x dpy-5*) *unc-13*/ (*let-x dpy-5*) *unc-13*. The parentheses indicate that the *let* gene could map either to the left or the right of *dpy-5*. These strains have an Unc-13 phenotype and segregate a single fertile phenotype (only Unc-13s).

(iv) *Recombination mapping and complementation testing*

Recombination mapping was done using procedures recommended by Rose & Baillie (1979). In order to generate the appropriate heterozygous individuals, N2 males were crossed to Unc-13 hermaphrodites from each of the lethal-bearing strains.

Wild-type heterozygotes which do not carry the duplication are required for mapping. Since the non-duplication-carrying progeny are the first to reach adulthood, they are easily selected. Occasionally 'Wts' carrying *sDp2* are accidentally set up. These were easily identified because they segregated approximately 12% Unc-13 progeny, whereas the heterozygotes lacking the duplication produced less than 1% Unc-13 progeny, the result of recombination events in the *dpy-5 unc-13* interval. The self progeny of appropriate out-cross hermaphrodites (+ + +/ (*let-x dpy-5*) *unc-13*) were scored. For those lethal mutations to the left of *dpy-5*, fertile Dpy Unc recombinants were recovered in a frequency proportional to the distance to the lethal. Unc-13 recombinants were recovered at the frequency expected for the *dpy-5 unc-13* interval. For those lethal mutations to the right of *dpy-5*, Dpy-5 and Unc-13 recombinants were recovered. The total number of progeny was calculated as 4/3 (total viable progeny); the number of recombinants was calculated as two times one recombinant class. The recombination fraction, R, was calculated as (number of recombinants)/ (total progeny). The frequency or recombination, p, was calculated as $1-\sqrt{(1-2R)}$ (Brenner, 1974). In this way both right–left positioning relative to *dpy-5* and two-factor recombination frequencies were obtained for each of the lethal mutations.

Heterozygous males from crosses similar to those described above were used for complementation testing. These males were mated to Unc-13 hermaphrodites from each of the lethal-bearing strains. The *sDp2*-carrying males develop more slowly and are less effective at mating than males lacking the duplication (Rose, Baillie & Curran, 1984). The out-cross progeny were scored. The presence of Dpy Unc males and fertile Dpy Unc hermaphrodites indicated complementation. For lethals tightly linked to *dpy-5*, the presence of Dpy Unc progeny was diagnostic. For lethals which mapped ten or more map units to the left of *dpy-5*, out-cross progeny were scored to ensure that Dpy Unc offspring were present in excess of the number expected from recombination between the lethal and *dpy-5*.

Table 1. *Recovery of lethal mutations*

Chromosomes tested		Lethals recovered	%
12 mM ethylmethane sulphonate			
sDp2			
Exp 1	1650	25	1·5
Exp 2	1933	28	1·5
Total	3583	53	1·5
*eT1**	831	55	6·6
1500 r gamma radiation			
sDp2	6005	29	0·5
*eT1**	1636	74	4·5

* Data from Rosenbluth, Cuddeford & Baillie (1983).

3. Results

In order to recover lethal mutations we used the strain KR235 (+(*sDp2*)/ *dpy-5*+*unc-13*/ *dpy-5* *unc-15*+). F_1s carrying a lethal were identified by the absence of Dpy Unc-13 F_2 progeny. An Unc-13 individual was isolated from each of these F_1s in order to maintain mutations induced on the *dpy-5 unc-13* chromosome within the *sDp2* region. These lethal strains had the genotype, + +(*sDp2*)/(*let-x dpy-5*)*unc-13*/(*let-x dpy-5*) *unc-13*. Since the duplication does not carry the wild-type allele of *unc-13*, these strains had an Unc-13 phenotype and segregated Unc and developmentally arrested Dpy Unc animals. Because a lethal mutation is present on both normal homologues, only those mutations having a wild-type allele on the duplication were recovered in this way.

(i) *Induction frequency*

To determine the relative efficiency of the screen, we compared our recovery to that of the *eT1* system (Rosenbluth, Cuddeford & Baillie, 1983). If our system were as efficient as *eT1* at recovering lethals, we would expect to recover 17 mu/45 mu (approximately one-third) as many lethals as with *eT1*. The results with both systems are summarized in Table 1. With EMS we observed $\frac{1}{4}$ as many lethals (1·5/6·6) as reported for *eT1*; and with gamma radiation $\frac{1}{9}$ the number of lethals (0·5/4·5).

Most of the lethals described above which were recovered by our screening method arrest during larval development. In order to determine if sterile adults were being recovered, we isolated mature Dpy Uncs and examined them a generation or two later. In this screen 12 adult steriles and three F_2 (maternal-effect) lethals were recovered in 800 tested chromosomes. These are not described further in this paper.

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Table 2. *Lethal mapping data*

Gene	Allele*	N†	Progeny‡	Dpy Unc	Dpy	Unc	*P*
A							
dpy-5 unc-13/++		30	6435	1422	52	51	0·016
B: EMS-induced lethals to the left of *dpy-5*							
let-362	*h86*	9	2099	148	1§	20	0·154
let-358	*h92*	8	1551	72	3§	5	0·102
let-360	*h96*	10	1716	39	0	10	0·046
let-365	*h108*	10	2663	56	2§	20	0·044
let-357	*h89*	8	1681	24	1§	14	0·030
let-356	*h83*	9	1356	15	1§	16	0·024
let-351	*h43*	7	1041	12	0	16	0·023
let-368	*h121*	10	1899	19	0	12	0·020
let-366	*h112*	9	1647	15	1§	20	0·020
let-372	*h126*	16	3413	31	4§	24	0·020
let-369	*h125*	8	868	7	0	4	0·016
let-354	*h79*	12	2577	16	1§	24	0·013
let-353	*h46*	6	872	4	0	12	0·009
him-1	*h134*	9	1719	4	0	18	0·005
let-361	*h97*	10	2263	5	0	19	0·004
let-363	*h111*	10	1883	3	0	14	0·003
let-364	*h104*	10	2337	3	0	26	0·003
let-352	*h45*	6	1360	1	0	12	0·001
let-371	*h123*	16	2367	2	0	16	0·002
let-359	*h94*	16	2891	1	0	23	0·001
C: EMS-induced lethals to the right of *dpy-5*							
let-376	*h130*	34	5768	0	1	44	0·0003
let-377	*h110*	10	2088	0	2	16	0·002
let-378	*h124*	10	2156	0	2	14	0·002
let-379	*h127*	9	2043	0	4	10	0·004
let-388	*h88*	8	2051	0	4	14	0·004
let-380	*h80*	9	1380	0	5	4	0·007
let-384	*h84*	9	1447	0	5	8	0·007
let-387	*h87*	8	1937	0	8	10	0·008
let-382	*h82*	11	2345	0	13	2	0·011
let-391	*h91*	10	2332	0	13	15	0·011
	h42	19	4116	0	22	21	0·011
let-385	*h85*	9	2349	0	14	1	0·012
let-381	*h107*	10	2429	0	14	14	0·012
let-383	*h115*	10	1972	0	12	4	0·012
let-386	*h117*	10	2176	0	13	9	0·012
let-389	*h106*	10	2096	0	14	4	0·013
let-392	*h120*	8	1525	0	11	8	0·014
let-390	*h44*	5	887	0	7	2	0·016
D: EMS-induced lethals inseparable from *dpy-5*							
let-355	*h81*	31	7532	0	0	52	0
let-367	*h119*	26	5356	0	0	35	0
let-370	*h128*	26	5267	0	0	45	0

* Lethal strain outcrossed to N_2 males.
† Number of heterozygotes.
‡ Calculated as 4/3 (wild types plus Dpys) for section A and 4/3 (viable progeny) for remainder of Table 2.
§ +*dpy-5 unc-13*/*let-x dpy-5*+.

(ii) *Mapping*

In order to characterize this initial set of lethals, 53 EMS-induced mutations from the screens described in Table 1 and 5 from a subsequent screen were mapped. Two-factor and three-factor data are reported in Table 2. The recombination frequency for the *dpy-5 unc-13* interval in a non-lethal strain is given in the first line of the table. These data were obtained from the strain KR236 (+(*sDp2*)/ *dpy-5 unc-13*/ *dpy-5 unc-13*), derived from a segregant of KR235. Unc-13 hermaphrodites from KR236 were crossed to N2 males and the recombination frequency in the resulting heterozygotes calculated from the Unc-13 recombinant class. In a similar manner, recombination frequencies were obtained for each of the lethal strains (identified in Table 2 by the allele carried). Data are reported only for the designated canonical allele of each comp-

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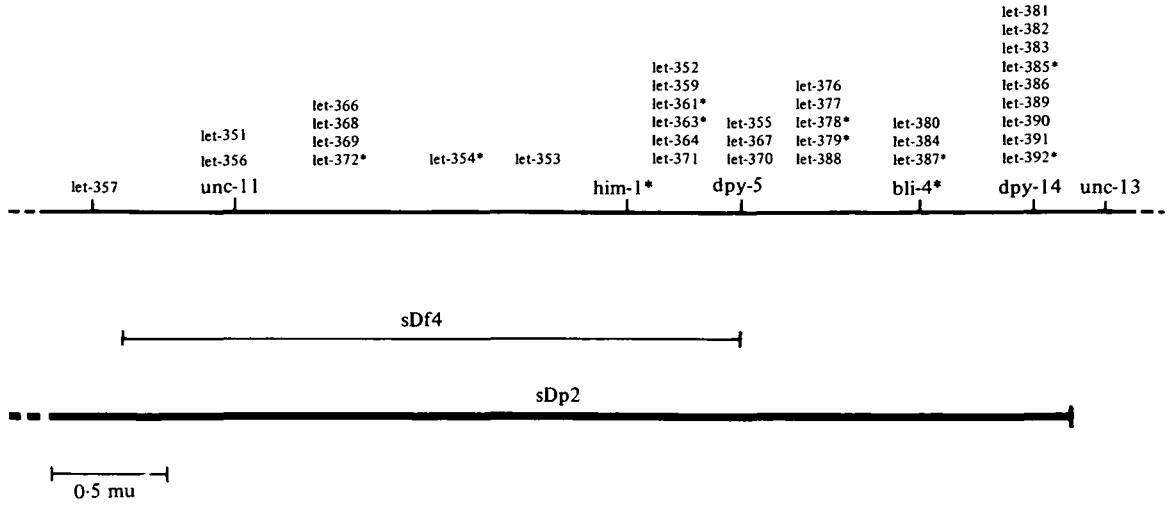

Fig. 3. Genetic map of the *sDp2* region emphasizing the 4 mu interval comprising the *unc-11 unc-13* region. *let-* genes are drawn in groups at 0·5 map unit intervals according to the data in Table 2. Four *let-* genes to the left of *unc-11* are not shown. Asterisks denote complementation groups represented by more than one EMS-induced allele.

lementation group. There were no anomalies in map position of alleles, i.e. all alleles of a gene mapped close to each other. For the lethal mutations which mapped to the left of *dpy-5*, Dpy Unc and Unc recombinant were expected. This was the case for 30 of the EMS-induced mutations. The distance the lethal maps from *dpy-5* was calculated from the Dpy Unc recombinant class. Section B of Table 2 gives the mapping data for the EMS mutations that were to the left of *dpy-5*. Dpy recombinants are not expected for this group of lethals since the cross-over chromosome (*let-x dpy-5* +) would carry the lethal and would not survive if fertilized by a *let-x dpy-5 unc-13* chromosome. In a few cases one cross-over chromosome *let-x dpy-5*+ was fertilized by another cross-over chromosome (+*dpy-5 unc-13*). Fourteen Dpy recombinants of this type were observed. A second possible type of Dpy recombinant could be the result of a double cross-over event (+*dpy-5* + /*let dpy-5 unc-13*). No Dpy recombinants of this type were observed, presumably the result of positive chromosomal interference. None of the Dpy recombinants listed in Section B of Table 2 of the latter type.

Section C shows the data for lethals mapping to the right of *dpy-5*. In these cases the distance to *dpy-5* was calculated from the Dpy-5 recombinant class. The distance from the lethal to *unc-13* was also calculated from the Unc-13 class (data not shown), and no anomaly was observed. Section D shows the data for the lethals that were inseparable from *dpy-5*.

(iii) *Complementation analysis*

All the EMS-induced lethal mutations that mapped to the left of *dpy-5*, and some of those that mapped to the right, were crossed to *sDf4* to test for complementation (see Fig. 3 for position of *sDf4*). Strains heterozygous for *sDf4* grow very slowly (even after back-crossing) and have low viability. A control cross was scored in order to determine the proper criteria for assessing complementation with *sDf4*. Heterozygous + + / *dpy-5 unc-13* males were generated by crossing N2 males to Unc-13 hermaphrodites from the KR236 strain. These males were mated to *sDf4*/ *bli-4 dpy-14* hermaphrodites and the out-cross progeny were scored. Sixteen Dpy-5 and 208 wild-type males were observed giving a control segregation ratio of 13:1 (predicted 3:1). Comparable numbers of wild-type and Dpy hermaphrodites were also present. The Dpy hermaphrodites grew slowly and gave few progeny. In complementation tests with the lethal-carrying strains, more than 50 wild-type males were scored in order to ensure that apparent allelism was not the result of *sDf4*-heterozygote inviability. Twenty of the lethals to the left of, and three inseparable from, *dpy-5* failed to complement *sDf4*, whereas all the tested lethals to the right of *dpy-5* complement *sDf4*. Since *sDf4* had been induced on a chromosome that was marked with *dpy-5* (*e61*), it is not known where the right breakpoint of *sDf4* is with respect to *dpy-5*. The right breakpoint of *sDf4* is to the left of *bli-4* (Rose, 1980). *sDf4* must not extend more than 0·03 map units to the right of *dpy-5* since *let-376* complements the deficiency (see Table 2, section C). *let-357*, which maps 3 mu to the left of *dpy-5*, is outside the deleted region, placing the left breakpoint of *sDf4* between *let-357* and *unc-11* (see Figure 3).

The 58 EMS-induced lethal mutations (the 53 described above and an additional 5 from a subsequent screen) were divided into the following three regions for the purposes of complementation analysis: (1) from the left end of the chromosome to the left breakpoint of *sDf4*; (2) inside *sDf4*; (3) from the right breakpoint of *sDf4* to the end of *sDp2*. All *inter se* complementation tests were carried out within each set (data not shown). The 9 lethal mutations in

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region 1 identify 5 complementation groups, 4 of which have 2 alleles. The 24 lethal mutations in region 2 define 18 complementation groups, 3 of which have 2 alleles and one which has 4. The 25 mutations in region 3 define 17 complementation groups, 4 of which have 2 alleles and one which has 4.

The 58 mutations define 40 genes. *let-363* is represented by four alleles, *h98*, *h111*, *h114* and *h131*. *let-385* is also represented by four alleles, *h85*, *h109*, *h135* and *h202*. Eleven are represented by two alleles, *let-354* (*h79*, *h90*), *let-357* (*h89*, *h132*), *let-358* (*h92*, *h99*), *let-361* (*h97*, *h113*), *let-362* (*h86*, *h93*), *let-365* (*h108*, *h129*), *let-372* (*h126*, *h234*), *let-378* (*h124*, *h181*), *let-379* (*h127*, *h186*), *let-387* (*h87*, *h183*) and *let-392* (*h120*, *h122*). The remaining 28 complementation groups are represented by one mutation.

Of the twenty visible genes in the region, four have been complemented to *sDp2*-rescued lethals. No lethal allele of *dpy-14* or *unc-87* was recovered. *h134* was found to be a lethal allele of *him-1* (Hodgkin, Horvitz & Brenner, 1979). All of the lethal mutations which mapped to the right of *dpy-5* were tested for complementation with *let-75*, *let-76*, *let-77* and *let-78* (Rose & Baillie, 1980). *h42* fails to complement *let-77*(*s90*) and *bli-4*(*e937*), but *let-77* and *bli-4* complement each other (K. Peters, unpublished results). Thus *h42* may be a small deletion, a two-hit event, or *s90* and *e937* are complementing alleles. It appears that *him-1* is an essential gene which was previously identified by a non-lethal allele.

Figure 3 is a map of the lethals described above. Four *let*-genes which map to the left of *let-357* are not shown. The *let-* genes are grouped in 0·5 map unit intervals according to the data in Table 2. The positions indicated are approximate, as left–right positioning between lethals has not yet been established. Asterisks denote genes which are represented by a canonical allele, and at least one additional *sDp2*-rescued EMS-induced lethal allele.

(iv) *Gamma-induced lethal mutations*

An initial analysis was done on a set of 29 lethal mutations induced with 1500 r of gamma radiation. Twelve lethal-bearing strains failed to yield informative recombinants in mapping experiments. Many of these gave extremely low brood sizes, which suggested that they could be carrying complex chromosomal rearrangements. These strains grew so poorly that they have not been further characterized.

Of the remaining 17 gamma-induced lethal mutations, 12 map to the left of *dpy-5* and five map to the right. Extensive complementation testing has been carried out with the mutations to the left against some visible markers and all the *sDp2*-rescued EMS-induced lethal mutations. None of these fails to complement more than one EMS-defined complementation group. *h330* is an allele of *unc-11* which was recovered as a lethal with *dpy-5 unc-13*; *h55* is a lethal allele of *him-1*; *h54* is an allele of *let-353*; *h60* is an allele of *let-363*: *h72* of *let-354*; and *h323* of *let-361*. Some lethal mutations mapped to a single site and complemented other lethals in the region; however, complementation group assignments have been made using only EMS-induced alleles.

4. Discussion

In this paper we report the development of a duplication system for identifying, maintaining and characterizing lethal mutations on the left third of LG *I* of *C. elegans*. Using this system we have recovered 87 mutations, and an initial analysis of 58 EMS-induced lethals has been presented. Thirty-three lethals mapping inseparably from or to the left of *dpy-5* represent 23 essential genes (in 15 mu). The twenty-five lethals mapping to the right of *dpy-5* represent seventeen essential genes (in 1·5 mu). This analysis has demonstrated the feasibility of using a free duplication as a genetic balancer for lethal mutations.

We have tested the effectiveness of the free duplication, *sDp2*, as a lethal rescue system. Lethals are induced in a strain designed to allow detection of new mutations in the F_2 generation. The KR235 strain can be easily maintained owing to the tight linkage between *unc-15* and *unc-13* (Waterston, Fishpool & Brenner, 1977; Rose & Baillie, 1980) and between *unc-15* and the *sDp2* breakpoint (Rose, Baillie & Curran, 1984). Over 29000 chromosomes have now been screened using this strain, with no indication of alteration in its genetic composition (data not shown). Although recombination between the normal homologues is considerably reduced in the presence of *sDp2*, it can occur (Rose, Baillie & Curran, 1984). Therefore, lethals ten or more map units to the left of *dpy-5* are not recovered about 5% of the time due to exchange between the lethal and *unc-13*.

It is a straightforward procedure to produce males with this system, because all the males resulting from an out-cross have the lethal-bearing chromosome. Those that carry the duplication are slow-developing, inefficient in mating and do not interfere with the analysis (Rose, Baillie & Curran, 1984). This simplifies and speeds up complementation testing by eliminating the need to maintain male strains that are heterozygous for the lethal or to identify the lethal-bearing males.

Our lethal mutations are maintained in a strain with an Unc-13 phenotype and that segregates only Unc progeny, all of which have an identical genotype. Thus these strains are self-maintaining over any number of generations, and transfer of the lethal-bearing individuals does not require prior familiarity with the phenotype of the strain. Furthermore, since *C. elegans* strains can be frozen, it should be possible to accumulate sufficient lethal mutations to approach saturation of the region.

Our efficiency of recovering gamma-induced mu-

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tations is considerably lower than that of *eT1*. Rosenbluth, Cuddeford & Baillie (1985) have analysed the range of mutational events generated with gamma radiation and shown that duplications, translocations, deletions and 'point' mutations are recovered with the *eT1* system. With the *sDp2* system we expect to recover only those lethal events that are rescued with wild-type alleles on the duplication. This would eliminate any deletions or translocations with one lethal breakpoint outside *sDp2*. It is unlikely that a second duplication of this region would be recovered, since this would produce a partial tetrasomic genome and it is known that individuals with two copies of *sDp2* are inviable (Rose, Curran & Baillie, 1984). In view of the very different nature of the *sDp2* and the *eT1* systems, and the non-uniform distribution of both lethals and visibles on the *C. elegans* map, the comparison of the relative induction rates may not be meaningful. A better comparison would be between *sDp2* and the reciprocal translocation *szT1* (Fodor & Deak, 1985), which balances the same region of LG*I*. Certain lethal mutations may not be recoverable by one wild-type allele in the presence of two mutant alleles. A comparison of the types of events recovered with the *sDp2* and *szT1* balancers is under way.

We would expect between 100 and 300 essential genes in the *sDp2* region, which extends for 17 map units from the *bli-3* end of LG *I* to *dpy-14* inclusive and comprises 5% of the genetic map, based on previous estimates of the number of genes in *C. elegans* (Brenner, 1974; Moerman & Baillie, 1979; Rogalski & Baillie, 1985). Thirty-nine previously unidentified complementation groups are described in this paper. We have currently recovered over 250 lethals for analysis using the *sDp2* system, and thus lethal saturation of this region appears to be a feasible goal.

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References

Anderson, P. & Brenner, S. (1984). A selection for myosin heavy chain mutants in the nematode *Caenorhabditis elegans*. *Proceedings of the National Academy of Sciences, USA* **81**, 4470–4474.

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

Fodor, A. & Deak, P. (1985). The isolation and genetic analysis of a *C. elegans* translocation (*szT1*) strain bearing an *X*-chromosome balancer. *Journal of Genetics* **64**, 143–157.

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

Judd, B. H., Shen, M. W. & Kaufman, T. C. (1972). The anatomy and function of a segment of the *X*-chromosome of *Drosophila melanogaster*. *Genetics* **71**, 139–156.

Meneely, P. M. & Herman, R. K. (1979). Lethals, steriles, and deficiencies in a region of the *X*-chromosome of *Caenorhabditis elegans*. *Genetics* **92**, 99–115.

Rogalski, T. R. & Baillie, D. L. (1985). Genetic organization of the *unc-22 IV* region of *Caenorhabditis elegans*. *Molecular and General Genetics* **201**, 409–414.

Rogalski, T. M., Moerman, D. G. & Baillie, D. L. (1982). Essential genes and deficiencies in the *unc-22* region of *Caenorhabditis elegans*. *Genetics* **102**, 725–736.

Rose, A. M. (1980). Genetic studies on the gene coding for paramyosin in *Caenorhabditis elegans*: *unc-15* and the adjacent region. Ph.D. thesis, Simon Fraser University, Burnaby, B.C., Canada.

Rose, A. M. & Baillie, D. L. (1979). The effect of temperature and parental age on recombination and nondisjunction in *Caenorhabditis elegans*. *Genetics* **92**, 21–30.

Rose, A. M. & Baillie, D. L. (1980). Genetic organization of the region around *unc-15* (*I*), a gene affecting paramyosin in *Caenorhabditis elegans*. *Genetics* **96**, 639-648.

Rose, A. M., Baillie, D. L. & Curran, J. (1984). Meiotic pairing behavior of two free duplications of linkage group *I* in *Caenorhabditis elegans*. *Molecular and General Genetics* **195**, 52–56.

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

Rosenbluth, R. E. & Baillie, D. L. (1981). Analysis of a reciprocal translocation, *eT1*(*III*;*V*), in *Caenorhabditis elegans*. *Genetics* **99**, 415–428.

Sigurdson, D. C., Spanier, G. J. & Herman, R. K. (1984). *C. elegans* deficiency mapping. *Genetics* **108**, 331–345.

Waterston, R. K., Fishpool, R. M. & Brenner, S. (1977). Mutants affecting paramyosin in *Caenorhabditis elegans*. *Journal of Molecular Biology* **117**, 679–698.

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